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Tunable Drug Conjugates: A Differentiated Drug Conjugate Platform

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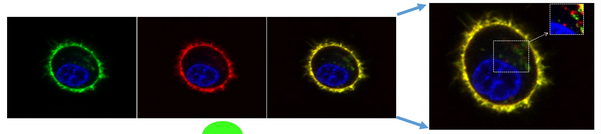
Introduction

We have innovated a family of novel targeting, linker and payload technologies called **Tunable Drug Conjugates** (TDCs) that differentiate from other Drug Conjugation approaches via a rapid payload release/rapid systemic clearance paradigm designed to drive high payload concentrations within tumor cells while minimizing toxicities to patients and enabling better management of side-effects. TDCs use proprietary Silicon-based linker chemistries **(SiLinkers)** in novel **Payload Cassettes (PC)** that enable the rapid and uniform release of multiple and/or mixed payloads both in the endosome after internalization and within the necrotic microenvironment of tumors. Payload Cassettes can incorporate multiple copies of the same payload or optimal ratios of different therapeutic agents that work together synergistically to kill cancer cells.

Another technology – a **Dual Variable Domain-Fab (DVD-Fab)** targeting capability provides a proprietary means of specifically targeting a broad array of cell surface proteins while maintaining the rapid clearance characteristics with $t_{1/2}$ measured in hours (rather than days for the traditional ADCs). We have validated the TDC technology via imaging, cellular and *in vivo* Xenograft studies.

SiLinkers Demonstrate Rapid Release of Payload after Internalization

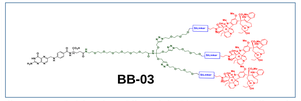


Linker cleavage at endosomal pH separates the two dyes

- An imaging construct using folic acid as the ligand and with two different fluorescent dyes (BODIPY and Rhodamine) positioned on either side of a proprietary disilylether linker was designed and synthesized.
- At 30 minutes, pictures show overlap of the dyes at the cell surface but separation of red and green dyes inside cells - consistent with the rapid cleavage rate of our pH sensitive linkers

Prototype Small Molecule TDCs (SM-TDCs)

BB-01



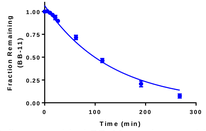
BB-03

- SM-TDCs containing folic acid as the targeting ligand & Vinblastine Silanol (VS) or MMAF Silanol (MMAF-S) as the payloads were designed and synthesized to validate SiLinker performance
- Triple Payload Cassette constructs (BB-03, BB-11 and BB-20) were included to demonstrate the impact of a higher Payload:Conjugate ratio in PoC studies

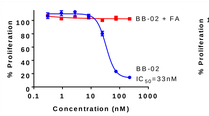
In Vitro Validation of SiLinker and PC Platform

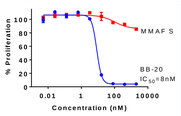
Compound	EC_{50} (nM) in KB cells
VH	4.2
VS	6.0
MMAF	307
MMAF S	345

Silanol analogs of cytotoxic payloads retain the activity of the parent drugs: VH = Vinblastine Hydrazide; MMAF = Monomethyl Auristatin F



Fully assembled BB-11 displays rapid linker hydrolysis at pH 5.0 with $t_{1/2}$ = 90 min and is stable at pH 7.4 in aqueous buffer with $t_{1/2} \geq 24$ hours

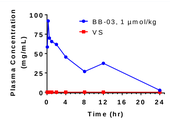




Blocking of cellular effects in the presence of excess folic acid unequivocally establishes targeted drug delivery approach

Potent cellular activity of BB-20 demonstrates the selective release of MMAF-S inside target cells

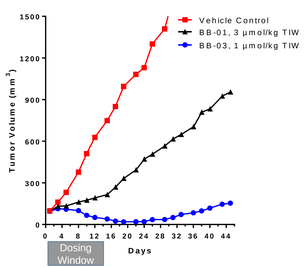
Cell Proliferation inhibition was assayed in KB cells using a 2 hour treatment followed by washout and a 70 hour chase



