**Molecular Cancer Therapeutics**

**Supplemental Information**

**MMAE delivery using the *Bicycle* toxin conjugate BT5528**

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Figure S1: Plasma concentration-time curves of BT5528 and MMAE following IV dosing of BT5528 1 in mouse, rat, and cynomolgus Monkey at 1 mg/kg (n=3 per species). Related to *in vivo* PK.

Figure S2: EphA2 expression and tumour growth inhibition in CDX and PDX xenograft models are correlated. BT5528 3 mg/kg qw dosing has a range of anti-tumour activities across different cell-line derived and patient-derived xenograft models (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 2way ANOVA from D0 to last day of vehicle tumour measurement).Data used to generate Figure 3.

Figure S3: EphA2 Immunohistochemistry staining of CDX and PDX xenograft models. A range of EphA2 expression is observed in stained tumour tissue from mouse xenograft models. Related to figure 3.

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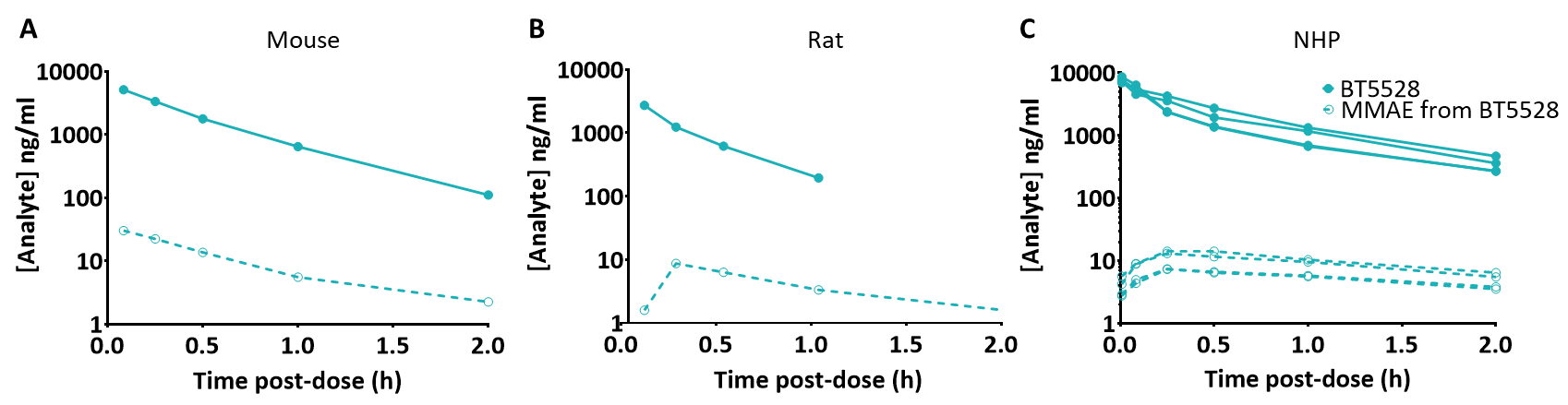
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Table T5a: Anti-Human EphA2 Antibody Binding Sites on CDX Cell Lines. Expression data used to generate Figure 3.

Table T5b:Anti-Human EphA2 Antibody Binding Sites on PDX Cell Lines. Expression data used to generate Figure 3.

Table T6: Doses of BT5528 and corresponding toxin (MMAE) used in preclinical toxicology studies compared with the clinical dose of MED-547 and corresponding toxin (MMAF)1. Related to the discussion (ADC versus BTC toxicology responses).

