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Site-specific and hydrophilic ADCs through disulfide-bridged linker and branched PEG

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ABSTRACT

Kadcyla® (T-DM1), an antibody–drug conjugates (ADCs) for HER2+ breast cancer treatment, has been approved by the Food and Drug Administration (FDA) in 2013. An ADC of random lysine conjugation, it has difficulties in DAR control and unsatisfactory PK due to uneven DAR distribution. It also gives rise to aggregation during conjugation because of the hydrophobicity nature of the cytotoxin, DM1. The linker-drug in T-DM1, SMCC-DM1 is hydrophobic and requires certain percentage of organic solvent such as DMA in the conjugation solution, limiting the manufacturing process in an organic-solvent-compatible device and adding extra costs. To address these problems, a site-specific conjugation method was developed involving full reduction of antibody and full conjugation with the bridge-like conjugator-drug, based on the work of Caddick and co-workers, to obtain a site-directed antibody-drug conjugate with DAR 4. The bridge-like conjugator was assembled with SMCC-DM1 and different lengths of hydrophilic polyethylene glycol (PEG) moiety. By applying PEG moiety in the side chain of the linker-drug, the organic solvent used in the conjugation can be reduced. When the PEG length is about 26 units, organic solvent is no longer needed in the conjugation. Reducing the amount of organic solvent in conjugation could also diminish the aggregation occurrence during the conjugation. Moreover, the conjugation configuration with the designed conjugator was also discussed in the article. The binding affinity of the resulting ADCs did not show significant decrease and the cell based assay and animal study have shown the comparable results with T-DM1.

Antibody-drug conjugate (ADC) is a group of rapidly developed cancer-cell-targeting drugs which combine antibody to potent drug(s), or cytotoxin(s), through a chemical linker.[1–3] An ADC exerts its efficacy by specifically binds to a target on the cell surface, be internalized and releases the high-potent drug which kills the cell.[4–7] Therefore, the antibody used in ADCs should have a high binding affinity and specific recognition to the over-expressed antigen on the cancer cells. The high potent drug conjugated with the antibody through the chemical linker should not only be stable in blood circulation but also be rapidly released after being internalized into the cancer cells.[8] Adcetris® (brentuximab vedotin) for Hodgkin lymphoma therapy and Kadcyla® (T-DM1) for HER2 + breast cancer treatment have been approved by the Food and Drug Administration (FDA) in recent years.[9–12] Brentuximab vedotin transports the microtubulin inhibitor MMAE to the target cancer cell through brentuximab binding to CD30 protein on the target cell membrane. The cleavable peptide-based linker bond can make it stable in plasma, preventing it from killing the healthy cell and make it released rapidly in the intracellular environment of the cancer cell.[13] T-DM1 is now used as a second-line drug for HER2-positive, advanced breast cancer in clinical practice.[14] Through the binding of antibody Trastuzumab to target protein HER2, T-DM1 transports the tubulin inhibitor DM1 to the target cancer cells and kills them.[15,16]

Two conventional methods are frequently used to covalently conjugate cytotoxic agents to antibodies using the amino side

Abbreviations: ADC, antibody–drug conjugates; MMAE, monomethyl auristatin E; DAR, dhrug-to-antibody ratio; DTT, ditiothreitol; TCEP, (tris(2-carboxyethyl) phosphine; T-DM1, Trastuzumab emtansine; DM1, Mertansine; SMCC, Succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; R&D, Research and development.

