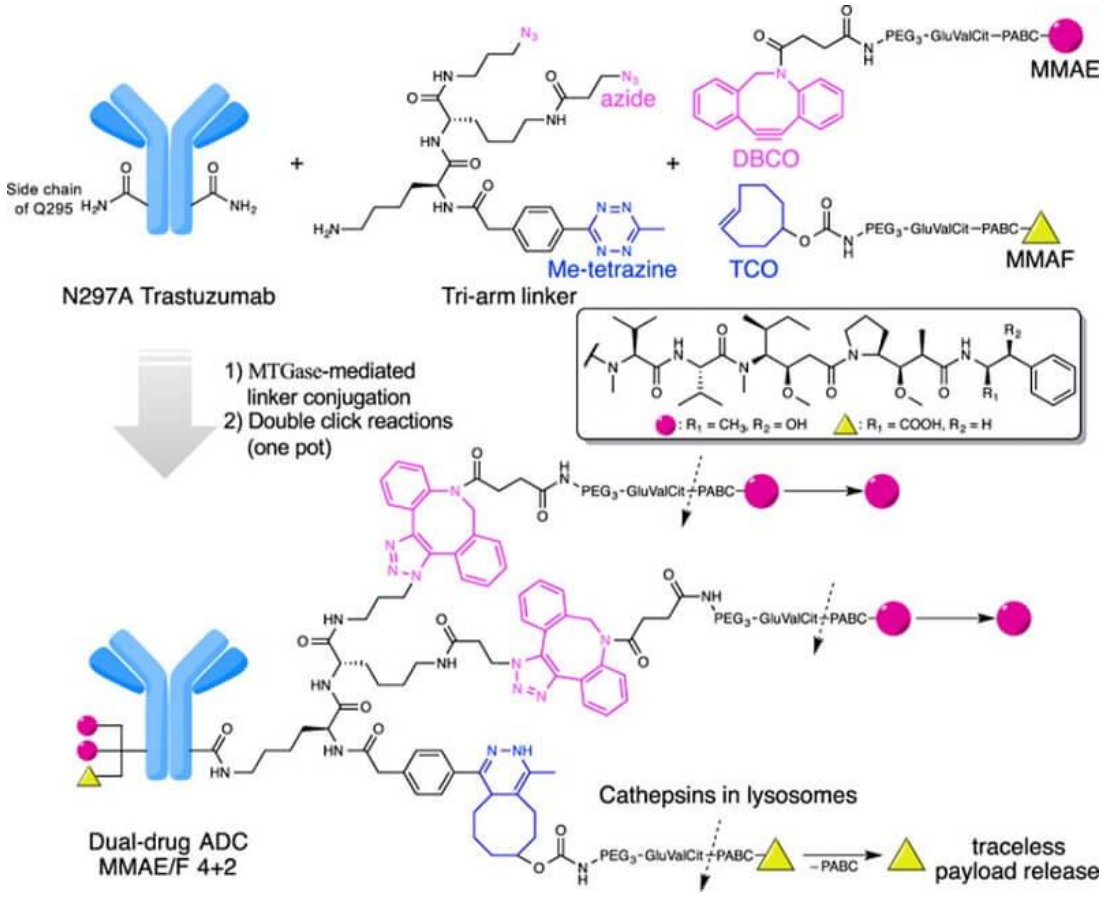
Antibody-Drug Conjugates With Dual Payloads Against Heterogeneous Tumors

Breast tumors often consist of distinct cell populations with distinct gene expression profiles. Breast tumor heterogeneity is a major factor leading to drug resistance, recurrence and metastasis after chemotherapy. Currently approved ADCs such as T-DM1 for HER2-positive breast cancer have good efficacy, but often have problems related to the inability to combat intratumoral heterogeneity.

Professor Zhiqiang An and Associate Professor Kyoji Tsuchikama from the University of Texas Health Science Center at Houston, USA, published a paper entitled "Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance" on Nature Communications. They combined their expertise in antibody and click chemistry to build a "dual-payload ADC". The DAR can be flexibly adjusted to 2 + 2, 4 + 2 and 2 + 4, thereby fine-tuning the physicochemical properties, efficacy and toxicity profile of the ADC.