Figure S1: Plasma concentration-time curves of BT5528 and MMAE following IV dosing of BT5528 1 in mouse, rat, and cynomolgus Monkey at 1 mg/kg (n=3 per species)



Abbreviations: h=hour, IV=intravenous, MMAE=monomethyl auristatin E, NHP=non-human primate.  
n=3 per species

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| **Figure S2: EphA2 expression and tumour growth inhibition in CDX and PDX xenograft models are correlated.** BT5528 3 mg/kg qw dosing has a range of anti-tumour activities across different cell-line derived and patient-derived xenograft models (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 2way ANOVA from D0 to last day of vehicle tumour measurement). |
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| **Figure S3: EphA2 Immunohistochemistry staining of CDX and PDX xenograft models.** A range of EphA2 expression is observed in stained tumour tissue from mouse xenograft models. |
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Table T1: Ki values for BT5528, 1C1-mcMMAF and control compounds binding to human, mouse and rat Epha2 receptor

|  |  |  |  |
| --- | --- | --- | --- |
|  | Human (Ki nM) | Mouse (Ki nM) | Rat (Ki nM) |
| BT5528 | 1.94 ± 0.91, n=31 | 5.60 ± 1.96, n=20 | 2.16 ± 1.25, n=14 |
| BCY6099 | 5.74 ± 11.08, n=28 | 5.64 ± 3.14, n=11 | 2.23 ± 0.80, n=6 |
| BCY6164 | 5.0 ± 0.8, n=2 | ND | ND |
| BCY6079 | >5000 (n=14) | >5000 (n=6) | >5000 (n=4) |
| BCY6063 | 22.2 ± 8.13 n=12 |  |  |
| BCY8245 | >5000 n=2 |  |  |
| BCY10188 | 2.35 n=1 |  |  |
| 1C1-mcMMAF | 22.44 ± 12.96, n=7 | 12.60 ± 4.14, n=3 | 3.73 ± 1.52, n=2 |

Table T2: Binding affinities for BT5528 binding to EphA and EphB tyrosine kinases receptors determined by Surface Plasmon Resonance

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ephrin domain | % identity to human EpHA2 | Binding affinity (SPR KD nM) | | | |
|  |  | Human | Mouse | Rat | Cynomolgus |
| EphA2 | 100 | 0.88 ± 0.40, n=2 | 2.07 ± 0.61, n=3 | 2.67 ± 0.42, n=2 | 1.01 n=1 |
| EphA1 | 54 | > 5000 |  |  |  |
| EphA3 | 58 | > 5000 | > 5000 | > 5000 |  |
| EphA4 | 55 | > 5000 | > 5000 | > 5000 |  |
| EphA5 | 56 | > 5000 |  |  |  |
| EphA6 | 56 | > 5000 |  |  |  |
| EphA7 | 56 | > 5000 |  |  |  |
| EpHb1 | 49 |  |  | > 5000 |  |
| EphB4 | 39 | > 5000 |  |  |  |

Table T3: *In vitro* ADME properties of BT5528 in mouse, rat, NHP and human: plasma protein binding, plasma stability and metabolic stability in hepatocytes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Mouse | Rat | NHP | Human |
| Plasma Protein Binding (fu,p) | 0.49 | 0.38 | 0.42 | 0.32 |
| Stability in plasma (t½, h) | 1.6 | >14.5 | >14.5 | >14.5 |
| Stability in hepatocytes (CL,int mL/min/g liver) | 2.7 | 0.75 | 0.77 | 0.89 |

Table T4: Summary of Calculated PK Parameters for BT5528 and MMAE Following IV Dosing of 1 mg/kg BT5528 in Mouse, Rat and Cynomolgus Monkey

|  |  |  |  |
| --- | --- | --- | --- |
| **Mouse** | PK Parameters | BT5528 | MMAE |
| Cmax (ng/mL) | 6321 | 30.2 |
| T1/2 (h) | 0.377 | 0.539 |
| Vdss (L/kg) | 0.180 | -- |
| Cl (mL/min/kg) | 6.17 | -- |
| AUC0-last (ng.h/mL) | 2643 | 18.1 |
| AUC0-inf (ng.h/mL) | 2703 | 19.9 |
| **Rat** | PK Parameters | BT5528 | MMAE |
| Cmax (ng/mL) | 4048 | 8.69 |
| T1/2 (h) | 0.285 | 0.736 |
| Vdss (L/kg) | 0.329 | -- |
| Cl (mL/min/kg) | 15.5 | -- |
| AUC0-last (ng.h/mL) | 998 | 6.67 |
| AUC0-inf (ng.h/mL) | 1078 | 9.23 |
| **Cynomolgus Monkey** | TK Parameters | BT5528 | MMAE |
| Cmax (ng/mL) | 7643 | 11 |
| T1/2 (h) | ~0.6 |  |
| Vdss (L/kg) | 0.21 | -- |
| Cl (mL/min/kg) | 4.9 | -- |
| AUC0-last (ng.h/mL) | 3516 | 23 |