Single dose PK data in C57-Black mice show that maximum systemic release of VS is between 0.8-3% over 24 hours using the most labile SiLinker prototype

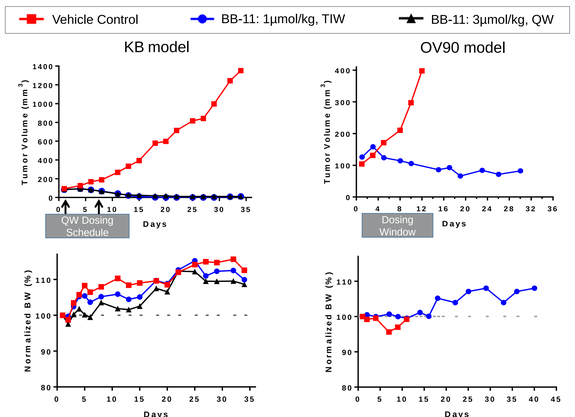
In Vivo Validation of SiLinker and PC Platform

- PC constructs were consistently more efficacious than single payload constructs at doses which delivered equivalent payload amounts
- BB-01 (single payload) and BB-03 (triple payload) have comparable PK parameters
- This superior efficacy of BB-03 supports our rapid release/high payload concentration approach



BB-11 Demonstrates Robust *In Vivo* Efficacy & Safety

- BB-11 is a more potent Triple Payload Cassette Analog than our BB-03 prototype
- BB-11 is very well tolerated and demonstrates regression and cures
- A TIW dosing regimen resulted in 5/5 cures & a once weekly dosing regimen resulted in 4/5 cures
- Promising efficacy was evident in the moderate FRα expressing OV90 model in addition to the high FRα expressing KB model for this unoptimized lead molecule TDC



DVD-Fabs Broaden Targeting Capability and DVD-Fab-TDCs May Provide Optimal Tumor Residence Time

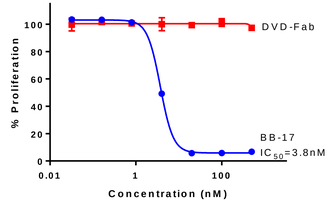
- **Enhanced Tumor Penetration**: DVD-Fab TDCs are smaller than ADCs & may more effectively penetrate the tumor, enhancing efficacy
- **Optimum Tumor Residence Time**: Balance of tumor penetration and clearance of parent DC is an important factor in the optimal binding of the DC to targeted tumor cells, with literature data suggesting that an optimal systemic clearance rate between 3-12 hours will allow for adequate tumor exposure and superior tumor/blood ratios

Cellular Activity of Prototype DVD-Fab TDC

- BB-17 is a single Auristatin Silanol-containing FRα targeting DVD-Fab TDC
- Auristatin Silanol (AF S) is a highly potent Auristatin F (AF) analog

Compound	EC_{50} (nM) in KB cells
AF	88.2
AF S	0.3

- DVD-Fab does not kill cells on its own
- DVD-Fab TDC shows strong cell kill



Summary

- We have assembled a proprietary suite of Drug Conjugate technologies with a differentiated rapid payload release/rapid systemic clearance profile
- Tunable PK Profile allows for systemic clearance in hours (vs. days-to-weeks for ADCs) enabling clinical management of side-effects through dose and schedule adjustments, thereby enhancing safety
- Broad Targeting Capability using DVD-Fab targeting ligands may address any targeted surface protein that an antibody can be raised against
- Rapidly Cleaving and Tunable SiLinkers payload release kinetics allows for tailoring of TDC payload release
- Payload Cassette Technology enables endosomal release of both mixed payloads and high stoichiometric Payload:Conjugate ratios
- SiLinkers and Payload Cassettes have been validated with strong *in vivo* efficacy data using FRα targeting SM-TDCs

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