CRISPR-Cas9 MEDIATED CELL LINE ENGINEERING OF APOPTOSIS PATHWAYS INCREASES ANTIBODY EXPRESSION WITH SITE-SPECIFIC MODIFICATIONS FOR ANTIBODY DRUG CONJUGATION

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Key Words: cell line engineering, cell line development, site -specific amino acid substitution, conjugation, amber suppressor tRNA, tRNA synthetase, CRISPR, process development, CHO

New generation of antibody drug conjugates (ADCs) have expanded the repertoire of antibody drugs in the clinic and the market for cancer and inflammation indications by using highly stable linkers to attach potent small-molecule drug to various targeting antibodies. The drug and site of drug linkage to the antibody can have profound impact on the physiochemical properties and pharmacological profile of the ADC. Ambrx has developed a technology, Eukaryotic Chemical Orthogonal Directed Engineering (EuCODE), which allows non-natural amino acids with diverse physicochemical and biological properties to be genetically encoded and site-specifically incorporated into proteins/antibodies in mammalian cells. The non-natural amino acid provides a handle for the attachment of a small-molecule drug to generate homogenous ADC with a defined Drug-to-Antibody Ratio (DAR). To establish a CHO expression system for high production of monoclonal antibodies (mAbs) containing non-natural amino acids, we successfully generated a EuCODE platform cell line stably expressing engineered amber suppressor tRNA and its cognate tRNA synthetase specific for non-natural amino acid *para*-acetyl phenylalanine (*p*AF). When transfected with antibody of interest engineered with amber nonsense codon (TAG) at selected sites suitable for drug conjugation, this EuCODE platform cell line generates stable cell lines producing *p*AF containing mAbs for site-specifically conjugated ADC. In order to improve production titers of *p*AF containing antibody and achieve a robust platform, the platform cell line and stable cell lines were further evolved using CRISPR/Cas9 genome editing technology to sequentially knock out selected genes in glutamine synthesis and apoptosis pathways to improve selection efficiency and prevent loss of viable cell mass in production cultures, respectively. Inhibition of apoptosis pathway leads to dramatic increase in viable cell mass and results in extended production time and increased productivity. Phenotypic and genetic properties of these CRISPR engineered cell lines and product quality of the antibody will be discussed in the context of using the platform to develop a commercial manufacturing cell line.