Table T5a: Anti-Human EphA2 Antibody Binding Sites on CDX Cell Lines

|  |  |
| --- | --- |
| **Cell line** | **Antibody binding sites** |
| SK-OV-3 | 20525 ± 6986, n=3 |
| OE-21 | 12107 ± 5766, n=7 |
| NCI-N87 | 18548 ± 1740, n=3 |
| MDA-MB-231 | 24740 ± 3499, n=4 |
| NCI-H1975 | 15872 ± 7412, n=7 |
| HT-1080 | 30750 ± 8679, n=5 |
| PC-3 | 57158 ± 14303 >ULOQ, n=4 |
| NCI-H292 | 60 ± 82.5 <LLOQ, n=4 |
| MDA-MB-468 | 1051 ± 352.6, n=3 |
| HCC1806 | 17037, n=1 |
| HCT-15 | 8931, n=1 |
| HT-29 | 9187, n=1 |
| SNU-16 | 1601 ± 1763, n=5 |
| EBC-1 | 24685, n=1 |
| MOLP-8 | 6 <LLOQ, n=1 |
| NCI-H322 | 2155 ± 797.2, n=3 |
| HT-1376 | 6865 ± 2023, n=3 |
| NCI-H526 | 387.2 ± 95.1<LLOQ, n=3 |

LLOQ: Lower Limit of Quantitation

ULOQ: Upper Limit of Quantitation

All data are mean antibody binding sites± standard deviation and n= number of replicates. <LLOQ = values below lower limit of quantitation

Table T5b:Anti-Human EphA2 Antibody Binding Sites on PDX Cell Lines

|  |  |
| --- | --- |
| **PDX Line** | **Antibody binding sites** |
| LU-01-0046 purified | 1500 ± 288.9, n=4 |
| LU-01-0251 purified | 8627 ± 12103, n=4 |
| LU-01-0486 purified | 843.9 ± 324, n=2 |
| LU-01-0412 (purified) | 139.5 ± 7.78 <LLOQ, n=2 |
| LU-01-0007 purified | 876.5 ± 317.5, n=2 |
| LU-01-0130 purified | 58 >LLOQ, n=1 |
| PC-07-0013 purified | 10399, n=1 |
| PC-07-0022 purified | 984, n=1 |
| PC-07-0018 purified | 478 ± 289.1 <LLOQ n=4 |
| HN-13-0001 purified | 2028, n=1 |
| ST-02-0248 purified | 365.7 ± 326.8 <LLOQ, n=3 |
| ES-06-0130 purified | 58.33 ± 29.14 <LLOQ, n=3 |
| ES-06-0012 purified | 94.83 ± 69.12 <LLOQ, n=3 |
| ES-06-0104 purified | 476.3 ± 291.4 <LLOQ, n=2 |

All data are mean antibody binding sites± standard deviation and n= number of replicates. <LLOQ = values below lower limit of quantitation

Table T6: Doses of BT5528 and corresponding toxin (MMAE) used in preclinical toxicology studies compared with the clinical dose of MED-547 and corresponding toxin (MMAF)1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | | | |
| Treatment | ADC/BTC (mg/kg) | ADC/BTC (mg/m2) | Toxin  (mg/kg) | Toxin (mg/m2) |
| BT5528 (Rat) | 4 | 24.00 | 0.65\* | 3.91\* |
| BT5528 (Monkey) | 1.5 | 18.00 | 0.24\* | 2.88\* |
| MEDI-547 (Human) | 0.08 | 2.96 | 0.002\*\* | 0.07\*\* |

\*MMAE, \*\*MMAF

1. Annunziata, C. M. *et al.* Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumors. *Invest New Drugs* **31**, 77–84 (2013).