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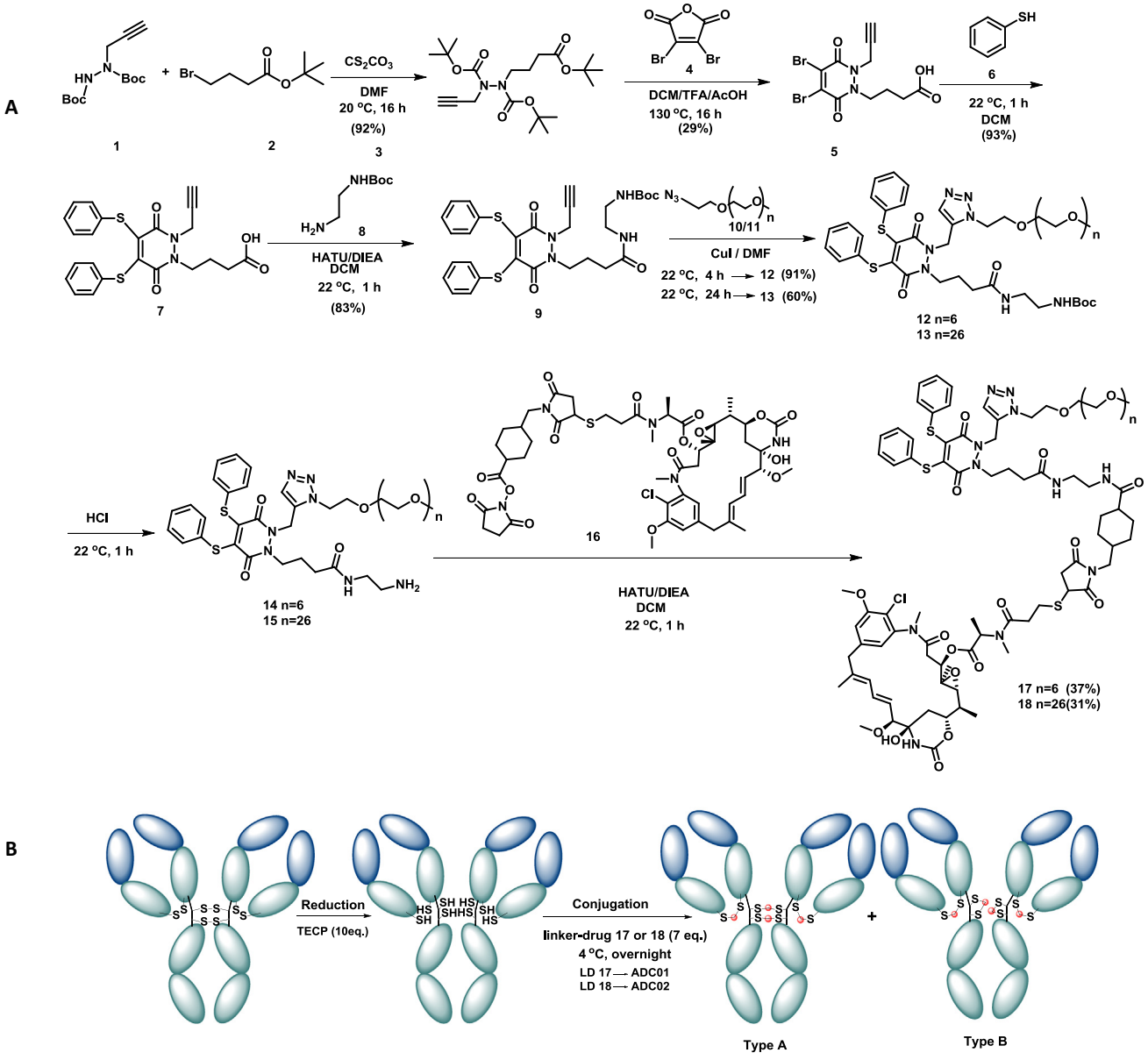
chains of lysine residues or the thiol side chains of cysteines on the antibody. As in the case of Kadcyla®, SMCC linker contains an activated ester group which can be nucleophilically attacked by the-NH_3^+-amino group of lysine residues to form amide bond with antibody.[11] This conjugation method often produces a highly heterogeneous mixture of ADCs with wide drug-to-antibody distribution ranging from 0 to 10 since there are 20–30 active surface-exposed lysine existing on the antibody.[17,18] On the other hand, Adcetris uses the thiol-malemide Michael addition reaction to achieve the linkage with antibody.[9,18] Partial reduction with TCEP or DTT to generate 0–8 free thiols from antibody interchain disulfide bonds and conjugate with excess maleimide linker-drug leading to the ADC with Drug to Antibody Ratio (DAR) 0, 2, 4, 6 and 8 mixture.[19,20] In the heterogeneous ADCs, the antibody conjugated with different numbers of drugs has different pharmacokinetic (PK) and pharmacodynamic (PD) properties.[6,21,22] ADCs with high DAR, have shown high activities in in vitro assays, but appear to have high toxicity and early clearance in liver and kidney. ADCs with low DAR, however, are not efficacious enough to achieve the active therapeutic effect.[23,24] In addition, heterogeneous ADCs bring numerous difficulties in quality control in the manufacturing and they require the intricate characterization. By contrast, homogeneous ADCs, having explicit DAR value and site-directed drug linkage, are better behaved in clinics (efficacy and PK), as well as in manufacturing processes (quality control and analysis).[25–27]

Current efforts in ADC R&D have been directed towards developing methodologies to obtain homogeneous products using site specific conjugation. Methods include drug conjugation to thiomabs,[28–30] unnatural-amino-acid incorporated mabs,[31–35] and engineered mAbs with enzyme assistance.[28–37] However, all of these technologies require additional bio-engineering work, and antibody tier produced by these methods are usually only in moderate levels.

Homogenous ADC alternatively can be achieved *via* chemical way, and the disulfide bridge modifications of antibody are frequently applied.[38] Antibody bioengineering or enzyme-assisted conjugations would not be needed and the post-conjugation purifications are easier leading to more cost-effective ADC production



Scheme 1. (A) Synthesis of LD17 and 18. (B) Conjugation reaction between Trastuzumab with LD 17 and 18.