**ABSTRACT**

**BT8009, a Bicycle® Toxin Conjugate targeting Nectin-4, shows target selectivity, and efficacy in preclinical in large and small tumor models.**

**METHODS**

Bicycle binders are identified using phage display technology. The Bicycle was synthesised by standard Fmoc solid phase synthesis and a proprietary cyclization step. Amino acid monomer libraries were made to optimise affinity, stability and hydrophilicity. The toxin-linker, valine-citrulline p-aminobenzoate (v-PABC-MMAE), was conjugated to the Bicycle to generate BT8009. Surface Plasmon Resonance (SPR) was used to confirm affinities, leading to Nectin-4 and Nectin-4-like members. High content imaging and immunocytochemistry demonstrated BT8009 binding to cell surface Nectin-4 expressing cells (MDA-MB-468). Expression of Nectin-4 in tumor cells, or patient-derived tumor samples was evaluated by FACS. Where protein was not BT8009 shown by IHA expression was provided by the SDS soluble fraction. Efficacy was evaluated using a range of xenograft models in nude mice. Xenografts were established as subcutaneous cell lines. In vivo and in vitro models were selected for “null” and increasing Nectin-4 expression. Antitumor activity was determined by tumor volume measurements following drug administration. The Bicycle phage display platform was used to identify a Nectin-4 binding parent Bicycle which was optimized for affinity and hydrophilicity. Conjugation of this Bicycle peptide, through a cleavable linker, to MMAE results in the Bicycle targeting Nectin-4. BT8009 targets Nectin-4 and releases MMAE on cleavage by the enzymes upregulated in the tumor micro-environment, in order to kill adjacent tumor cells.

**RESULTS**

Bicycle binders are identified using phage display technology. The Bicycle was synthesised by standard Fmoc solid phase synthesis and a proprietary cyclization step. Amino acid monomer libraries were made to optimise affinity, stability and hydrophilicity. The toxin-linker, valine-citrulline p-aminobenzoate (v-PABC-MMAE), was conjugated to the Bicycle to generate BT8009. Surface Plasmon Resonance (SPR) was used to confirm affinities, leading to Nectin-4 and Nectin-4-like members. High content imaging and immunocytochemistry demonstrated BT8009 binding to cell surface Nectin-4 expressing cells (MDA-MB-468). Expression of Nectin-4 in tumor cells, or patient-derived tumor samples was evaluated by FACS. Where protein was not BT8009 shown by IHA expression was provided by the SDS soluble fraction. Efficacy was evaluated using a range of xenograft models in nude mice. Xenografts were established as subcutaneous cell lines. In vivo and in vitro models were selected for “null” and increasing Nectin-4 expression. Antitumor activity was determined by tumor volume measurements following drug administration. The Bicycle phage display platform was used to identify a Nectin-4 binding parent Bicycle which was optimized for affinity and hydrophilicity. Conjugation of this Bicycle peptide, through a cleavable linker, to MMAE results in the Bicycle targeting Nectin-4. BT8009 targets Nectin-4 and releases MMAE on cleavage by the enzymes upregulated in the tumor micro-environment, in order to kill adjacent tumor cells.

**CONCLUSION/SUMMARY**

Using phage display technology a Nectin-4 binding Bicycle was identified. The Parent Bicycle was optimized for affinity, stability and hydrophilicity. BT8009 was synthesized by conjugation through an inert spacer and a cleavable linker to the toxin MMAE. The binding peptide and the Bicycle are highly selective for the target protein. The pharmacokinetic profile of BT8009 enables a rapid attainment of high tumor levels of MMAE, with corresponding reduced systemic exposure. BT8009 shows excellent efficacy in large tumor CDX and PDX models expressing Nectin-4 targets. IND enabling studies for BT8009 are ongoing.

**REFERENCES**


**Figure 6:** MMAE is retained in tumor but rapidly cleared from plasma, thereby reducing systemic exposure.

**Figure 3:** BT8009 binds Nectin-4 on MDA-MB-468 cells. Cells preincubated with BT8009, MMAE, non-binding BSA were washed and retained MMAE detected with anti-MMAE antibody. BT8009 remains bound to cells.

**Figure 4:** Efficacy tracks Nectin-4 expression determined by FACS. Models were selected for “null” and increasing expression of Nectin-4 by FACS. High expression models show excellent efficacy.

**Figure 5:** Efficacy tracks Nectin-4 expression as determined by IHC. BT8009 was tested on the 5 NSCLC PDX models at 3 mg/kg qw.

**Figure 7:** BT8009 delivers outstanding efficacy against large CDX and PDX tumors, with rapid tumor regression.

**Figure 2:** Identification and optimization of Nectin-4 binding Bicycle.

**Figure 1:** Bicycle Toxin Conjugates BT8009