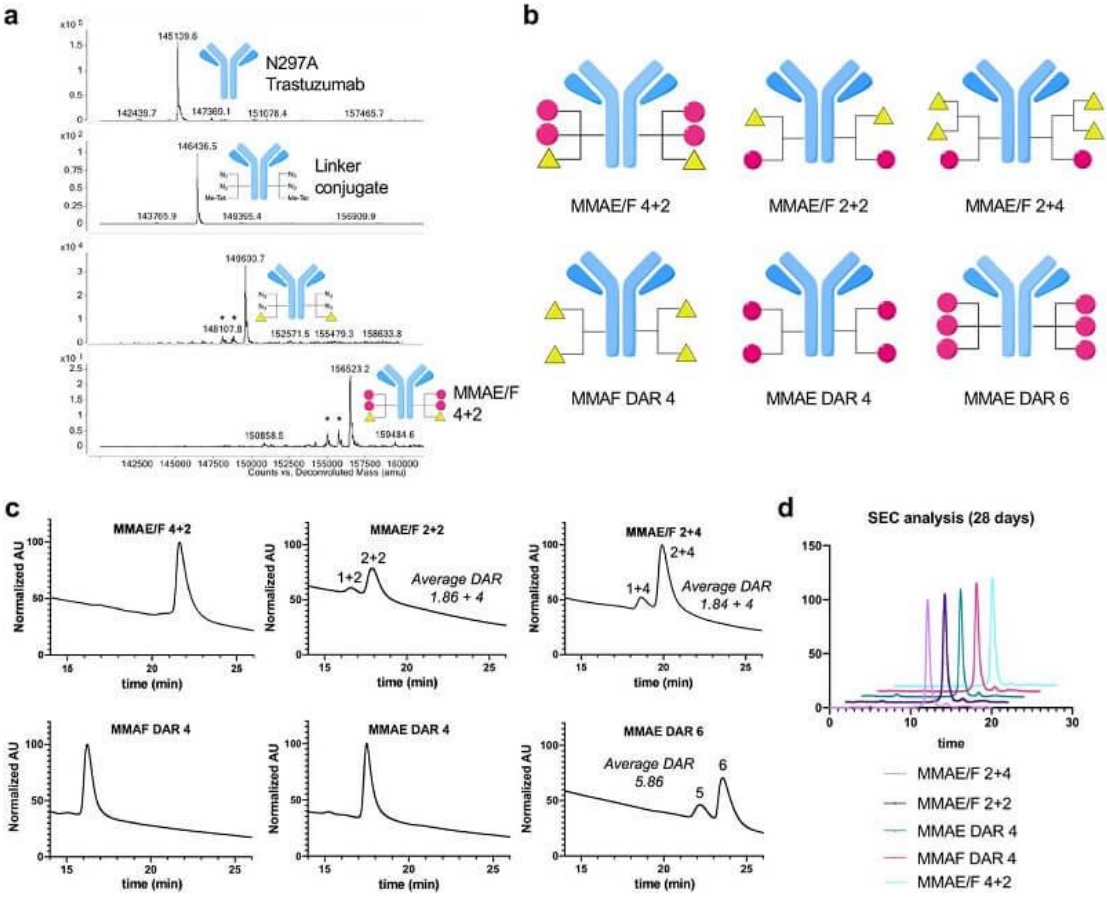
Structure Design of "Dual-Payload ADC"

The antibody uses Trastuzumab, which targets HER2. The side chain is a trifurcated branch chain synthesized by the researchers, which has two joints of "N3" and one joint of "me-tetrazine", in which "N3+ dibenzocyclooctyne DBCO" is a click chemical pair, and "me-tetrazine+ trans-cyclooctyne (TCO)" is a click chemical pair. And it is confirmed that the two click chemical pairs do not cross - couple. Meanwhile, the DBCO of the former was coupled with "glutamine-valine-citrulline (GluValCit) -PABC split-MMAE". The latter TCO is coupled with the "GluValCit-PABC connector -MMAF". Through this structure design, a "double load ADC" based on click chemistry is synthesized.



DAR Flexibility of "Dual-Payload ADC"

