8. GENOTOXICITY: IN VITRO

Table 8.1 cys-mcMMAF (SGD-1362): Bacterial Mutation Assay (Ames Test)

Report Title: Bacterial Reverse Mutation Assay

Study No: AE01BX.502ICH.BTL

GSK Document No: 2019N406489

Location in CTD: m4.2.3.3.1

Test Article: cys-mcMMAF (SGD-1362)

Batch Number: [redacted]

Test for Induction of: Reverse mutation in bacterial cells

Species/Strain: *S. typhimurium, E. coli*

Metabolizing System: Aroclor-induced rat liver S9

Vehicle for Test Article: DMSO

Treatment: Plate incorporation

Cytotoxic Effects: None

Genotoxic Effects: None

No. of Independent Assays: 1

No. of Replicate Cultures: 3

No. Cells Analyzed/Culture: 1.4 to 3.7 x 10^8 cells / plate

GLP Compliance: Yes

Vehicle for Positive Controls: DMSO, except sterile water for sodium azide

Date of Treatment: 28 October 2014

cys-mcMMAF (SGD-1362): Bacterial Mutation Assay (Ames Test) (Continued)

Study No: AE01BX.502ICH.BTL　　Test Article: SGD-1362

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mutagenicity Assay Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvrA*
Without Activation	DMSO	100 µL/plate	15 ± 7	88 ± 16	14 ± 4	5 ± 2	17 ± 8
	SGD-1362	50	10 ± 2	83 ± 17	11 ± 5	3 ± 2	19 ± 6
		150	11 ± 4	88 ± 9	15 ± 9	4 ± 4	22 ± 4
		500	8 ± 1	88 ± 5	13 ± 4	3 ± 3	22 ± 12
		1500	6 ± 3	90 ± 10	10 ± 5	6 ± 4	17 ± 4
		5000	13 ± 9	83 ± 15	12 ± 3	5 ± 2	19 ± 6
	2NF	1.0	118 ± 18				
	SA	1.0		409 ± 6	470 ± 12		
	9AAD	75				274 ± 96	
	MMS	1000					299 ± 40
With Activation	DMSO	100 µL/plate	23 ± 6	110 ± 4	16 ± 6	8 ± 2	29 ± 8
	SGD-1362	50	21 ± 3	106 ± 6	13 ± 2	4 ± 2	21 ± 3
		150	21 ± 10	115 ± 25	13 ± 6	6 ± 2	27 ± 3
		500	18 ± 8	115 ± 9	19 ± 2	8 ± 3	30 ± 5
		1500	22 ± 7	104 ± 3	15 ± 3	7 ± 2	22 ± 2
		5000	21 ± 6	106 ± 10	18 ± 1	7 ± 2	23 ± 8
	2AA	1.0	378 ± 82		99 ± 14		
	2AA	2.0		659 ± 73		46 ± 7	
	2AA	15					268 ± 37

Key to Positive Controls

SA	sodium azide
2AA	2-aminoanthracene
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate

Table 8.2 cys-mcMMAF (SGD-1362): In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Report Title: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Test Article: cys-mcMMAF (SGD-1362)
Batch Number: [redacted]

Study No: AE01BX.704ICH.BTL

GSK Document No: 2019N406496

Location in CTD: m4.2.3.3.1

Test for Induction of: Forward mutations at TK locus

No. of Independent Assays: 1

Study No.: AE01BX.704ICH.BTL

Strain: L5178Y

No. of Replicate Cultures: 2 for vehicle and test article, 1 for positive control

No. of Cells Analyzed/Culture: 3X10^6

Metabolizing System: Aroclor-induced rat liver S9

GLP Compliance: Yes

Test Article Vehicle: DMSO

Positive controls: Non-activated: Methyl methanesulfonate (MMS) in sterile, distilled water; S9-activated: 7, 12 Dimethylbenz(a)anthracene (DMBA) in DMSO

Treatment: 4 hours with and without S9 activation, 24 hours without activation

Date of Treatment: 04 November 2014

Cytotoxic Effects: In the mutagenesis assay, the relative total growth (RTG) of the cloned cultures ranged from 22 to 79% (4-hour treatment with S9), 14 to 85% (4-hour treatment without S9) and 14 to 95% (24-hour treatment without S9).

Genotoxic Effects: No increases in induced mutant frequency ≥90 mutants per 10^6 clonable cells were observed under any treatment condition.

cys-mcMMAF (SGD-1362): *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay (Continued)

Study No: AE01BX.704ICH.BTL　　Test Article: SGD-1362

Metabolic Activation and Exposure Time	Test or Control Article	Concentration (µg/mL)	Relative Total Growth (% of Control)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
Without Activation 4 Hours	DMSO	NA	100	35	NA
		NA		44	
	SGD-1362	4	82	53	13
		4	85	32	-7
		5.34	****	****	****
		5.34	****	****	****
		7.12	72	52	13
		7.12	72	55	15
		9.49	****	****	****
		9.49	****	****	****
		12.7	33	59	19
		12.7	30	69	29
		16.9	14	103	64
		16.9	18	57	17
		22.5	3	++	++
		22.5	6	++	++
		30	+	+	+
		30	+	+	+
	MMS	20	5	366	327
		15	11	398	358

**** - Discarded prior to cloning because a sufficient number of higher concentrations was available
+ - Discarded prior to cloning due to excessive toxicity
++ - Excluded from evaluation of mutagenicity due to excessive toxicity

cys-mcMMAF (SGD-1362): *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay (Continued)

Study No: AE01BX.704ICH.BTL Test Article: SGD-1362

Metabolic Activation and Exposure Time	Test or Control Article	Concentration (µg/mL)	Relative Total Growth (% of Control)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
With S9 Activation 4 Hours	DMSO	NA	100	66	NA
		NA		43	
	SGD-1362	4	****	****	****
		4	****	****	****
		5.34	64	56	1
		5.34	79	44	-11
		7.12	****	****	****
		7.12	****	****	****
		9.49	55	40	-15
		9.49	49	61	6
		12.7	27	90	35
		12.7	42	68	13
		16.9	22	86	31
		16.9	25	41	-14
		22.5	2	++	++
		22.5	6	++	++
		30	2	++	++
		30	3	++	++
	DMBA	1.5	8	543	488
		1.0	26	320	266

**** - Discarded prior to cloning because a sufficient number of higher concentrations was available
++ - Excluded from evaluation of mutagenicity due to excessive toxicity

cys-mcMMAF (SGD-1362): *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay (Continued)

Study No: AE01BX.704ICH.BTL Test Article: SGD-1362

Metabolic Activation and Exposure Time	Test or Control Article	Concentration (µg/mL)	Relative Total Growth (% of Control)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
Without Activation 24 Hours	DMSO	NA	100	38	NA
		NA		39	
	SGD-1362	0.119	****	****	****
		0.119	****	****	****
		0.238	****	****	****
		0.238	****	****	****
		0.475	89	31	-7
		0.475	95	32	-7
		0.95	****	****	****
		0.95	****	****	****
		1.27	62	44	5
		1.27	90	19	-20
		1.69	57	43	4
		1.69	67	36	-2
		2.25	39	44	5
		2.25	45	37	-2
		3	19	56	17
		3	14	69	30
		4	+	+	+
		4	+	+	+
		5.34	+	+	+
		5.34	+	+	+
		7.12	+	+	+
		7.12	+	+	+
	MMS	7.5	16	432	393
		5	10	276	237

**** - Discarded prior to cloning because a sufficient number of higher concentrations was available
+ - Discarded prior to cloning due to excessive toxicity

Table 8.3 Belantamab Mafodotin: In vitro Micronucleus Assay using Cultured Human Peripheral Blood Lymphocytes (Screening Study)

Study Type: Genetic Toxicology and Photosafety In Vitro

Report Title: GSK2857916: In vitro Micronucleus Assay using Cultured Human Peripheral Blood Lymphocytes (screening study)

Test Article: Belantamab mafodotin

Batch Number: [redacted]

Test for Induction of: Micronuclei in binucleate cells

No. of Independent Tests: Two tests comprising 24 hour treatment in the absence of S9-mix, with no recovery or with 24 hour recovery

Study No: hVMNT56

GSK Document No: 2019N416146

Cell Type: Human Peripheral Blood Lymphocytes

No. of Replicate Cultures: 3 (vehicle); 1 (treatment and positive control)

Location in CTD: m4.2.3.3.1

Metabolising System: not applicable

Vehicles

GLP Compliance: No

Test Article: 25 mM aqueous citrate buffer containing 0.05 mM EDTA, 200 mM Trehalose and 0.02% Polysorbate 80 (pH 6.2)

Positive Controls: DMSO

Treatment: Treatment for 24 hours in the absence of S9-mix with no recovery; treatment for 24 hours in the absence of S9-mix with 24 hour recovery.

