Tunable Drug Conjugates: A Differentiated Drug Conjugate (DC) Platform

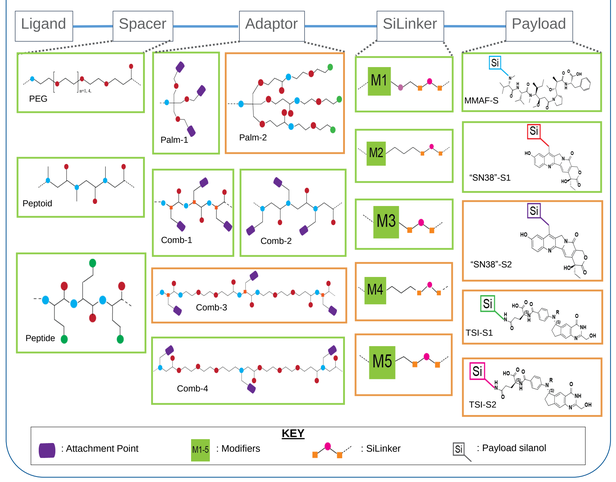
Vinayak Gupta[1], Sara C. Hickey[1], Hanh N. Nguyen[1] Christoph Rader[2], Michael K. Rood[1], Jutta Wanner[1] and Douglas S. Werner[1]

[1]BlinkBio, Inc., 130 Scripps Way, Jupiter FL 33458. [2]The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458.

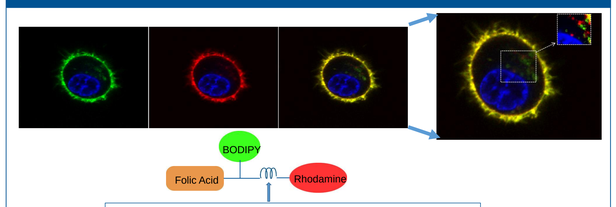
Introduction

We integrated a family of innovative technologies with an aligned set of properties into Tunable Drug Conjugates (TDCs). Our goal is to improve Therapeutic Index and dosing flexibility by creating TDCs around a rapid payload release/rapid systemic clearance approach. TDCs employ small molecule and proprietary antibody fragment ligands (DVD-Fabs); proprietary Silicon-based linkers (SiLinkers); and, novel Payload Cassette (PC) designs that are preferentially coupled to Conditionally Activated Payloads (CAPs). Our SiLinker/CAP constructs are designed to concentrate payload in the targeted tumor cell (and associated tumor microenvironment) while minimizing systemic payload release and rapidly clearing the parent TDC. We believe the properties of our TDCs will differentiate from other Drug Conjugates (DCs) and could provide a compelling addition to the existing array of DC technologies. We use a modular approach for the flexible assembly and evaluation of TDCs. We designed multiple families of ligands, spacers, Payload Cassette adapters, SiLinkers and CAPs to establish a 'toolbox' of components allowing the rapid exploration of targets. We assembled SiLinker- based TDCs to target the folate receptor α (FRα) using folic acid or a DVD-Fab as the targeting ligands and various silanol analogs of cytotoxic payloads. We used some historically deployed payloads for early benchmarking of the technology. We established the pH-dependent hydrolytic behavior of preferred SiLinkers. They exhibit good stability at 37°C in pH7.4 buffer (and plasma) and show rapid hydrolysis at pH ~5.5 to 6.5 ($t_{1/2}$ = 15 - 120 min). We have validated our TDC technologies in imaging, cellular and *in vivo* Xenograft studies.

Components of Modular Toolbox Enable Assembly of Differentiated TDCs



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 disilyl ether 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

SiLinkers Exhibit Tunable pH-Dependent Hydrolysis Profiles

	Pyrimidine	Amide	Phenyl	Carboxamide	Homologous Pyrimidine	Triazole	Benzyl
pH 7.4 $t_{1/2}$ (mins)	>600	6080	6170	>7920	9850	9134	>11520
pH 5 $t_{1/2}$ (mins)	2	77	307	95	480	445	1550

* Hydrolysis studies were carried out in 50 mM HEPES buffer (pH 7.4 or pH 5) at 37 °C

- SiLinkers are stable at pH 7.4 (and in plasma), and hydrolyze rapidly at more acidic pHs.
- Hydrolysis profiles can be tuned by incorporating different Modifiers

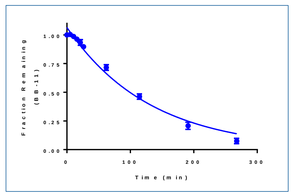
Folate Receptor Targeting Small Molecule TDCs (FA-SM-TDCs)

- Early prototypes of SM-TDCs contained folic acid as the targeting ligand & Vinblastine Silanol (VS) as a payload for benchmarking against published data. Triple Payload Cassette constructs (BB-03, BB-11) were included to demonstrate the impact of higher Payload / Conjugate ratio in PoC studies in comparison to single payload BB-01.
- SM-TDC optimization focuses on incorporation of CAPs as payloads, and employing our modular toolbox to optimize all elements of any candidate molecule. For this, a prototype SM-TDC, BB-42, used MMAF Silanol (MMAF-S) as the payloads to validate the CAP concept.