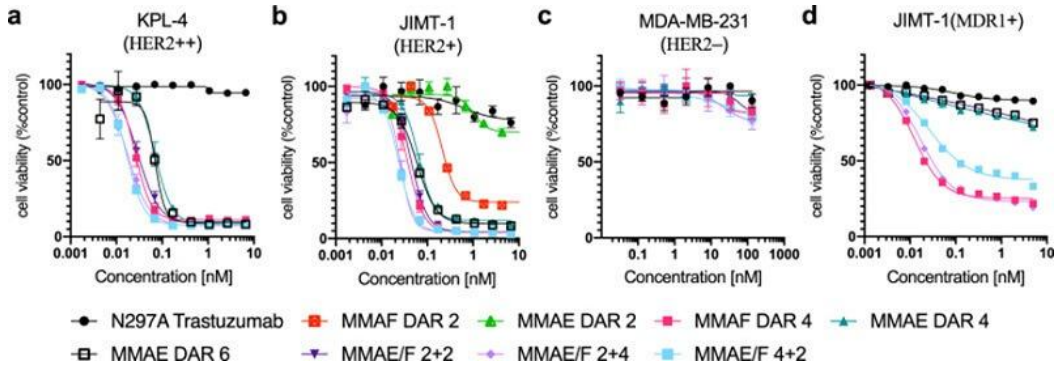
By adjusting the structure of the branched chain and the click chemical reaction pair, the flexible recombination of the dual-payload ADC can be achieved, and the physicochemical properties, efficacy and toxicity characteristics of the ADC can be fine-tuned. Liquid chromatography (LC)-mass spectrometry (MS) showed that this dual-load coupling did not generate impurity conjugates. After incubation of each conjugate in PBS (pH 7.4) at 37°C for 28 days, no significant degradation or aggregation of the ADC was observed.



Cell Killing Toxicity of "Dual-Payload ADC"

The "double-loaded ADC" showed high cytotoxicity only in HER2-expressing cell lines, with EC50 values of 0.017 -- 0.029 nM in KPL-4 cells and 0.024 -- 0.045 nM in JIMT-1 cells. No significant toxicity was observed in HER2-negative cell lines.

The MDR1 gene, the multi-drug resistance gene, exhibited effective cytotoxicity against this highly resistant cell type in both single-drug and "double-loaded ADCs" containing MMAF, but not in MMAE single-drug ADCs. This result suggests that the "combined binding of MMAF" can help hydrophobic chemotherapy drugs (MMAE) overcome breast tumors with high resistance.

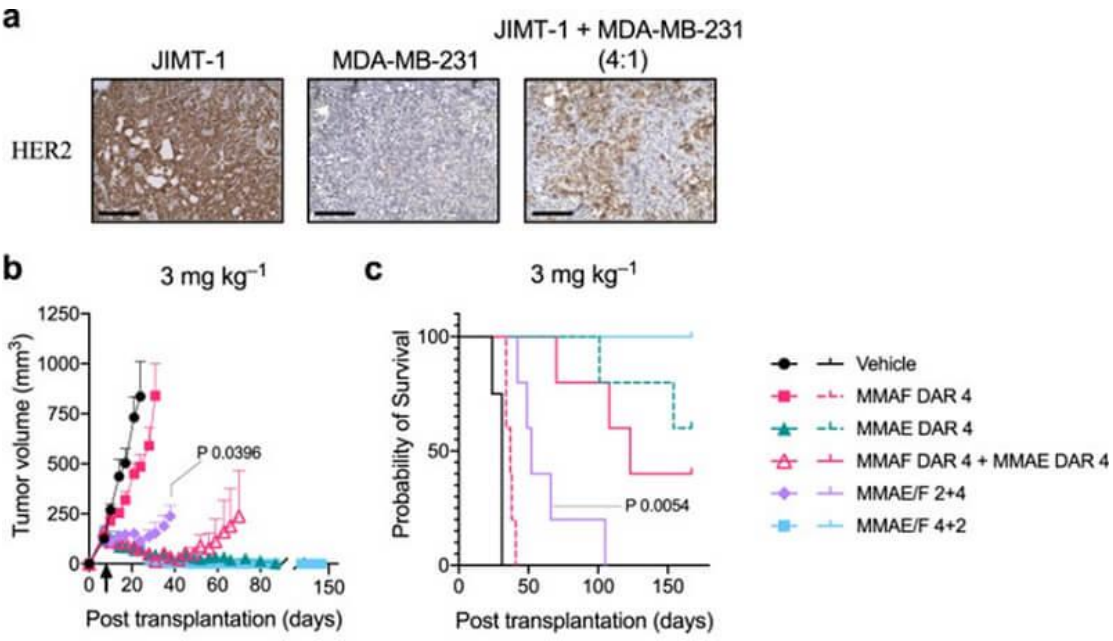


"Dual-Payload ADC" Has Great Potential For The Treatment of HER2 Heterogeneous/Resistant Breast Tumors

The researchers established the transfer of HER2-positive JIMT-1 cells and HER2-negative 231 cells (4:1) into immunodeficient PDX mice. This mixed tumor grew rapidly, and immunohistochemistry revealed a heterogeneous distribution of HER2-positive and negative cells within the tumor.