Date of Treatment(s): July 2019

Cytotoxic Effects: None

Genotoxic Effects: Genotoxic following treatment for 24 hours in the absence of S9-mix with 24 hour recovery

Test Article	Nominal Concentration[a]	24 hour treatment with no recovery, -S9-mix			24 hour treatment with 24 hour recovery, -S9-mix		
	µg/mL	Mean %MN[b]	Fold Increase	% Cytotoxicity[c]	Mean %MN[b]	Fold Increase	% Cytotoxicity[c]
Dimethyl sulphoxide	0	0.60	-	-	0.53	-	-
Belantamab mafodotin	577	1.19[e]	2.0	7	1.42[e,f]	2.7	0
Carbendazim	2	3.21[d]	5.4	26	2.43[d]	4.6	31
Methyl Methane Sulphonate	20	4.90[d]	8.2	22	2.85[d]	5.4	0

Key:

a = Concentration expressed in terms of parent compound.
b = Based on 2000 binucleate cells analysed.
c = Cytotoxicity calculated from Cytochalasin B Proliferation Index values (i.e. % reduction compared with the concurrent vehicle control)
d = Positive control induced an acceptable response.
e = Statistically significant ($p<0.05$) increase in micronucleated binucleate cells (Chi square test, one sided with Yates' correction)
f = Exceeded the laboratory historical control range.
MN Micronuclei (in binucleate cells)

9. GENOTOXICITY: IN VIVO

Table 9.1 In Vivo Micronucleus Assay in Rats with SGD-1362 (cys-mcMMAF)

Test for Induction of: Bone marrow micronuclei

Species/Strain: Sprague-Dawley (Hsd:SD) rats

Age: 6 weeks

Treatment Schedule: Once

Sample Time: Approximately 24 or 48 hours post dose

Method of Administration:

Test Article and Vehicle: Single intravenous bolus injection, 5 mL/kg

Positive Control: Oral gavage, 10 mL/kg

Study Number: AE01BX.125M012ICH.BTL

Lot Number: [redacted]

GLP Compliance: Yes
GSK Document No: 2019N406497
Location in CTD: m4.2.3.3.2

Cells Evaluated: PCE and Total erythrocytes

Vehicle for Test Article Formulation: Phosphate buffered saline

Vehicle/Positive Control: 0.9% saline/Cyclophosphamide

Date of Treatment: 29 September 2014

Number of Cells Analyzed/Animal: ~ 2000 PCEs/animal

Special Features: Dose Formulation Analysis

Toxic/Cytotoxic Effects: No mortality occurred at any dose level during the course of the definitive assay. Clinical signs included piloerection, which was observed at 25 mg/kg. Piloerection and lethargy were also observed in the positive control-treated group. All other rats appeared normal throughout the observation period.

Genotoxic Effects: No statistically significant increase in the incidence of mnPCEs in the test-article treated groups was observed relative to the negative control group ($p > 0.05$, Kastenbaum-Bowman tables). The positive control induced a statistically significant increase in the incidence of mnPCEs ($p < 0.05$, Kastenbaum-Bowman tables). The number of mnPCEs in the vehicle control groups did not exceed the historical control range.

Evidence of Exposure: N/A

In Vivo Micronucleus Assay in Rats with SGD-1362 (Continued)

Sampling Time	Controls/Test Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Sex/No. of Animals/Group	PCE/ Total Erythrocyte (Mean± SD)	Number mnPCE/PCE scored
24 hrs post-dose	1X PBS	0	5	Males 5	0.514 ± 0.01	1 / 10,000
	SGD-1362	10	5	Males 5	0.518 ± 0.01	3 / 10,000
	SGD-1362	17.5	5	Males 5	0.521 ± 0.01	3 / 10,000
	SGD-1362	25	5	Males 5	0.519 ± 0.01	3 / 10,000
	Cyclophosphamide (40 mg/kg)	40	10	Males 5	0.560 ± 0.01	*277 / 10,000
48 hrs post-dose	1X PBS	0	5	Males 5	0.522 ± 0.01	2 / 10,000
	SGD-1362	25	5	Males 5	0.521 ± 0.01	5 / 10,000

*Statistically significant increase, $p \leq 0.05$ (Kastenbaum-Bowman Tables, binomial distribution)
PCE: Polychromatic Erythrocytes; mnPCE: Micronucleated Polychromatic Erythrocytes

10. CARCINOGENICITY

No studies of this type were conducted.

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: NON-PIVOTAL STUDIES

No studies of this type were conducted.

12. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION (PIVOTAL)

No studies of this type were conducted.

13. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON EMBRYOFETAL DEVELOPMENT (PIVOTAL)

No studies of this type were conducted.

14. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT INCLUDING MATERNAL FUNCTION (PIVOTAL)

No studies of this type were conducted.

15. STUDIES IN JUVENILE ANIMALS

No studies of this type were conducted.

16. LOCAL TOLERANCE

No stand-alone studies have been conducted.

17. OTHER TOXICITY STUDIES

Table 17.1 Evaluation of Cytokine Release in an in vitro Human Peripheral Blood Mononuclear Cell Assay

Study Type: Other Toxicity	**Report Title:** Evaluation of Cytokine Release in an in vitro Human Peripheral Blood Mononuclear Cell Assay	**Test Article:** GSK2857914 **Batch No.:** [redacted]
Species/Strain: Human Peripheral Blood Mononuclear Cells	**Duration of Dosing:** N/A **Route/Frequency:** Ex Vivo/In vitro	**Study No.:** V70385N **GSK Document Number:** 2013N173704 **Location in CTD:** m4.2.3.7.2
Initial Age: N/A	Vehicle/Formulation: NA	**Study in Compliance with GLP:** No

Data Collected: In vitro cytokine stimulation, H929 depletion by flow cytometry, NFκβ signalling by H929 cells

Conclusions: This study evaluated the potential of GSK2857914 to induce an in vitro cytokine response (IL-6, IL-8, IL-10, IFN-γ, TNF-α, or MCP-1) in peripheral blood mononuclear cells (PBMCs) from 10 healthy human volunteer donors cultured with and without BCMA expressing H929 cells. GSK2857914 (up to 10 μg/mL) did not induce a cytokine response in human PBMCs in the absence of H929 cells. Furthermore, cytokine induction was not detected when H929 cells were stimulated with GSK2857914 in the absence of PBMCs, except for slight increase in IL-10. When PBMCs were co-cultured with H929 target cells, GSK2857914 induced IL-8, IFN-γ, and TNF-α at concentrations ≥0.1 μg/mL (up to 21.5X, 39.5X, and 23.8X, respectively, relative to unstimulated control). Based on intracellular cytokine staining of cell cultures stimulated with GSK2857014, IFN-γ, and TNF-α were produced predominantly by NK cells and a small proportion of CD8+ cells, while IL-8 was produced by monocytes. These data indicate that pronounced cytokine release in vitro requires the presence of both target and effector cells, and that the under the conditions of this assay, cytokine is released from effector cells. However, GSK2857914, when cross linked with anti-human IgG antibodies, induced BMCA signalling in H929 cells as measured by increased NFκβ activation, indicating that GSK2857914 has potential to impart agonist activity on BCMA positive target cells as well.

