# Rapid accumulation of cytotoxic payload in tumor tissue drives BT5528 activity in tumor models

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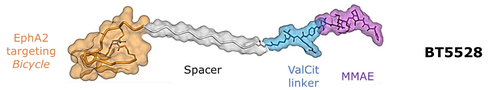
## ABSTRACT

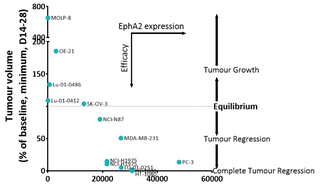

- BT5528 was developed as a Bicycle® toxin conjugate (BTC) to deliver monomethyl auristatin E (MMAE) - payload to EphA2 overexpressing tumors. It consists of a bicyclic peptide targeting the tumor antigen EphA2, linked to the cytotoxin MMAE via a molecular spacer and cleavable linker. Administration of BT5528 results in rapid uptake of payload into EphA2 overexpressing xenograft tumors associated with persistent toxin levels in tumor tissue and limited systemic exposure of both parent drug and payload (1).
- We have used EphA2 overexpressing tumor xenograft models in both mice and rats to elucidate the relative differences between toxin payload delivery and systemic exposure of BT5528. Comparison of payload-delivery and systemic exposure from EphA2 -targeted Bicycle toxin conjugate and antibody-drug conjugate (ADC) demonstrate the differentiation of these toxin-payload delivery platforms. ADC payload delivery depends upon a sustained plateau-like pharmacokinetic (PK) profile that exposes target and potentially nontarget organs to intact ADC for several days. In contrast, BT5528 achieves prolonged toxin delivery to tumors, following transient exposure in plasma of up to an hour.
- Initial pre-clinical PK/PD/Efficacy –studies with BT5528 were performed in EPhA2+ tumor models in mice (1), but extension of this work into rats, a relevant safety –species, is a powerful strategy to gain confidence around estimated tumor payload delivery as well as the pre-clinically estimated therapeutic index.
- BT5528 showed a favorable preclinical profile supporting the initiation of a first-in-human Phase I/II study (NCT04180371) to investigate safety and efficacy of BT5528 in indications with evidence of EphA2 expression including non-small-cell lung cancer (NSCLC), ovarian cancer, triple-negative breast cancer (TNBC), gastric/upper gastrointestinal (GI), pancreatic and urothelial cancers (2). Doses administered to date appear tolerable with manageable adverse events.


## INTRODUCTION

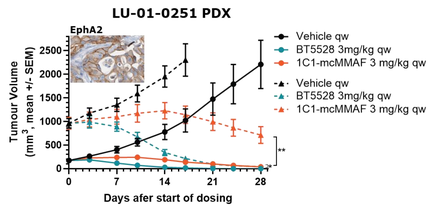



**BT5528, a Bicycle toxin conjugate is a fully synthetic ~4.4 kDa molecule consisting of a bicyclic peptide targeting the tumor antigen EphA2 ($K_D$: 1.9 ± 0.9 nM), linked to the cytotoxin MMAE via a molecular spacer and cleavable (ValCit) linker. The EphA2 Bicycle cross reacts with mouse ($K_D$: 5.6 ± 2.0 nM) and rat ($K_D$: 2.2 ± 1.3 nM) EphA2.**

### EphA2 expression and BT5528 anti-tumor activity in CDX and PDX xenograft models are correlated




### BT5528 Displayed superior activity to EphA2-ADC in large tumor xenografts




**(LEFT) Anti-tumor activity of BT5528 was determined across several cell line derived xenograft (CDX) and patient derived xenograft (PDX) models showing a correlation with EphA2 expression levels. (RIGHT) Both BT5528 (3 mg/kg qw) and EphA2-ADC (1C1-mcMMAF; 3 mg/kg qw) show broadly equivalent anti-tumor activity in small (~200 mm³) NSCLC PDX tumors (LU-01-0251) but BT5528 is much more effective than 1C1-mcMMAF in combating the growth of large (~1000 mm³) NSCLC PDX tumors.**

## RESULTS

### Rapid accumulation of cytotoxic payload MMAE in tumor tissue is seen after BT5528 administration into EphA2+ tumor bearing mice

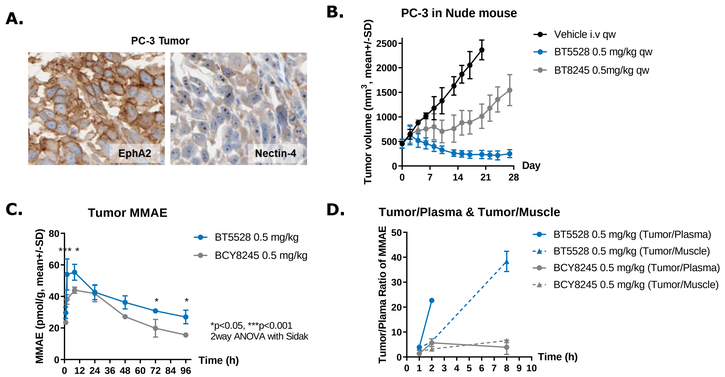



**Figure 1: MMAE accumulation in tumor tissue after BT5528 dosing. (A)** PC-3 tumor xenografts have high cell surface expression of EphA2 whereas they have no cell surface expression of Nectin-4 by IHC. **(B)** Weekly 0.5 mg/kg BT5528 administration into PC-3 bearing tumor mice leads to rapid tumor regressions whereas a Nectin-4 –targeted BTC BCY8245 leads to a suboptimal anti-tumor activity at the same dose. (C) 0.5 mg/kg dose of BT5528 leads to rapid ($C_{MAX}$ at 4-8 hours) increase of MMAE in tumor tissue that persists at least until 96 hours after dosing. MMAE levels delivered by BCY8945 are significantly lower. (D) Tumor to plasma and tumor to muscle ratios of MMAE are much higher in the BT5528 -treated mice when compared to BCY8945 –treated mice.