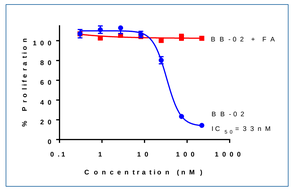
In Vitro Validation of SiLinker and Payload Cassette Components

Compound	EC_{50} (nM) in KB cells
VH	4.2
VS	6.0
AF	88
AF-S	0.3
MMAF	307
MMAF S	345
10-hydroxycamptothecin	14
SN38-S	179

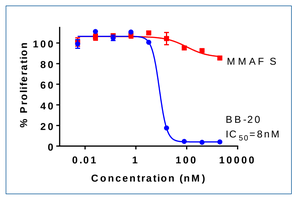
Exemplary Silanol analogs of cytotoxic payloads retain comparable activity to the parent drugs: VH = Vinblastine Hydrazide; AF = Auristatin F; MMAF = Monomethyl Auristatin F; SN-38-S = Silanol analog of 10-hydroxycamptothecin



Fully assembled SM-TDCs maintain the pH-dependent hydrolysis profile. Exemplified with BB-11 which 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 $t_{1/2}$ ≥ 24 hours



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



The charged state of CAPs at physiological pH of 7.4 prevents cellular permeability and cell kill. In the acidic tumor microenvironment and endosome, high permeability is achieved, leading to selected cell kill The potent cellular activity of BB-20 demonstrates the selective release of MMAF-S inside target cells

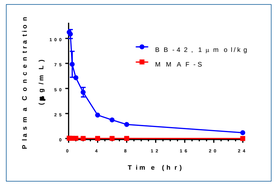
Cell Proliferation inhibition was assayed in KB cells using a 2 hour treatment followed by washout and a 70 hour chase

DMPK Profiling of SM-TDCs

SM-TDCs exhibit good to excellent plasma stability, excellent exposures, low volume of distributions, and clearance rates between 3-12 hours

SM-TDC	Modifier	Plasma Stability (H/R/M) % Remaining*	Dose (mg/kg)	$T_{1/2}$ (h)	CL (mL/min/kg)	AUC (h*ng/mL)	Vss (L/kg)
BB-01	Pyrimidine	*TBD*	5	3.3	0.12	717,557	0.02
BB-03	Pyrimidine	*TBD*	5	4.2	0.11	770,689	0.05
BB-11	Pyrimidine	25/36/77	5	2.8	0.08	1,016,127	0.02
BB-20	Phenyl	100/100/100	5	11	0.12	716,717	0.10
BB-42	Pyrimidine	100/100/91	5	11	0.16	534,897	0.12

* At 24 hours

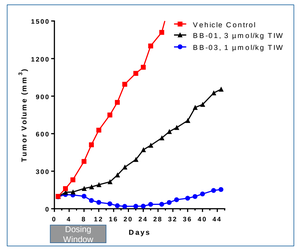


Single dose PK data in C57-Black mice show that maximum systemic release of payload is between 0.8-3% over 24 hours using the most labile SiLinker prototypes with the exception of BB11 which released 9%.*

*Compound handling may also influence amounts of MMAF-S measured in plasma samples

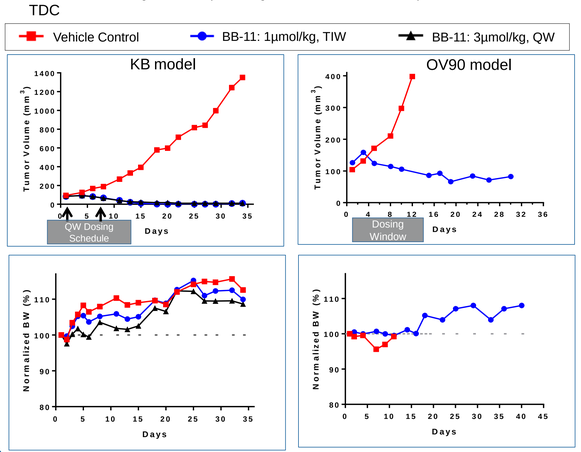
In Vivo Validation of SiLinker and PC Platform

- 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

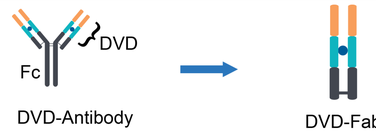


BB-11 Demonstrates Robust *In Vivo* Efficacy & Safety

- 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



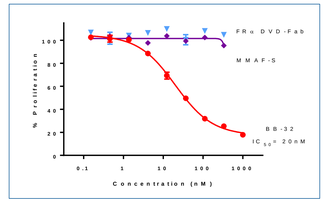
DVD-Fabs Broaden Targeting Capability and DVD-Fab-TDCs May Provide Optimal Tumor Residence Time



- **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 TDCs

- CAPs also can be employed for DVD-Fab TDCs
- BB-32 is a potent single MMAF Silanol-containing FRα targeting DVD-Fab TDC
- DVD-Fab does not kill cells on its own
- DVD-Fab TDC shows strong cell kill



Summary

- We have innovated TDCs from a family of novel technologies with an aligned set of properties designed to provide a differentiated rapid payload release/rapid systemic clearance approach that we believe can provide a compelling addition to existing Drug Conjugate technologies
- Our modular toolbox allows for the rapid synthesis and systematic evaluation of a series of targeted TDCs to facilitate lead optimization and candidate selection
- Conditionally Active Payloads (CAPs) are ideally suited as payloads for TDCs, because aligned pH properties can further increase Therapeutic Index (TI)
- 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
- Broad Targeting Capability using DVD-Fab targeting ligands may address many targeted surface proteins that antibodies can be raised against
- Tunable rapid cleavage and SiLinkers payload release kinetics allows tailoring of TDC payload release
- Payload Cassette Technology can enable endosomal release of both mixed payloads and high stoichiometric Payload:Conjugate ratios
- SiLinkers and Payload Cassette components of the TDC toolbox have been validated with strong *in vivo* efficacy data using FRα targeting SM-TDCs

Acknowledgements:

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