Table 17.2 Investigation of Uptake of Vivotag-680 Labelled GSK2857916A into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial Cells

Report No.: 2019N409936
Study No.:
Location in CTD: m4.2.2.3

Compounds: Belantamab mafodotin or GSK2857914

Batch Number: Belantamab mafodotin [redacted]
GSK2857914 [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: HCEC or RPTEC

Data Collected: Cellular Uptake [Fluorescent probe (Vivotag 680 XL); Fluorescence microscopy]

Conclusion: The uptake of belantamab mafodotin into HCEC was significantly reduced by 25.9% following treatment with EIPA [5-(N-Ethyl-N-isopropyl)amiloride; commonly used as a macropinocytosis inhibitor]. EIPA appeared to have no effect on uptake of belantamab mafodotin into RPTEC. Further, EIPA did not significantly inhibit 10,000 MW Alexa488-labelled dextran uptake in HCEC or RPTEC. These findings suggest that an uptake route of belantamab mafodotin into HCEC was micropinocytosis mediated. Intracellular co-localisation of vivotag-680 labelled belantamab mafodotin within lysosomes of HCEC and RPTEC was demonstrated using lysotracker green as a marker of lysosomes.

HCEC and RPTEC were incubated with 100µg/mL belantamab mafodotin or GSK2857914 for 48 hours. Cys-mcMMAF was not detected in the untreated control or the GSK2857914-dosed control, but it was detected for all biological replicates in belantamab mafodotin-dosed cells, both RPTEC and HCEC. Thus, demonstrating the capacity of the cells to catabolise belantamab mafodotin, releasing cys-mcMMAF.

Investigation of Uptake of Vivotag-680 Labelled GSK2857916A into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial Cells (Continued)

Species	Noteworthy findings
Human	Uptake of Vivotag-680 labelled belantamab mafodotin and GSK2857914 into Human Corneal Epithelial Cells (HCEC) as well as Renal Proximal Tubule Epithelial cells (RPTEC) was detected by spinning disk confocal microscopy. Uptake was detected after 20 hours or 44 hours at two concentrations, 10 µg/mL and 100 µg/mL. Uptake was found to be concentration dependent as well as time dependent in HCEC and RPTEC. Overall, uptake of belantamab mafodotin was higher in RPTEC compared to HCEC and there was a greater difference in compound fluorescence for RPTEC incubated with 10 µg/mL and 100 µg/mL of compound compared to HCEC incubated with 10 µg/mL and 100 µg/mL of compound. There was no cell count change for RPTEC after 20 hours of treatment with belantamab mafodotin and no reduction of cell count after 44 hours compared to the GSK2857914 control. A disruption of the cell layer, however, was observed in RPTEC following incubation with belantamab mafodotin when compared to control cells, indicating a deleterious effect on the health of these cells. Cells that were disrupted were retained in the cell layer which could explain why there was no change in the cell number. In HCEC, a time dependent decrease in the number of cells was observed, with 75% of cells present after 20 hours and 68% of cells after 44 hours when treated with belantamab mafodotin. No visible effects were seen on microscopy images of HCEC incubated with belantamab mafodotin and no disruption of the cell layer was observed indicating that dead cells had been washed off before imaging. For both cell types, no adverse effects on cell health were observed following incubation with GSK2857914. Donor variability was tested for HCEC donors CE435B1 and CE9060216B1. No difference between donors was seen for the uptake of Vivotag-680 labelled belantamab mafodotin and GSK2857914 into cells, which was concentration and time dependent. The nuclei count after treatment was also consistent between donors, which showed lower nuclei count when cells were treated with belantamab mafodotin compared to cells treated with GSK2857914 or control cells. EIPA (12.5 µM) treatment of HCEC incubated with belantamab mafodotin led to a significant reduction of fluorescent signal inside the cells indicating a reduction in uptake of belantamab mafodotin. However, treatment of HCEC with EIPA did not alter Dextran uptake, which was used as a positive control marker for macropinocytosis. Cell count was not significantly affected when compared to EIPA treated control HCEC. However, treatment with EIPA alone reduced the cell count when compared to non-treated control HCEC. As fluorescence was measured as mean fluorescent intensity per cell different cell numbers did not affect quantification. At 12.5 µM, EIPA did not affect the uptake of belantamab mafodotin in RPTEC or affect the cell number. However, treatment of RPTEC with EIPA also did not alter Dextran uptake, which was used as a positive control marker for macropinocytosis, possibly because the concentration EIPA at 12.5 µM was too low. Cell associated cys-mcMMAF was quantified using LC-MS/MS from bulk HCEC and RPTEC cultures. Following incubation of belantamab mafodotin with HCEC and RPTEC cells, there was evidence for co-localisation with lysosomes and catabolism releasing cys-mcMMAF was observed

Key:
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).
HCEC = Human corneal epithelial cells
LC/MS/MS = Liquid chromatography with mass spectrometry.
RPTEC = Renal Proximal Tubule Epithelial Cells

Table 17.3 Investigations to Assess the Cytotoxicity of Belantamab Mafodotin (GSK2857916) in Primary Human Renal Proximal Tubule Epithelial (RPTEC) and Corneal Epithelial Cells (HCEC)

Report No.: 2019N410330
Study No.: N70517-1
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin, GSK2857914, Vinblastine sulphate

Batch Number: Belantamab mafodotin [redacted] and GSK2857914 [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Cytotoxicity Assays (Caspase 3/7, CellTiter-Blue)

Conclusion: Cytotoxicity of belantamab mafodotin (0.1-577 µg/mL) to HCEC and RPTEC primary cells as assessed using caspase 3/7 and CellTiter-Blue assays was demonstrated at concentrations of ≥30 µg/mL and ≥100 µg/mL, respectively, at 48 hours post treatment. Experiments conducted in monolayers of human primary corneal epithelial and renal proximal tubule epithelial cells indicate that belantamab mafodotin exposure results in significant concentration-dependent increases in apoptosis in both cell types in vitro. This is consistent with the mechanism through which mcMMAF, the cytotoxic conjugate payload, is believed to induce cell death. The effects were observed in multiple donors for each cell type and there is a trend for HCECs being slightly more sensitive than RPTECs when assessing the lowest tested concentrations that caused a significant increase in apoptosis over vehicle controls (belantamab mafodotin concentrations of 30 µg/mL in HCECs and 100 µg/mL in RPTECs at 48 hours). However, inter-donor variability was higher in HCECs and when defining the belantamab mafodotin 'minimum effective concentration' (MEC) only a marginal difference was noted, and confidence intervals overlapped for both cell types (HCEC MEC = 50.4 µg/mL [95% CI 16.0 –202.0]; RPTECMEC = 70.7 µg/mL [95% CI 32.2-100.3]. Mechanistic experiments were conducted to explore what effect the macropinocytosis inhibitor5-(N-Ethyl-N-isopropyl)amiloride (EIPA) had on belantamab mafodotin-mediated cytotoxicity. These investigations demonstrated that pre/co-treatment with EIPA significantly reduced belantamab mafodotin-mediated apoptosis in RPTECs (three donors) and there was a similar trend in HCECs (single donor assessed on three occasions). These experiments included the negative control, GSK2857914 (afucosylated parent antibody, which also has enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity) and vinblastine sulphate (comparator tubulin inhibitor). Treatment with GSK2857914 showed no significant change in cytotoxicity (apoptosis or cell viability) for both cell types after 48-hours.

The findings of this study are consistent with belantamab mafodotin-mediated cytotoxicity via apoptosis. There was a trend for HCEC cells showing slightly greater sensitivity than RPTECs, although donor-to-donor variability would necessitate additional studies to determine whether this is a true difference. Given the lack of significant BCMA expression confirmed in HCEC sand RPTECs, a non-specific pathway of belantamab mafodotin uptake is likely involved in this cytotoxicity. Based on the clear trend for a reduction belantamab mafodotin-mediated apoptosis with EIPA pre/co-treatment, the evidence suggests that macropinocytosis plays a role in this non-specific uptake in HCECs and RPTECs in vitro.