### Higher plasma:tumor payload ratio is seen after BT5528 administration in comparison to EphA2-ADC administration in EphA2+ tumor bearing mice

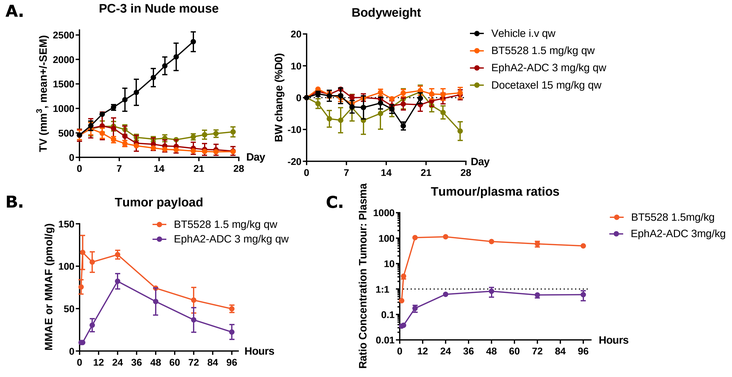



**Figure 2: Payload accumulation in tumor tissue after BT5528 and EphA2-ADC dosing. (A)** QW 1.5 mg/kg BT5528 or 3 mg/kg EphA2-ADC (1C1-mcMMAF) administration into PC-3 bearing tumor mice leads to rapid tumor regressions. 15 mg/kg QW Docetaxel showed anti-tumor activity but was poorly tolerated. **(B)** In contrast to maximum payload accumulation around 24 hour timepoint from EphA2-ADC, BT5528 delivers the payload into tumor tissue at much earlier timepoint ($C_{MAX}$ at 2 hours). **(C)** Comparison of tumour and plasma shows significant accumulation of toxin in tumour after dosing BT5528 (compared to toxin present as parent drug in plasma), while concentrations of toxin in tumour after dosing ADC always remain below the concentration of toxin in plasma (as parent drug).

## RESULTS

### Rapid accumulation of cytotoxic payload MMAE in tumor tissue is seen after BT5528 administration into EphA2+ tumor bearing rats

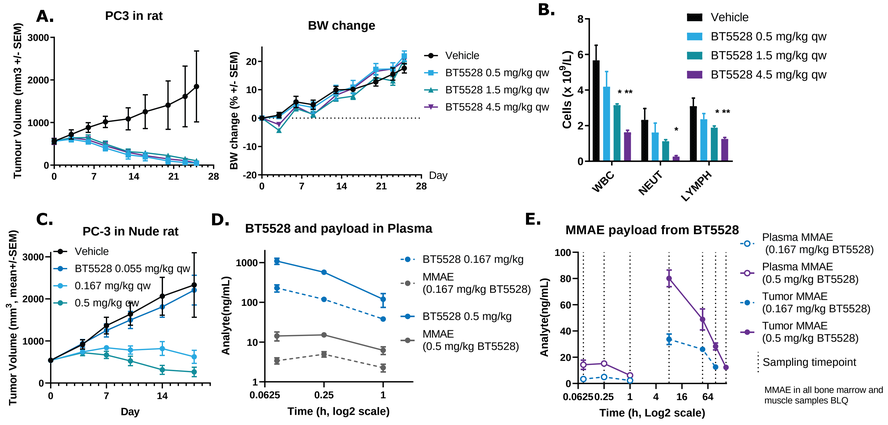



**Figure 3: MMAE accumulation in tumor tissue after BT5528 dosing in rat. (A)** Weekly (QW) BT5528 administration into PC-3 bearing tumor mice leads to rapid tumor regressions with as low a dose as 0.5 mg/kg. 0.5-4.5 mg/kg dosing was well tolerated in nude rat as observed by the weight gain of the rats. (B) Neutropenia and lymphocytopenia can be observed at the end of the treatment period with 1.5 and 4.5 mg/kg dosing cohorts. (C) Significant anti-tumor activity could be observed with as low a dose as 0.167 mg/kg BT5528, 0.5 mg/kg BT5528 dose saturates the activity. (D) consider rephasing: BT5528 and MMAE levels in plasma after BT5528 dosing: both BT5528 and MMAE levels below LLOQ after 1 h. (E) MMAE levels in plasma are below LLOQ after 1 h. Highest MMAE concentration in tumor tissue was at 8 hours (the earliest timepoint analyzed) and MMAE was detected up to 168 hours (last timepoint analyzed) after BT5528 administration.

## CONCLUSIONS/SUMMARY

- BT5528 delivers MMAE to EphA2+ tumor tissue rapidly, with peak concentrations of MMAE observed at 2 hours post BT5528 dose. MMAE is sequestered in tumor tissue, in contrast to the rapid elimination of BT5528 from the systemic circulation.
- Tumor delivery of MMAE payload from BT5528 happens faster than from EphA2-ADC, likely reflecting the faster tissue distribution of BT5528 (~4 kDa) when compared to the ADC (~150kDa) and the efficient release of the MMAE payload in the protease rich tumor microenvironment.
- Rapid tumor MMAE accumulation and fast plasma clearance can be observed in rats after BT5528 administration.

References: [1] Bennett et al, *Mol Cancer Ther* (2020); 2] Bennett et alTPS3655 *JCO* 38, no. 15_suppl

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