# Dual-payload TME-activated ADC platform

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# 1805

## Introduction

The combination of Antibody-Drug Conjugates (ADCs) and Immuno-Oncology (IO) agents is regarded as one of the most exciting and promising trends in the rapidly evolving field of cancer treatment. Affinity Biopharma has developed the Tumor MicroEnviroment Activated (TMEA) platform, with proprietary TMEA linkers applicable in both Small Molecule Drug Conjugates (SMDCs), ADCs and dual-payload ADCs.

The TMEA linkers could achieve extracellular activation of payloads in the Tumor MicroEnviroment (TME) by a tumor-specific protease Legumain (AEP), which is an acid endopeptidase with tumor site over-expression and a strict specificity for one particular amino acid (Asn), making it a first-in-class, highly specific and pan-solid tumor target in novel drug design.

### Dual-Payload TMEA-ADC Structure (8-DAR dual-payload design)

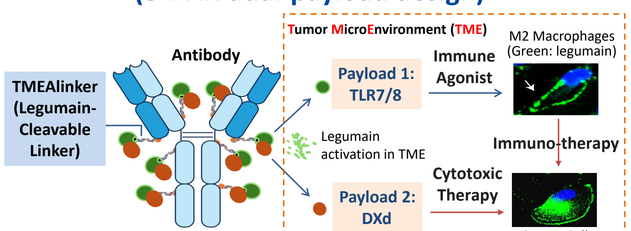



Figure 1: For dual-payload TMEA-ADCs, each conjugated group is paired with two insoluble payloads via the hydrophilic TMEAlinker. Payloads, including TLR7/8 agonists and topoisomerase I inhibitors, are shielded by the TMEAlinkers to reach comparable maximum tolerated doses (MTD) in both mice and monkeys. The homogeneous formation of 8-DAR (16-Drug) ADCs is achieved through single conjugation to the antibody, which significantly simplifies the CMC processes.

### Activating Protease: Legumain

Legumain is highly expressed by tumor cells and by TAMs (M2 macrophages) in almost all tumor tissues. Legumain could be secreted onto the tumor cells surface and into the tumor-cell cultured medium, achieving extracellular activation of payloads under acidic environment in the TME. The activation of the linker in the TME can be assessed by evaluating whether the IgG-linker-toxin conjugate exhibits efficacy without requiring targeting or endocytosis.

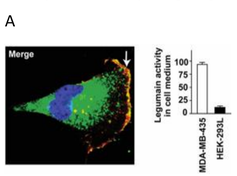



Figure 2A[1]: Legumain is co-localized with integrin αVβ3 on tumor cell surface, and the tumor cell cultured medium of MDA-MB435 has legumain activities.

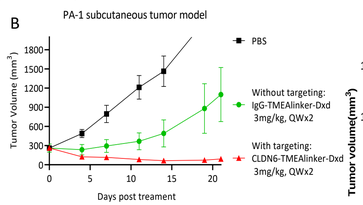



Figure 2B: IgG-TMEAlinker-DXd demonstrated efficacy, indicating that legumain can release cell-permeable DXd in the TME without the need for antibody targeting or endocytosis. CLDN6-TMEAlinker-DXd further enhanced efficacy by increased targeting, particularly in the large tumors (300 mm³) treatment.

## Results

### TMEAlinker vs. GGFG Linker

#### Highly stable in human plasma

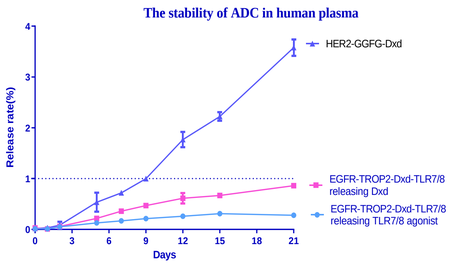



Figure 3: Compared to GGFG linkers cleaved by lysosomal enzymes such as cathepsins B and K, the TMEA linkers exhibited superior stability in human plasma. For EGFR-TROP2-TMEAlinker-DXd-TLR7/8 ADC, the 21-day release rate of free DXd and TLR7/8 in human plasma (<1%) was significantly lower than DXd release rate for DS8201 and Dato-DXd in the literature (~3%-4%).