Investigations to Assess the Cytotoxicity of Belantamab Mafodotin in Primary Human Renal Proximal Tubule Epithelial (RPTEC) and Corneal Epithelial Cells (HCEC) (Continued)

Species	Noteworthy findings
Human	Experiments conducted in HCEC and RPTECs indicate that belantamab mafodotin exposure results in significant concentration-dependent increases in apoptosis in both cell types. There was a trend for HCECs being slightly more sensitive than RPTECs when assessing the lowest tested concentrations that caused a significant increase in apoptosis over vehicle controls at 48 hours (30 μg/mL in HCECs and 100 μg/mL in RPTECs). However, inter-donor variability was higher in HCECs and when defining the MEC only a marginal difference was noted, and confidence intervals overlapped for both cell types (HCEC MEC = 50.4 μg/mL [95% CI 16.0 – 202.0]; RPTEC MEC = 70.7 μg/mL [95% CI 32.2-100.3]. Mechanistic experiments demonstrated that pre/co-treatment with the macropinocytosis inhibitor EIPA significantly reduced belantamab mafodotin-mediated apoptosis in RPTECs (three donors) and there was a similar trend in HCECs (single donor assessed on three occasions).

Key:
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).

Table 17.4 Repeat of Investigation into the Effect of GSK2857916 on Primary Human Corneal Epithelial Cells (HCEC) and the Impact that Inhibition of Macropinocytosis has on GSK2857916-mediated Cytotoxicity and Apoptosis

Report No.: 2020N437131
Study No.: N70517-6
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin, GSK2857914, Vinblastine sulphate

Batch Number: Belantamab mafodotin [redacted] and GSK2857914 [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Cytotoxicity Assays (Caspase 3/7, CellTiter-Blue)

Conclusion: Experiments were conducted to confirm what effect the macropinocytosis inhibitor EIPA had on belantamab mafodotin-mediated cytotoxicity in HCEC. These investigations demonstrated that pre/co-treatment with EIPA significantly reduced belantamab mafodotin-mediated apoptosis in HCECs (all four donors assessed on a single occasion at passage 3). These experiments included the negative control, GSK2857914 (afucosylated parent antibody, which also has enhanced ADCC activity) and vinblastine sulphate (comparator tubulin inhibitor).

The findings of this study are consistent with those concluded in Report 2019N410330, m4.2.3.7.3, confirming belantamab mafodotin-mediated cytotoxicity via apoptosis is observed. Given the lack of significant BCMA expression confirmed in HCECs [Report 2019N410329, m4.3], a non-specific pathway of belantamab mafodotin uptake is likely involved in this cytotoxicity. Based on the clear trend for a reduction belantamab mafodotin-mediated apoptosis with EIPA pre/co-treatment, the evidence strongly suggests that macropinocytosis plays a role in this non-specific uptake in HCECs in vitro.

Species	Noteworthy findings
Human	Experiments confirmed that pre/co-treatment with the macropinocytosis inhibitor ≥12.5 µM EIPA significantly reduced belantamab mafodotin-mediated apoptosis at ≥ 10 µg/mL in HCECs when combined with data previously obtained from HCECs.

Key:
Belantamab mafodotin = GSK2857916.
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).

Table 17.5 Investigation of the cytotoxic effect of the uptake of Cys-mcMMAF (SGD-1362) into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial Cells

Report No.: 2021N478152
Study No.: ELN23187
Location in CTD: m4.2.3.7.3

Compounds: cys-mcMMAF

Batch Number: cys-mcMMAF (SGD-1362) [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Fluorescence microscopy (Hoechst nuclear stain, CellTracker Green)

Conclusion:

Cytotoxicity drug responses following treatment with cys-mcMMAF as determined by a reduction in cell nuclei was observed in both cell lines, HCEC and RPTEC, however, the effect was more marked in HCEC. For HCEC the cytotoxic effect was seen at 0.5 µg/mL (32% reduction) while for RPTEC it was only observed at 10 µg/mL (24% reduction). Therefore, the cytotoxic effect of cys-mcMMAF was 20 times greater in HCEC compared with HCEC. Furthermore, the degree of cellular toxicity was greater in HCEC compared with RPTEC, approximately 70% of HCEC died after treatment with cys-mcMMAF at 5 µg/mL while for RPTEC the number was only approximately 25% at 100 µg/mL.

Species	Noteworthy findings
Human	The cytotoxic effect of cys-mcMMAF was assessed following uptake into HCEC (5 donors) and RPTEC (1 donor). Nuclei count in HCEC and RPTEC was tested following cys-mcMMAF treatment for 48 hours at concentrations of 0.1, 0.5, 1, 5, 10, 50 and 100 µg/mL. HCEC appear to be more sensitive to the effects of cys-mcMMAF, as cytotoxicity determined by a reduction in Hoechst stained nuclei was observed at concentrations as low as 0.5 µg/mL whilst for RPTEC the cytotoxic effect was only statistically significant at 10 µg/mL and 100 µg/mL. There was also a significant difference in the degree of the cytotoxicity observed. Sixty-eight percent of HCEC died after treatment with cys-mcMMAF at 5µg/mL while for RPTEC the number was only approximately 26% at 100 µg/mL. A similar dose response was seen for all donors from ATCC and MatTek, however no response was seen in the Merck/Millipore donor.

Key:
HCEC = Human corneal epithelial cells
RPTEC = Renal proximal tubule epithelial cells.

Table 17.6 Investigation of the cytotoxic effect of the uptake of Cys-mcMMAF and MMAE into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial Cells

Report No.: 2022N506460
Study No.: ELN55873/ 55667
Location in CTD: m4.2.3.7.3

Compounds: cys-mcMMAF, MMAE

Batch Number: GSK4025948C N15 labelled cys-mcMMAF ([redacted]) MMAE [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Fluorescence microscopy (Hoechst nuclear stain, CellTracker Green)

Conclusion:

This study sought to compare the cytotoxic effect of cys-mcMMAF and MMAE in human corneal epithelial cells and renal proximal tubule epithelial cells following exposure to concentrations of 5 ng/mL to 50 µg/mL. Concentration responses, as determined by a reduction in cell nuclei, were observed for both HCEC and RPTEC following treatment with cys-mcMMAF, however, the effect was significantly more marked in HCEC. A cytotoxic effect was observed in HCEC cells dosed with at 500 ng/mL cys-mcMMAF, and the decrease in nuclei numbers was statistically significant ranging between 34.7% and 79.9%, with a clear concentration response. For RPTEC, cell counts showed a concentration response from 5 µg/mL, with 32.1% and 43.4% decreases reaching statistical significance at 10 and 50 µg/mL, respectively. These findings demonstrate a cytotoxic effect of cys-mcMMAF on HCEC and RPTEC. When comparing the cytotoxic concentration of cys-mcMMAF, in HCEC the MMAE cytotoxicity was at least 100 times greater than cys-mcMMAF, leading to reductions in nuclei count up to 82% at concentrations ≥5 ng/mL, and in RPTEC it was 1000 times greater, leading to reductions in nuclei count up to 69.2% at concentrations ≥10 ng/mL, reflecting the lower cell permeability of cys-mcMMAF.