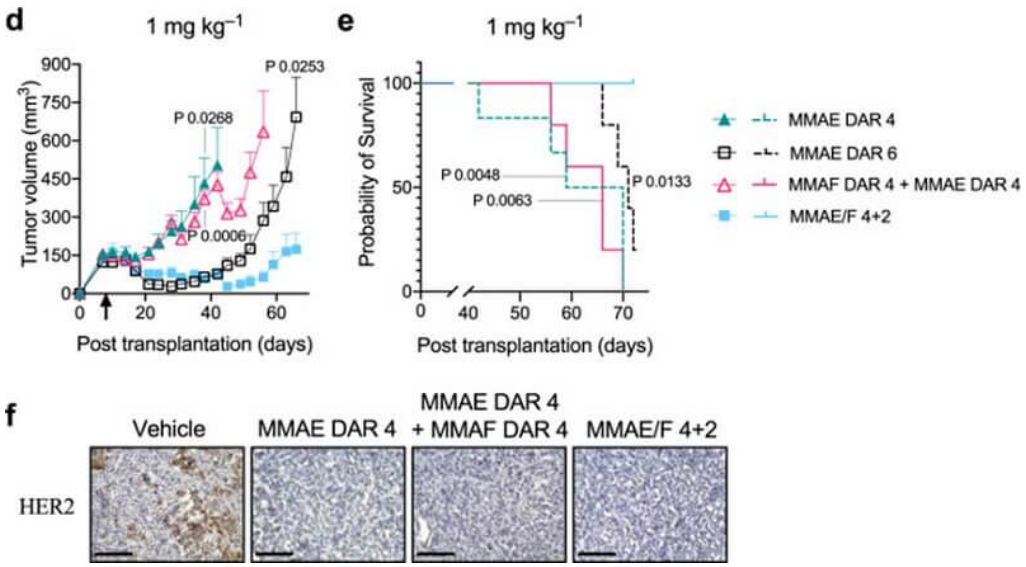
The MMAF DAR 4 ADC showed only limited inhibition of tumor growth in this HER2 heterogeneous model. This result is in stark contrast to our previous reports on the remarkable efficacy of JIMT-1 single-cell lines in providing complete remission, which may be due to the absence of bystander effect in MMAF alone, which may not be effective in vivo.

The "dual-payload ADC" of MMAE/F 4+2 completely eliminated the heterotumor, and no death occurred in this group of mice.



In order to make the above experimental results clearer, the researchers used a lower dose of 1mg/kg for treatment, and found that the therapeutic effect of MMAE/F 4 + 2 "dual-payload ADC" was further highlighted. This result also highlights the advantages of "MMAF co-combination" in the treatment of drug resistance in breast tumors. At the same time, the curative effect of the cocktail therapy "MMAF DAR4+MMAE DAR4" is poor, and the tumor growth is still rapid.

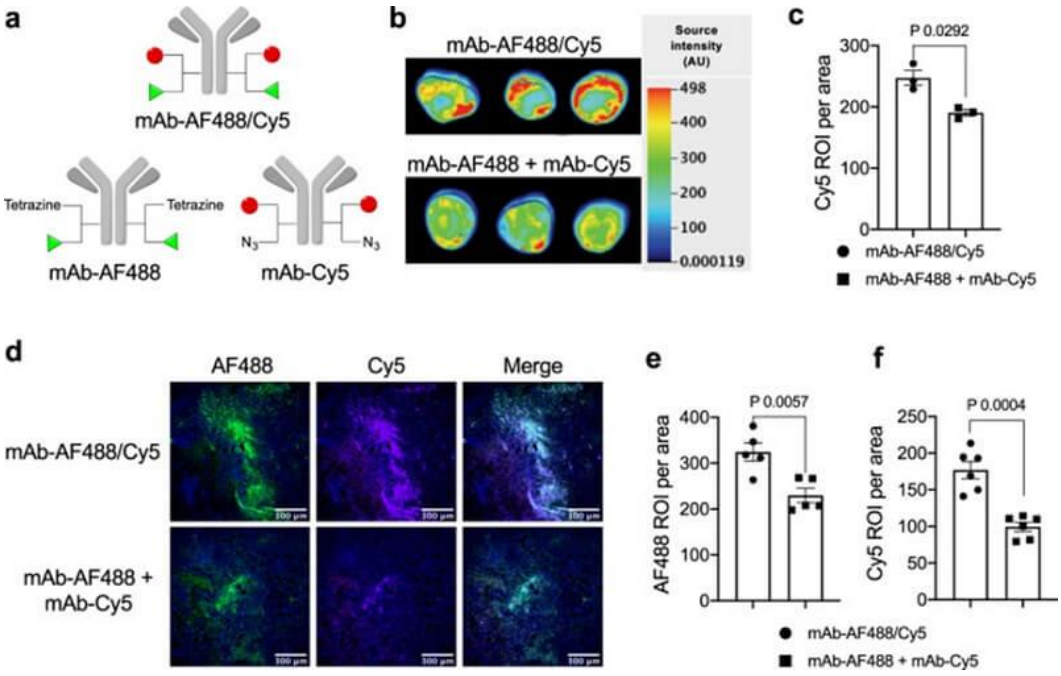
At the end of the experiment, immunohistochemistry of tumors showed that all tumors were composed of HER2 negative cells. No HER2 positive cells were detected. These results suggest that the proliferation of MDA-MB-231 cells is a major factor in tumor recurrence in this model.