#### Safety Profile in Primate

| Antibodies | PD-L1 | EGFR-TROP2 | ROR1 | CLDN6 |
|---|---|---|---|---|
| Linker | TMEAlinker | TMEAlinker | TMEAlinker | TMEAlinker |
| 8-DAR Payloads | TLR7/8 agonist | DXd & TLR7/8 | DXd & TLR7/8 | DXd & TLR7/8 |
| Safety Test in Primate | 50mg/k Not reaching HNSTD* | 60mg/k Not reaching HNSTD* | 80mg/kg Not reaching HNSTD* | 60mg/kg Not reaching HNSTD* |

*HNSTD: Highest non-severely toxic dose

Table 1: In non-human primate (NHP) studies, TMEAlinker and dual-payload TMEA-ADCs showed excellent stability and safety profile in circulation, reducing DLT (dose-limiting toxicities) and indicating a greatly expanded therapeutic window.

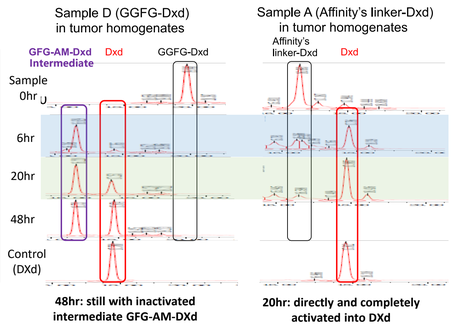



Figure 4: TMEAlinker showed higher cleavage efficiency compared to GGFG-linker under identical condition in tumor homogenates (pH=6.5), generating a clean cleavage with minimal to no inactive intermediates. The payload DXd was largely released after around 6 hrs in Sample A, while it took around 20-48 hrs in Sample D.

### ROR1-TMEAlinker-DXd-TLR7/8 (IMD2358)

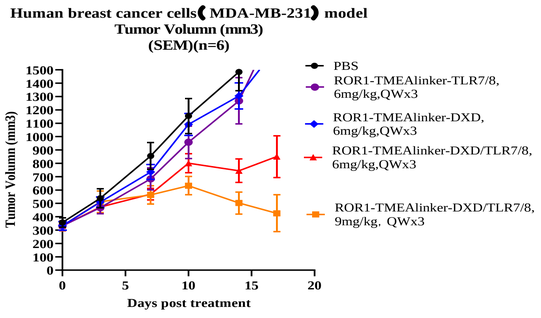



Figure 7: Single payload ADCs showed no efficacy against tumors larger than 350mm³ in human breast cancel cells model. In contrast, IMD2358 demonstrated dose-dependent efficacy and tumor shrinkage in large tumors > 350mm³ in size. (IMD2358 MTD >120mg/kg QW*3 in mice)

### Her2-TMEAlinker-DXd-TLR7/8 (IMD526): Toxin-ISAC Dual Payload ADC

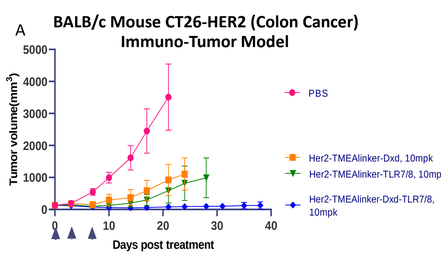
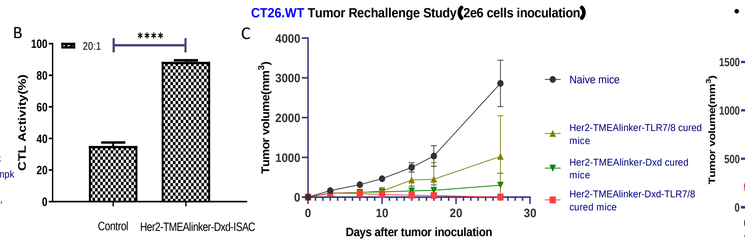



Figure 5A: When conjugated to HER2 antibody, dual-payload ADC IMD526 was superior to two single-payload-ADCs in efficacy in in-vivo CT26-HER+ syngeneic mouse model, and long-term elimination of detectable tumors was achieved.