Species	Noteworthy findings
Human	This study was conducted to compare the cytotoxic effect of cys-mcMMAF and MMAE following uptake into HCEC (up to 6 donors) from different suppliers and RPTEC (up to 3 donors). Nuclei count was determined in HCEC or RPTEC following cys-mcMMAF or MMAE treatment for 48 hours at concentrations of 5, 10, 20, 50, 100, 200 and 500 ng/mL, and 1, 5, 10, 50 µg/mL. The results indicate that the cytotoxicity of cys-mcMMAF in HCEC (n=6 donors) and RPTEC (n= 3 donors) follows a concentration response curve, while MMAE was cytotoxic at all concentrations tested. In both instances, treatment with cys-mcMMAF and MMAE, HCEC were more sensitive than RPTEC to their cytotoxic effects. In HCEC, when treated with cys-mcMMAF, statistically significant cytotoxicity was observed at concentrations ≥0.5 µg/mL leading to a reduction in cell number of between 34.7% and 79.9%. In RPTEC statistically significant cytotoxicity following treatment with cys-mcMMAF was observed at concentrations of 10 µg/mL and 50 µg/mL leading to a reduction in cell number of 32.1% and 43.4%, respectively. Therefore, HCEC were 20 times more sensitive to cys-mcMMAF treatment than RPTEC in these experiments. MMAE was significantly more cytotoxic to both HCEC and RPTEC. In HCEC (n=4 donors), concentrations of ≥5ng/mL led to a reduction in cell number of up to 82%. Whilst for RPTEC (n=1 donor) significant cytotoxicity was noted at ≥10ng/mL with a reduction in cell number of up to 69.2%. When comparing the cytotoxic concentration of cys-mcMMAF and MMAE, in HCEC the MMAE cytotoxicity was 100 times greater that cys-mcMMAF, and in RPTEC it was 1000 times greater.

Key:
HCEC = Human corneal epithelial cells
RPTEC = Renal proximal tubule epithelial cells.

Table 17.7 Investigation into the effect of belantamab mafodotin on Primary Human Corneal Epithelial cells and the impact of using Immunoglobulins in excess, has on belantamab mafodotin-mediated cytotoxicity and apoptosis in multiple donors

Report No.: 2022N507716
Study No.: ELN43728
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin, GSK2857914

Batch Number: Belantamab mafodotin [redacted], GSK2857914[redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Cytotoxicity Assays (Caspase 3/7 (48 hours), CellTiter-Blue (72 hours)

Conclusion:

Experiments in primary HCEC were conducted to investigate the effect that using excess immunoglobulins (IVIG and GSK2857914) had on belantamab mafodotin-mediated cytotoxicity after a 48- or 72-hour treatment period. The test hypothesis was that pre/co-treatment of HCECs with an excess of immunoglobulins could competitively reduce uptake of belantamab mafodotin and ameliorate associated cytotoxicity. The findings of this study are consistent with the previous study [2020N437131], confirming belantamab mafodotin -mediated cytotoxicity via apoptosis is observed after 48 hours of treatment. Also consistent with previous studies, treatment of HCEC with belantamab mafodotin at up to 100 µg/mL for 72 hours (with/without the various pre/co-treatments) did not result in any clear impact on the cell viability endpoint. Pre/co-treatment with 15 mg/mL IVIG significantly reduced belantamab mafodotin -mediated apoptosis at 100 µg/mL, and 20 mg/mL IVIG significantly reduced belantamab mafodotin -mediated apoptosis at ≥50 µg/mL (with trends for reduction at the immediately preceding IVIG concentrations), in HCECs over 48 hours (at least three donors assessed on one occasion). For GSK2857914 as a competitive blocker, it was not possible to evaluate meaningful pre/co-treatment concentrations as ≥1.5 mg/mL was previously shown to cause cytotoxicity. These investigations demonstrated that pre/co-treatment with IVIG significantly reduced belantamab mafodotin -mediated apoptosis in HCECs in a concentration dependant manner. These data strongly suggest that competitively blocking uptake using high concentrations of immunoglobulin G ameliorates belantamab mafodotin -mediated cytotoxicity.

Species	Noteworthy findings
Human	Pre/co-treatment of HCEC with IVIG (≥8 mg/mL) resulted in a clear trend for concentration-dependent reduction in belantamab mafodotin -mediated apoptosis at ≥50 µg/mL belantamab mafodotin at 48 hours. At 50 µg/mL belantamab mafodotin, the trend for reduction in apoptosis was observed at ≥15 mg/mL IVIG, with the effect being statistically significant at 20 mg/mL IVIG. The trend was more pronounced following treatment with 100 µg/mL belantamab mafodotin, with a reduction in apoptosis observed at ≥8 mg/mL IVIG which was statistically significant at ≥15 mg/mL. Pre/co-treatment with 1 mg/mL GSK2857914 resulted in modest but significant decreases in belantamab mafodotin -mediated apoptosis at ≤10 µg/mL at 48 hours, although the trend did not continue at 100 µg/mL. Co-treatment with 1 mg/mL GSK2857914 (without pre-treatment) resulted in a significant but modest increase in apoptosis with 100 µg/mL belantamab mafodotin.

Key:
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).
HCEC = Human corneal epithelial cells; IVIG = Intravenous immunoglobulin; RPTEC = Renal proximal tubule epithelial cells.

Table 17.8 Concentration range finding studies of GSK2857914 and IVIG in primary human corneal epithelial cells with limited characterisation of renal proximal tubule epithelial cells

Report No.: 2022N508021
Study No.: ELN43729, 46186
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin, GSK2857914

Batch Number: GSK2857914 [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Cytotoxicity Assays (Caspase 3/7 (48 and 72 hours), CellTiter-Blue (48 and 72 hours), Imaging Assay (48 hours)

Conclusion:

Concentration response experiments using a range of endpoints in HCEC (apoptosis, cell viability and cell count assays) and RPTEC (cell count assay only) enabled the identification of sub-cytotoxic concentrations of GSK2857914 and IVIG that could be used in future mechanistic studies in these cell types.

In HCEC, concentrations of GSK2857914 ≤1 mg/mL for up to 72 hours were sub-cytotoxic, with a consistent response being observed across six donors for the cell count endpoint (48 hours assessment only). In RPTEC, no evidence of effect on cell count was observed at up to 10 mg/mL GSK2857914 at 48 hours, indicating these cells are less sensitive than HCEC. For IVIG, concentrations of ≤ 20 mg/mL were sub-cytotoxic in HCEC for up to 72 hours.

Species	Noteworthy findings
Human	Concentration response experiments, using apoptosis, cell viability (48 hours and 72 hours) and cell count (48 hours only) endpoints, with IVIG and GSK2857914 generated data to enable appropriate concentration selection for future studies in HCECs. Exposure of HCEC to GSK2857914, up to 1 mg/mL, did not result in an increase in apoptotic signal at 48 and 72 hours, with a clear effect observed at ≥5 mg/mL. Concentration-dependant decreases in cell viability and cell count were observed at 48 and 72 hours, with a clear effect at ≥2 mg/mL GSK2857914 with no effects observed at the lower concentrations ≤ 1 mg/mL. Exposure of RPTEC to GSK2857914, using the cell count assay endpoint only, did not result in a significant decrease in mean cell counts at concentrations ≤10 mg/mL GSK2857914 tested up to 48 hours. Exposure of HCEC to IVIG at up to 20 mg/mL did not induce any cytotoxic effect over a 72-hour treatment period, with a decrease in apoptosis signal observed at 25 mg/mL.

Key:
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).
HCEC = Human corneal epithelial cells; IVIG = Intravenous immunoglobulin; RPTEC = Renal proximal tubule epithelial cells.

Table 17.9 Investigation of uptake of Vivotag-680 labelled GSK2857916A and GSK2857914A into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial cells dosed with commercially available H1 inhibitors and IVIG

Report No.: 2023N528252
Study No.: N/A
Location in CTD: m4.2.2.3

Compounds: Belantamab mafodotin or GSK2857914

Batch Number: Belantamab mafodotin [redacted];
GSK2857914[redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: HCEC or RPTEC

Data Collected: Cellular Uptake [Fluorescent probe (Vivotag 680 XL); Fluorescence microscopy]

Conclusion:

An assessment of whether the co-administration of five commercially available H1 inhibitors (ketotifen 520 nM, levocabastine 1.8 µM, desloratadine 2.2 µM, levocetirizine 1.2 µM and fexofenadine 34.1 µM) or IVIG inhibited the uptake of Vivotag-680 labelled belantamab mafodotin or GSK2857914 into HCEC and RPTEC and reduced belantamab mafodotin-related cytotoxicity in up to 5 donors was conducted. Desloratadine, fexofenadine and ketotifen were shown to reduce the uptake of belantamab mafodotin in HCEC by 34%, 24.1% and 26.6%, respectively, but had no effect in reducing cytotoxicity (except for Desloratadine at 10ug/mL belantamab mafodotin). In general, no significant protective effect of H1 inhibitors in HCEC was observed as there was no reduction in intracellular fluorescence compound intensity nor increase in nuclei counts (cell number) when compared to cells treated with belantamab mafodotin alone. For RPTEC, none of the H1 inhibitors ameliorated the cytotoxicity associated to belantamab mafodotin. Co-treatment with IVIG did produce a protective effect, inhibiting uptake of belantamab mafodotin in HCEC as indicated by a reduction of intracellular fluorescence of almost 36%, which resulted in an increase in cell number of 23%, when compared to cells treated with belantamab mafodotin alone. The results were similar in RPTEC in which a reduction of 31.4% in cell-associated compound fluorescence was observed with a 28% increase in cell numbers, when compared to cells treated with belantamab mafodotin alone..

