# StarLinker (SL) Technology: A Novel Synthetic Linker Technology Platform With Robust and High DAR and Multiple-Agent ADC Capabilities

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## Background

Synthesis of ADCs is typically achieved by coupling a cytotoxic agent to an antibody using thiol specific linkers on the antibody surface. A typical linker-payload (LP) consists of one linker connecting to one payload (drug molecule). The Drug-to-Antibody Ratio (DAR) for the current marketed ADCs ranges from 2 to 8. To tackle some biological targets that have low expression levels and better address the heterogenous nature of the tumors, it is desirable to have ADCs with higher DARs to allow more effective cell tumor killing. Furthermore, there is an increasing demand focus on developing the next generation of ADCs with dual cytotoxic agents from different MOAs to produce synergistic effects. Moreover, hydrophilic linkers with low molecular weight (CMC friendly) would benefit and improve properties of ADC drug-likeness, such as reduced aggregation, improved solubility, improved ADC plasma clearance and pharmacokinetics, reduced immunogenicity, and finally improved therapeutic window.

Herein, CanWell has developed a novel chemical-defined StarLinker technology platform where each linker can load up to 4 units of therapeutic agents. It offers significant flexibility in generating ADCs with DARs ranging from 4 to 32. ADC with DAR up to 21 has been developed, while ADC with the maximum expected DAR of 32 are planned. More importantly, it has the capability to load 2 or more different therapeutic agents at different ratios. Our pre-clinical studies conducted to date show our ADCs generated under StarLinker platform have good hydrophilicity, little aggregation and satisfactory PK profile. StarLinker has the potential to become an important enabling technology in the development of the next generation ADCs with high DARs and/or dual/multi-drug ADCs. Further developments are currently ongoing, including partnering of the technology.

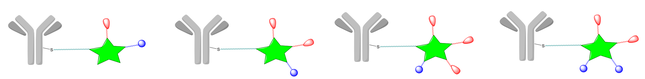
## Features of StarLinker-Payloads

- **Small molecules with MW 1500~6000**
- **Pure single compound prepared via synthetic chemistry**
- **1~4 Payloads linked on each linker.**
- **Payloads can be a variety of toxins or other small drugs;**
- **Dual payloads with the ratio precisely controlled by structure design**
- **Excellent and adjustable hydrophilicity**
- **StarLinker ADCs have favorable drug-likeness properties**

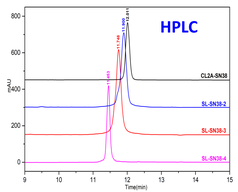
## StarLinker ADCs with 1-4 Identical or Different Payloads

**Starlinker ADCs with same payloads:**

**Starlinker ADCs with dual payloads:**



## Hydrophilicity of StarLinker-Payloads as Confirmed in HPLC



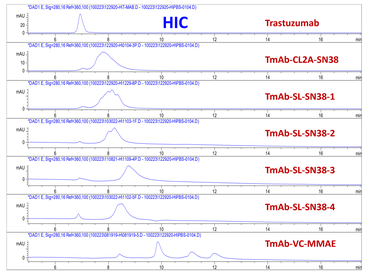

| Linker-Payload (LP) | HPLC Retention Time (min) |
|---|---|
| CL2A-SN38 | 12.01 |
| SL-SN38-2 | 11.90 |
| SL-SN38-3 | 11.75 |
| SL-SN38-4 | 11.45 |
| MC-GGFG-DXd (DS-8201 LP) | 13.17 |
| SL-DXd-1 | 12.12 |
| SL-DXd-2 | 13.24 |
| SL-DXd-3 | 13.56 |

StarLinker payloads with 1-4 of SN38s or 1-3 of Dxds were synthesized respectively. The HPLC retention time of all StarLinker-payloads are less than their corresponding reference LP; CL2A-SN38 or MC-GGFG-Dxd, indicating better hydrophilicity of the StarLinker-payloads.

| Linker-Payload (LP) | HPLC Retention Time (min) |
|---|---|
| VC-MMAE | 15.28 |
| SL-Exatecan-1 | 11.59 |
| SL-Exatecan-2 | 11.85 |
| SL-MMAE-Exatecan | 14.05 |
| SL-MMAE-DXd | 14.32 |
| SL-MMAE-SN38 | 13.83 |

StarLinker with 1 to 2 Exatecan, dual drugs (MMAE-Exatecan, MMAE-DXd, and MMAE-SN38) were synthesized. The HPLC retention time of the StarLinker-payloads are less than VC-MMAE, indicating improved hydrophilicity of the StarLinker-payloads.

