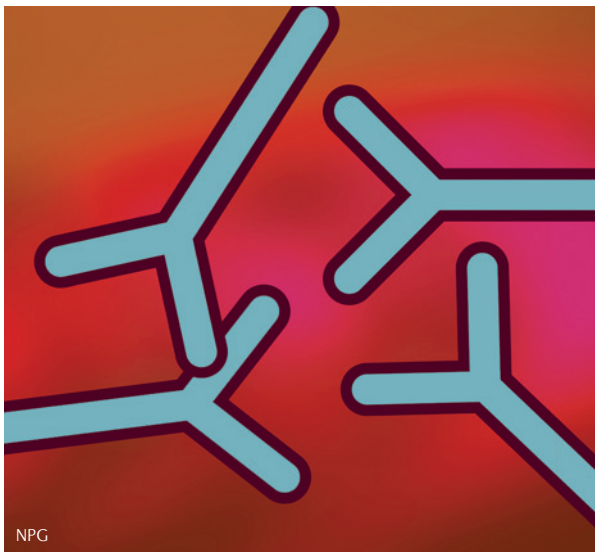
ANTIBODY ENGINEERING

Fine-tuning antibody–drug conjugates

Antibody–drug conjugates (ADCs), which harness the targeting ability of monoclonal antibodies (mAbs) to deliver highly toxic drugs to cancer cells, are emerging as a powerful new class of anticancer agents. However, it is difficult to control the site and stoichiometry of drug conjugation to the mAb, typically resulting in heterogeneous mixtures of ADCs that are difficult to optimize. Writing in *PNAS*, Tian and colleagues now report a new approach for generating site-specific ADCs (termed NDCs).

It had previously been shown that site specificity can be advantageous. However, technologies described before (such as site-specific cysteine conjugates (TDCs)) require a multi-step process for conjugation.

In order to develop an improved, general approach to site-specific conjugation, the authors devised a strategy to incorporate non-native amino acids into mAb scaffolds, allowing a conjugation chemistry that is not feasible with the 20 canonical amino acids.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In *Escherichia coli* expression systems, the incorporation of non-native amino acids is achieved by repurposing a little-used nonsense codon (TAG; the 'amber codon') to code for the new amino acid. Therapeutic mAbs, however, must be produced in eukaryotic protein expression systems to allow for correct glycosylation. It is more challenging to establish this technology in eukaryotic systems as these use the three nonsense (stop) codons with equal frequency, and the universal elongation of TAG-terminated host proteins can affect cell viability.

To engineer a viable eukaryotic cell line in which the amber codon codes for a new amino acid, Chinese hamster ovary (CHO) cells were stably transfected with an *E. coli*-derived tyrosyl-tRNA/aminoacyl-tRNA synthetase orthogonal pair. Subsequent transient transfection with a vector coding for amber-codon-disrupted green fluorescent protein (GFP), followed by single-cell fluorescence-activated cell sorting (FACS), led to the identification of a platform cell line that showed an optimal balance between amber suppression efficiency and cell viability.
The cell line was used for the production of various mAbs with genetically encoded *para*-acetylphenylalanine (*p*AF) residues. To assemble the NDC, the small-molecule drug auristatin D (a tubulin inhibitor) was coupled to a short linker containing a terminal alkoxyamine, which reacts with the aromatic ketone on *p*AF to form an oxime bond. The resulting NDCs have a drug:Ab ratio of 2.

In *in vitro* experiments, the NDCs were found to kill target cells much more efficiently than their TDC and conventional ADC counterparts, even though they had only half the drug load of the ADCs. The difference in potency was particularly apparent when the target cells had low antigen expression. NDCs also showed less cytotoxicity towards non-targeted cells.

In *in vivo* tumour xenograft models, the NDCs were well tolerated and more effective than their TDC and conventional ADC counterparts. Pharmacokinetic analysis revealed that the NDCs had higher serum stability than TDCs or conventional ADCs; this was attributed to the intrinsic chemical stability of the oxime bond, as well as greater homogeneity and the absence of fast-clearing high-drug-load species.

The authors note that the cell viability of their platform cell line is consistent with a process that can be used to support clinical-scale production, although the antibody titres achieved (about 1 g per l) still need to be improved. Ultimately, this approach could allow for much greater control over the structure of next-generation ADCs.

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ORIGINAL RESEARCH PAPER Tian, F. *et al.* A general approach to site-specific antibody drug conjugates. *Proc. Natl. Acad. Sci. USA* **111**, 1766–1771 (2014)