Investigation of uptake of Vivotag-680 labelled GSK2857916A and GSK2857914A into Human Corneal Epithelial Cells and Renal Proximal Tubule Epithelial cells dosed with commercially available H1 inhibitors and IVIG (Continued)

Species	Noteworthy findings
Human	
	Cytotoxicity of Desloratadine, Fexofenadine and Ketotifen, was noted at the highest concentration evaluated, in both HCEC and RPTEC ($p<0.001$). In addition, there was a significant reduction in cell numbers at the 22uM concentration for Desloratadine with a clear concentration response.
	Desloratadine was the only H1 inhibitor that when co-dosed with belantamab mafodotin reduced cytotoxicity (reduction in nuclei counts) but only when dosed at 10µg/mL in HCEC. Desloratadine, Ketotifen and Fexofenadine were shown to reduce the uptake of belantamab mafodotin in HCEC by 34%, 24.1% and 26.6%, respectively. For RPTEC, none of the H1 inhibitors ameliorated the cytotoxicity associated to belantamab mafodotin in a statistically significant way. There was an increase in uptake of belantamab mafodotin when codosed with Levocabastine of 27.8% (p-value of 0.0316) at the 100µg/mL belantamab mafodotin dose and 25.6% (p-value of 0.0463) at the 10 µg/mL belantamab mafodotin dose.
	For GSK2857914 the sole statistically significant result was a reduction of the compound cell uptake of 26.3% (p-value 0.0083) when co-dosed with Fexofenadine, but as expected this had no effect on cytotoxicity as GSK2857914 is not cytotoxic to HCEC or RPTEC.
	IVIG was shown to have a dose-related protective effect in HCEC and RPTEC when dosed with belantamab mafodotin. A reduction of intracellular fluorescence from Vivotag-680 labelled belantamab mafodotin up to 36% in HCEC and 31.4% in RPTEC was noted with an increase in cell numbers by 23% in HCEC and 28% in RPTEC, when compared to cells treated with 100ug/mL of belantamab mafodotin alone. When GSK2857914 was co-dosed with IVIG, there was no effect on the cytotoxicity as expected, but there is a statistically significant reduction in GSK2857914 uptake in both HCEC and RPTEC. In HCEC, the GSK2857914 uptake was decreased by 25.1% (pvalue 0.0203) at 8mg/mL IVIG, 40.7% (p-value 0.0000) at 15mg/mL IVIG and 47.6% (pvalue of 0.0000) at 20mg/mL, when compared to cells treated with belantamab mafodotin alone. The reduction in GSK2857914 uptake was less in RPTEC than HCEC; 15.9% (p-value 0.0061) at 8mg/mL IVIG, 25.8% (p-value 0.0000) for 15mg/mL IVIG and 35.3% (pvalue of 0.0000) at 20mg/mL IVIG, when compared to cells treated with belantamab mafodotin alone.

Key:
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody); HCEC = Human corneal epithelial cells;
IVIG = Intravenous immunoglobulin; LC/MS/MS = Liquid chromatography with mass spectrometry.
RPTEC = Renal Proximal Tubule Epithelial Cells

Table 17.10 Investigation into the effects of Histamine H1 Inhibitors on GSK2857916-mediated apoptosis in Human Corneal Epithelial Cells

Document No.: 2023N528622
Study No.: ELN109309
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin

Batch Number: [redacted] ([redacted])

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Data Collected: Cytotoxicity Assays (Caspase 3/7 (48 hours))

Conclusion:
In conclusion, experiments were conducted to investigate whether pre/co-treatment of human corneal epithelial cells (HCEC) with H1 inhibitors (desloratadine, fexofenadine, ketotifen, levocabastine and levocetirizine) could ameliorate belantamab mafodotin-mediated apoptosis, after 48 hours. H1 receptors expressed on the surface membrane of HCEC, when stimulated activate protein kinase C (PKC) which is hypothesised to induce macropinocytosis, a non-specific uptake pathway for belantamab mafodotin in BCMA negative cell types. These investigations demonstrated that pre/co-treatment with ketotifen, fexofenadine and desloratadine significantly reduced belantamab mafodotin-mediated apoptosis in HCECs in multiple donors in a concentration-dependent manner, with maximal effects when pre/co-treated with 25 µg/mL belantamab mafodotin for 48-hours. From these investigations, it was observed that the H1 inhibitors provided the greatest cytoprotective effect in the following order: ketotifen > fexofenadine > desloratadine > levocetirizine. As levocabastine was cytotoxic to HCEC at the concentrations tested it was not possible to interpret the effect of this H1 inhibitor.

Species	Noteworthy findings
Human	Pre/co-treatment of HCEC with ketotifen (52 µM), fexofenadine (341 µM) and desloratadine (2.2 µM) resulted in significantly reduced belantamab mafodotin-mediated apoptosis in HCECs in multiple donors in a concentration-dependent manner, with maximal effects when co-treated with 25 µg/mL belantamab mafodotin for 48-hours.

Key:
HCEC = Human corneal epithelial cells; RPTEC = Renal proximal tubule epithelial cells.

Table 17.11 Transcriptomic Evaluation of Cultured Primary Heterogenous Human Corneal Epithelial Cells

Report No.: 2022N502360
Study No.: V72052N
Location in CTD: m4.2.3.7.3

Compounds: Belantamab mafodotin, GSK2857914

Batch Number: Belantamab mafodotin [redacted]
GSK2857914 [redacted]

Route/Frequency: In vitro

Study in Compliance with GLP: No

Species/Strain: Human

Duration of Dosing:
Experiment 1: Not applicable
Experiment 2: 24 hours

Negative Control:
Experiment 1: Not applicable
Experiment 2: GSK2857914

Vehicle/Formulation:
Experiment 1:Not applicable
Experiment 2: Vivotag 680 XL fluorochrome in 25 mM aqueous citrate buffer containing 0.05 mM EDTA, 200 mM Trehalose and 0.02% Polysorbate 80 (pH 6.2).

Data Collected: Cell Counts and Transcriptomic Analysis using Affymetrix GeneChips

Conclusion:

In untreated primary cell culture of human corneal epithelial cells, gene expression analysis (via Affymetrix GeneChip analysis) no transcriptomic differences between small proliferative and large squamous cell populations; neither population of cells preferentially expressed limbal stem cell-like markers. Upon treatment with belantamab mafodotin or GSK2857914 (negative control), there was inconsistent expression of limbal stem cell like markers and other genes. Of note, in positive cell fractions of HCEC, CTNNB1 was downregulated in all three donors at 30 µg/mL (lowest concentration tested) and WDR74 was upregulated at ≥ 30 µg/mL with belantamab mafodotin treatment. In addition, TUBA1A, a microtubule-related gene, was downregulated in the majority of belantamab mafodotin -treated (positive and negative) cell fractions when compared to GSK2857914 (negative control).