## StarLinker ADCs with High DAR and Low Aggregation




| LP | Trastuzumab ADC | DAR | Agg% |
|---|---|---|---|
| CL2A-SN38 | TmAb-CL2A-SN38 | 4.9 | 2.0 |
| SL-SN38-1 | TmAb-SL-SN38-1 | 5.1 | 1.7 |
| SL-SN38-2 | Tmab-SL-SN38-2 | 10.3 | <1.0 |
| SL-SN38-3 | TmAb-SL-SN38-3 | 13.1 | <1.0 |
| SL-SN38-4 | TmAb-SL-SN38-4 | 20.7 | <1.0 |
| VC-MMAE | TmAb-VC-MMAE | 5.1 | <1.0 |

A number of ADCs were prepared from Trastuzumab and StarLinker Payloads. The excellent hydrophilicity of StarLinkers were confirmed by the retention times of the corresponding ADCs as shown in the Hydrophobic Interaction Chromatography (HIC). Aggregation level is very low across all StarLinker ADCs even with a high DAR of 20.7.

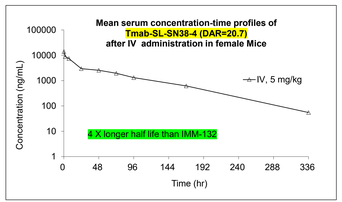
## In Vitro Test Results with StarLinker ADCs

| Compound | DAR | OVCAR-3 IC50 (nM) |
|---|---|---|
| SN38 | - | 63 |
| SL-SN38-1 | - | 453 |
| SL-SN38-3 | - | 108 |
| TmAb | - | NA |
| TmAb-CL2A-SN38 | 4.9 | 52 |
| TmAb-SL-SN38-1 | 5.1 | 44 |
| TmAb-SL-SN38-3 | 13.1 | 20 |

In Vitro experiment results indicate that SN38 in ADC is taken into cells by antibody and the payloads were released as expected.

## High-DAR StarLinker ADCs with Favorable Serum Stability and PK Properties

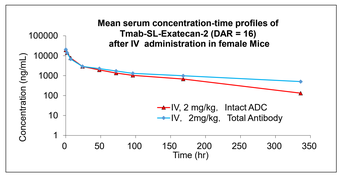
| ADC | DAR | Mouse Plasma Stability (T1/2, h) |
|---|---|---|
| Sacituzumab-CL2A-SN38 (IMM-132) | 7.6* | 17.5* |
| Tmab-CL2A-SN38 | 8 | 10 |
| Tmab-SL-SN38-3 | 13.1 | 55 |




| Parameter | Units | Estimate |
|---|---|---|
| $T_{1/2}$ | hr | 52.2 |
| $C_0$ | ng/mL | 14376 |
| $AUC_{last}$ | hr*ng/mL | 439031 |
| $AUC_{INF}$ | hr*ng/mL | 443185 |
| CL | L/hr/kg | 0.0113 |
| $MRT_{INF}$ | hr | 68.3 |
| $V_{ss}$ | L/kg | 0.771 |

* Cardillo *et al.* Bioconjug chem. , 2015, 919.

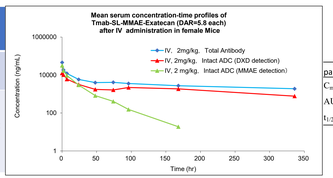
| ADC | DAR | Mouse Plasma Stability |
|---|---|---|
| TmAb-SL-Exatecan-2 | 16 | 100% remain @ 168 h |




| Parameter | Units | Estimate |
|---|---|---|
| $C_{max}$ | ng/mL | 19488 |
| $T_{max}$ | h | 0.083 |
| $AUC_{0-336}$ | h*ng/mL | 434719 |
| $t_{1/2}$ | h | 77.4 |

## Dual-Drug StarLinker ADC's Promising Serum Stability and PK Properties

| ADC | DAR | Mouse Plasma Stability | Human Plasma Stability |
|---|---|---|---|
| Ab095-VC-MMAE* | 4 | 75% remain @ 144h* | >99% remain @ 144h* |
| TmAb-SL-MMAE-Exatecan | 5.8 for each drug | • MMAE: 78% remain @ 168 h; • Exatecan: 100% remain @ 168 h | • MMAE: 100% remain @ 168 h; • Exatecan: 100% remain @ 168 h |




**Intact ADC-DXD**

| parameter | unit | estimate |
|---|---|---|
| $C_{max}$ | ng/mL | 12843 |
| $AUC_{0-336}$ | h*ng/mL | 657452 |
| $t_{1/2}$ | h | 153 |

* Durbin *et al.* ACS Omega, 2017, 4207.

* The higher MMAE levels in mouse plasma than in human plasma were expected due to the presence of carboxylesterase 1C in mouse plasma. This resulted with the shorter half life of the intact ADC in mouse PK. The intact ADC is expected to have longer half life in non-rodent PK.

## Conclusions

- StarLinker chemistry has been validated.
  - Ability to link 1-4 payloads; Payloads could be the same or different
  - Conjugation chemistry is validated
  - High DAR (8-21) and dual drugs payload achieved, potentially up to a DAR of 32
- StarLinker ADCs have favorable drug-likeness properties.
  - Excellent and adjustable hydrophilicity
  - Stable in vitro (PBS, plasma)
  - Potent in different cell lines and with payloads released as expected
  - Favorable DMPK properties
- Partnering or co-development is welcome!