Toxin-ISAC dual payload ADC demonstrates in situ vaccine characteristics:
Figure 5B: Enhanced CTL activities of T cells extracted from the spleen of mice cured by IMD526;
Figure 5C: In tumor re-challenge studies, all mice cured by IMD526 and later inoculated with CT26 tumor cells (2×10⁶) showed no relapse (exhibited immune memory), whereas a few cases of relapse were observed with single-payload ADC treatment.

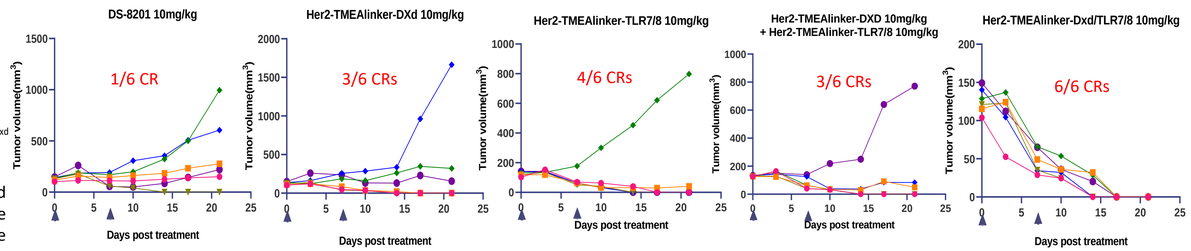



Figure 6: In preclinical animal models (N=6), equal molar dose of dual-payload ADC Her2-TMEAlinker-DXd-TLR7/8 (6/6 CRs) was superior in efficacy to DS8201 (1/6 CR) and two single-payload-ADCs, namely Her2-TMEAlinker-DXd and Her2-TMEAlinker-TLR7/8, and these two ADCs in combination.

### EGFR-TROP2-TMEAlinker-DXd-TLR7/8 (IMD2113)

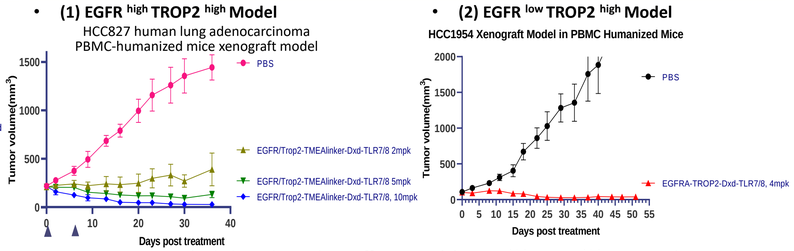



Figure 8: IMD2113 had dose-dependent efficacy in (1): EGFR$^{high}$ TROP2$^{high}$ HCC827 human lung cancer tumors, with an effective dose of 2 mg/kg in mice; (2) cured EGFR$^{low}$ TROP2$^{high}$ HCC1954 tumor model with 4mg/kg QWx2 dose. Long-term monitoring confirmed no tumor recurrence.

### PDL1-TLR7/8 ADC (IMD2109)

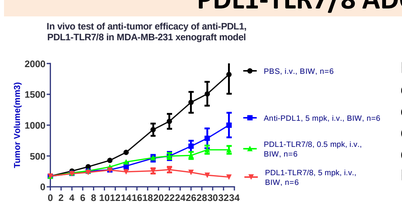



Figure 9: IMD2109 exhibited dose-dependent efficacy in human TNBC tumor model, outperforming PD-L1 antibody at equimolar doses. Notably, IMD2109 at 0.5 mg/kg demonstrated superior efficacy compared to PD-L1 at 5 mg/kg.

**Conclusion:** By integrating antibody targeting, TME activation, cytotoxic therapy, and I/O therapy into one single molecule, the dual-payload TMEA-ADCs have demonstrated the synergistic effects of ADCs conjugated with 16 insoluble payloads from 2 distinct mechanisms of actions in tumor models. And ICD inducing toxin-ISAC dual payload ADCs showed in situ cancer vaccine characteristics.

**References:**
1. Liu Y, Liu C, etc. Targeting cell surface alpha(v)beta(3) integrin increases therapeutic efficacies of a legumain protease-activated auristatin prodrug. Mol Pharm. 2012 Jan 1;9(1):168-75.

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