Species	Noteworthy findings
Human	Whole transcriptome gene expression analysis was performed using Affymetrix Gene Chip Human Gene 1.0 Microarrays on Human Corneal Epithelial Cells (HCEC) obtained from three donors on untreated heterogenous HCEC following separation by size using cell strainers into small proliferative (<20 µm) and large squamous (>20 µm) cell populations. Gene expression analysis was performed to understand the transcriptomic differences between the small proliferative (<20 µm) and large squamous (>20 µm) cell populations and to determine if any population of cells preferentially express limbal stem cell-like markers1. In addition, Whole transcriptome gene expression analysis was performed on HCEC treated with belantamab mafodotin or GSK2857914 (identical antibody to belantamab mafodotin minus the cys-mcMMAF, negative control) at 0, 30 and 100 µg/mL for 24 hours which were separated into positive or negative cell fractions using Flow Assisted Cell Sorting (FACS) based on uptake of fluorescently tagged (VivoTag 680) belantamab mafodotin and GSK2857914 (i.e., positive – uptake of VivoTag 680/compound or negative – no uptake of VivoTag/compound). Gene expression analysis was performed to

Transcriptomic Evaluation of Cultured Primary Heterogenous Human Corneal Epithelial Cells(Continued)

Species	Noteworthy findings
	investigate the transcriptional differences between HCEC following 24-hour treatment with 30 or 100 µg/mL of belantamab mafodotin or GSK2857914 and to determine if any population of cells preferentially express limbal stem cell-like markers. Gene expression patterns of 33 limbal stem cell-like markers evaluated were unchanged between untreated HCEC small proliferative (<20 µm) and large squamous (>20 µm) cell populations. In HCEC treated with ≥30 µg/mL belantamab mafodotin or GSK2857914, an inconsistent expression of limbal stem cell like markers as well as other genes was observed across samples in both treatments of positive and negative cell fractions. Beta-catenin (CTNNB1), a gene encoding an adherens junction protein is critical for the establishment and maintenance of epithelial layers by regulating cell growth and adhesion between cells, was downregulated across all three donors at the lowest concentration (30 µg/mL) in positive cell fractions of HCEC treated with belantamab mafodotin. WD Repeat domain containing 74 (WDR74), a gene involved in ribosome biogenesis, was upregulated in positive cell fractions for ATCC/70021863 and MatTek /CE9060216B1 donors at ≥30 µg/mL and at 100 µg/mL in donor ATCC/80915170 in positive cell fractions treated with belantamab mafodotin. Microtubule-related gene, Tubulin 1A (TUBA1A) was downregulated in the majority of HCEC treated with belantamab mafodotin when compared with GSK2857914.

Key:
CTNNB1 = Beta-catenin; EDTA = Ethylenediaminetetraacetic acid; FACS = Flow Assisted Cell Sorting
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).
HCEC = Human corneal epithelial cells; RPTEC = Renal proximal tubule epithelial cells; WDR74 = WD Repeat domain containing 74.

Table 17.12 Investigative Intravenous Tolerability and Ocular Toxicity Study in New Zealand White Rabbits

Species/Strain	Route (Vehicle/ Formulation/ Form and batch)	Duration of Dosing	Doses[a] (mg/kg/week)	Number of Animals/ Sex[b]	Noteworthy Findings	GSK Document Number (Study Number)
Rabbit (NZW)	Intravenous (25 mM Citrate Buffer containing 0.05 mM EDTA, 200 mM Trehalose and 0.02% (v/v) Polysorbate 80/Solution) Belantamab mafodotin (batch [redacted])	Weekly dosing for 2 weeks (Phase I) or 4 weeks (Phase II)	0 (Phase I) 30 (Phase I) 0 (Phase II) 15 (Phase II) 30 (Phase II)	3 3 3 3	After four doses of 30 mg/kg/week, corneal epithelial single cell necrosis in all 3 rabbits, potentially associated with superficial corneal haze and corneal opacity in one rabbit on Days 27 and 30, was observed. Increased mitoses in the corneal epithelium in 2 of 3 rabbits given 15 mg/kg/week for four weeks, was also seen. There was no test article related effect on tear production (measured using Schirmer tear test strips) in treated animals when compared to vehicle control. Bilateral retinal striations observed during ophthalmoscopy on Days 27 and 30 in a single animal, given 15 mg/kg/week for 4 weeks, were of uncertain relationship to treatment; this observation did not correlate with any microscopic findings in the retina following examination of multiple sections containing the retina from this animal. Animals given 30 mg/kg/week showed a reduction in group mean body weight gain following dosing for 2 or 4 weeks AUC0-last (mean) data for belantamab mafodotin (ADC) after a total of 4 weekly doses of 15 or 30 mg/kg were 36300 and 68400 µg.h/mL respectively and mean Cmax values were 458 and 924 µg/mL respectively. AUC0-last (mean) data for belantamab mafodotin (total mAb) after a total of 4 weekly doses of 15 or 30 mg/kg were 51700 and 90800 µg.h/mL respectively and mean Cmax values were 565 and 1120 µg/mL respectively. AUC0-last (mean) data for cys-mcMMAF (after a total of 4 weekly doses of belantamab mafodotin at 15 or 30 mg/kg) were 238 and 468 ng.h/mL respectively and mean Cmax values were 2.69 and 4.79 ng/mL respectively.	2018N385412 (L32295N) **Location in CTD:** m4.2.3.2[c]

Key:
NZW = New Zealand White.
a = Doses are expressed in terms of the parent compound.
b = Female animals only.
c = The report for this study has been placed in m4.2.3.2 rather than m4.2.3.7.3 for administrative purposes only.

Table 17.13 Investigative Intravenous Tolerability and Ocular Toxicity Study in Dutch Belted Rabbits

Species/ Strain	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg/week)	Number of Animals/ Sex[b]	Noteworthy Findings	GSK Document Number/ Study Number
Dutch Belted Rabbit (DB)	Intravenous (25 mM Citrate Buffer containing 0.05 mM EDTA, 200 mM Trehalose and 0.02% (v/v) Polysorbate 80/Solution)	Weekly dosing for 7 weeks	0 30 (Doses 1-4) 15 (Doses 5-7)	6 6	Dutch Belted rabbits (6 females/group) were given 0 mg/kg/week (7 doses) or 30 mg/kg/week belantamab mafodotin (doses 1-4) followed by 15 mg/kg/week belantamab mafodotin (doses 5 - 7) once weekly via intravenous administration. One animal given the vehicle control was found dead shortly after dosing on Day 8. Macroscopic assessment showed no abnormalities and the cause of death was not established. Little or no body weight gain and transient mean body weight loss was observed after the third and fourth doses of 30 mg/kg/week and therefore, the dose level was reduced to 15 mg/kg/week for the remainder of the study. Following a reduction in dose, weight gain generally increased in animals given belantamab mafodotin but overall mean weight gain was still slightly lower from Days 1 to 48 in treated animals compared with control animals. Superficial corneal haze and vascularisation were observed in one or both eyes of two belantamab mafodotin -treated animals from Day 30 following ophthalmic examination. In one of these animals the presence of corneal haze precluded the ability to visualise eye structures beyond the cornea in both eyes on Day 48 and conjunctival hyperemia was observed in both eyes from Days 41. Clinical observations comprising cloudy/opaque in appearance were noted for both eyes of this animal from Days 43 to 48.	2021N465838 (L71822N) **Location in CTD:** m4.2.3.2[c]

Investigative Intravenous Tolerability and Ocular Toxicity Study in Dutch Belted Rabbits(Continued)

Species/ Strain	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg/week)	Number of Animals/ Sex[b]	Noteworthy Findings	GSK Document Number/ Study Number
					An increase in the incidence and severity of interstitial inflammatory cell infiltration was noted in the Harderian glands of animals given the test article compared with concurrent controls. This change was unilateral or bilateral, and often did not correlate with the presence of changes in the ipsilateral cornea. Given that inflammatory cell infiltration or inflammation is a recognised background finding in the rabbit Harderian gland, and the lack of clear correlation with ocular change, this finding is considered of uncertain relationship to the test article. There were no notable test item-related findings in the limbal region or other areas of the eye.	