Why Is "Dual-Payload ADC" More Effective Than "ADC Combination"?

To explore what makes "dual-load ADCs" more effective than ADC combinations. The researchers prepared anti-HER2 mAb conjugated with Alexa Fluor® 488 or Cy5 as an alternative to single-agent ADC. In addition, an anti-HER2 mAb conjugated with two dyes was prepared as an alternative to "dual-payload ADC".

The results showed that the accumulation of dual-dye conjugations in the tumor was more fluorescent and concentrated than that of co-applied monodye antibodies. Based on this observation, the investigators speculated that combination therapy of two single-agent ADCs targeting the same antigen might cause binding competition, resulting in reduced delivery efficiency of each payload.



Summary and Prospect

1. Compared with MMAE=4, MMAE/F=4+2 is more resistant to drug resistance, and can exert a bystander effect at the same time

2. Compared with MMAF=4, MMAE/F=4+2 has a bystander effect, and has a significant tumor inhibitory effect in heterogeneous tumors.

3. Compared with MMAE=4+MMAF=4, MMAE/F=4+2 has higher delivery efficiency, and the former has competition

4. It shows the convenience, universality, stability and safety of click chemistry.

Click chemistry is a versatile tool, and its use is not limited to a specific situation. At present, many ADC companies have begun to deploy click chemistry in the field of ADC. This is a new direction worth exploring.

Table 1 | Selected biotech companies working with bioorthogonal chemistry for ADC preparation

Name	ADC components	Leading candidates (partner)	Target	Progress (date initiated)
Ambrx	HER2 targeting antibody + Amberstatin269	ARX788	Advanced cancers with HER2 expression	Phase 1 (March 2016)
Catalent/ Redwood Bioscience	Anti-CD22 antibody + maytansine	TRPH-222 (Triphase Accelerator Corp.)	Non-Hodgkin lymphoma	Phase 1 (April 2019)
Sutro Biopharma	Anti-CD74 IgG1 antibody (SP7219) + maytansine	STRO-001	Multiple myeloma and non-Hodgkin lymphoma	Phase 1 (April 2018)
Sutro Biopharma	Anti-FolRa human IgG1 antibody (SP8166) + 3-aminophenyl hemiasterlin	STRO-002	Ovarian and endometrial cancers	Phase 1 (March 2019)
Synaffix	Anti-AXL IgG1 antibody + pyrrolobenzodiazepine dimer SG3199	ADCT-601 (ADC Therapeutics)	Solid tumors with AXL expression	Phase 1 (January 2019)
Syndivia	Undisclosed	SDV1101	Breast cancer, liposarcoma	Preclinical: human trials expected 2021
Tagworks	CC49 fragment + monomethyl auristatin E	TAGW-110	Tumor-associated glycoprotein 72 (TAG72) in various cancers	Preclinical

As a professional PEG supplier, **Biopharma PEG** supplies high-purity PEG linkers in GMP or non-GMP grades for your ADC & PROTAC research and development. We can also provide custom PEG synthesis services to meet your needs. Contact us at sales@biochempeg.com for more information.

References:

[1].Yamazaki CM, Yamaguchi A, Anami Y. Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance. Nat Commun. 2021 Jun 10;12(1):3528.

[2]. umar A, et al. Synthesis of a heterotrifunctional linker for the site-specific preparation of antibody-drug conjugates with two distinct warheads. Bioorg. Med. Chem. Lett. 2018;28:3617–3621.

[3].Nilchan N, et al. Dual-mechanistic antibody-drug conjugate via site-specific selenocysteine/cysteine conjugation. Antib. Ther. 2019;2:71–78.

Related articles:

[1].Key To The Successful Design of ADC Drugs

[2]. ADC Drugs Global Sales of 2021 and Future Prospects

[3]. Learn More About ADCs From Its Structure

[4]. How To Choose The Best ADC Linker?

[5]. Antibody-drug Conjugates (ADCs) - Approvals & Clinical Trails