Investigative Intravenous Tolerability and Ocular Toxicity Study in Dutch Belted Rabbits(Continued)

Species/ Strain	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg/week)	Number of Animals/ Sex[b]	Noteworthy Findings	GSK Document Number/ Study Number
					Systemic exposure of belantamab mafodotin (ADC) and cys-mcMMAF (plasma AUC0-t and Cmax) was similar between 30 mg/kg/week (Day 1 and Day 22) and 15 mg/kg/week (Day 36) for each analyte; Tmax for belantamab mafodotin (ADC) and cys-mcMMAF was generally observed at 0.5 and 24 hours, respectively. Concentrations of belantamab mafodotin and cys-mcMMAF in tears were variable for each analyte. belantamab mafodotin was generally quantifiable in all tear samples whereas cys mcMMAF was only quantifiable in approximately 45% of tear samples. Concentrations of belantamab mafodotin in plasma samples were higher than tear samples taken at comparable times after dosing. Additionally, molar ratios of belantamab mafodotin:cys-mcMMAF in plasma and tear samples indicated that there was a consistently higher proportion of belantamab mafodotin in each matrix than cys mcMMAF. Where comparisons allowed, the relative molar proportion of cys mcMMAF (when compared to belantamab mafodotin) was higher in tears than in plasma.	

Key:

a = Doses are expressed in terms of the parent compound.

b = Female animals only.

c = The report for this study has been placed in m4.2.3.2 rather than m4.2.3.7.3 for administrative purposes only.

Table 17.14 Ex-vivo Study to Investigate the Cross Reactivity of the BCMA Antibodies GSK2857914 and GSK2857916 in Human and Cynomolgus Monkey Tissues

Study Type: Other Toxicity

Report Title: Ex-vivo Study to Investigate the Cross Reactivity of the BCMA antibodies GSK2857914 and GSK2857916 in Human and Cynomolgus Monkey Tissues

Test Article(s): Belantamab mafodotin and GSK2857914
Batch No.: [redacted] and [redacted] respectively

Species/Strain: Human, Cynomolgus monkey

Duration of Dosing: N/A

Study No.: V29775
GSK Document Number: 2013N169796
Location in CTD: m4.2.3.7.7

Initial Age: N/A

Route/Frequency: Ex vivo

Study in Compliance with GLP: No

Data Collected: Microscopic pathology [immunohistochemically (IHC) stained slides]

Conclusions: Specific staining by GSK2857914 and belantamab mafodotin of human spleen and BCMA coated beads indicates that the antibody dilution and IHC conditions used are suitable for detection of the positive control material. The level of background staining observed did not prevent evaluation for specific staining. No specific staining with BCMA absorbed GSK2857914 or belantamab mafodotin was observed in any of the human or cynomolgus tissues examined.

Ex-vivo Study to Investigate the Cross Reactivity of the BCMA Antibodies GSK2857914 and GSK2857916 in Human and Cynomolgus Monkey Tissues (Continued)

Species/Strain	Method of Administration	Duration of Dosing	Concentration	Number of Animals/Sex	Noteworthy Findings
BCMA coated bead	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin moderate specific staining was observed at concentrations between 10 and 1 μg/mL
Blank beads	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin no staining was observed at concentrations between 10 and 1 μg/mL
ARH77 10B5 cells	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin mild to moderate specific staining was observed at concentrations between 10 and 1 μg/mL
H929 cells	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin moderate specific staining was observed at concentrations between 10 and 1 μg/mL
Human MM bone marrow	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 moderate specific staining was observed at 10 μg/mL
Human tissues	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin mild to moderate specific staining was observed at 10g/mL in adrenal, heart, kidney, liver, lymph node, spleen, tonsil and adipose/skin. Non-specific staining with BCMA absorbed GSK2857914 or GSK2857916 was observed in colon
Cynomolgus monkey tissues	Ex vivo	NA	Up to 10 μg/mL	NA	GSK2857914 and belantamab mafodotin mild to moderate specific staining was observed at 10g/mL in adrenal, colon, kidney, liver, lymph node, spleen and tonsil. Non-specific staining with BCMA absorbed GSK2857914 or belantamab mafodotin was observed in colon

Key:
Belantamab mafodotin = GSK2857916.
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).
NA = Not applicable; MM = Multiple myeloma.

Table 17.15 Assessment of the Potential Cross Reactivity of GSK2857916 with a Selected Panel of Human Tissues

Study Type: Tissue Cross Reactivity

Report Title: GSK2857916: Assessment of the Potential Cross Reactivity of GSK2857916 (anti-BCMA ADC mAb) with a Selected Panel of Human Tissues

Test Article: Belantamab mafodotin
Batch No.: [redacted]
Positive Control Article: Elastin pre-absorbed belantamab mafodotin conjugate
Batch No.: N/A
Negative Control Article: BCMA pre-absorbed GSK2857916 conjugate
Batch No.: N/A

Species/Strain: Human

Duration of Dosing: N/A

Study No.: 8284607

Initial Age: N/A

Route/Frequency: Ex vivo

Location in CTD: m4.2.3.7.7

Date of First Dose: N/A

Vehicle/Formulation: N/A

Study in Compliance with GLP: Yes

GSK Document Number: 2013N176627

Data Collected: Microscopic pathology (immunohistochemically stained slides).

Conclusion: The potential cross-reactivity of belantamab mafodotin (an anti BCMA Antibody Drug Conjugate [ADC] mAb), prepared as a conjugate of belantamab mafodotin and Anti-Idiotype mAb S389105H11, with histologically-prepared cryo-sections from a selected panel of human tissues (3 donors per tissue) was assessed, using immunohistochemical techniques, at concentrations of 2.5, 5 or 10 μg/mL. Equivalent concentrations of the Positive Control Article (elastin pre-absorbed belantamab mafodotin conjugate) or the Negative Control Article (BCMA pre-absorbed belantamab mafodotin conjugate) were used as comparators.

Under the conditions of this study, antigen-specific binding of belantamab mafodotin conjugate was demonstrated in Positive Control Material (human spleen). Comparable staining was seen with the Positive Control Article in this material, but no comparable staining was observed with the Negative Control Article. Specific positive staining was observed with belantamab mafodotin conjugate in a number of the tissues examined and this was generally associated with individual or focal groups of cells, blood vessel walls/perivascular tissue and connective tissue. The majority of specific positive staining observed with belantamab mafodotin conjugate was cytoplasmic. In all tissues where specific positive staining with belantamab mafodotin conjugate was observed, comparable staining was seen with the Positive Control Article but not with the Negative Control Article.

All other observed staining was variable and considered to be non-specific.

Assessment of the Potential Cross Reactivity of Belantamab Mafodotin (GSK2857916) with a Selected Panel of Human Tissues (Continued)

Species/Strain and tissues	Method of Administration	Concentrations (µg/mL)	Gender and No./ Group	Noteworthy Findings
Human: adrenal, urinary bladder, blood cells, bone marrow, breast, brain (cerebellum and cortex), endothelium, eye, fallopian tube, gastrointestinal tract (oesophagus, gastric antrum, gastric body, duodenum, ileum, colon), heart, kidney, liver, lung, lymph node, ovary, pancreas, parathyroid, parotid, peripheral nerve, pituitary, placenta, prostate, skin, spinal cord, spleen, striated muscle, testis, thymus, thyroid, tonsil, ureter, uterus (cervix and endometrium).	Ex vivo	2,5, 5 or 10	Three donor tissue samples per organ	**Human Tissue Titration** Antigen-specific binding of belantamab mafodotin conjugate was demonstrated in Positive Control Material (human spleen). Comparable staining was seen with the Positive Control Article in this material, but no comparable staining was observed with the Negative Control Article. Specific positive staining was observed with belantamab mafodotin conjugate in a number of the tissues examined and this was generally associated with individual or focal groups of cells, blood vessel walls/perivascular tissue and connective tissue. The majority of specific positive staining observed with belantamab mafodotin conjugate was cytoplasmic. In all tissues where specific positive staining with belantamab mafodotin conjugate was observed, comparable staining was seen with the Positive Control Article but not with the Negative Control Article. All other observed staining was variable and considered to be non-specific.

Key:
Belantamab mafodotin = GSK2857916.
GSK2857914 = Unconjugated afucosylated mAb (parent anti-BCMA antibody).