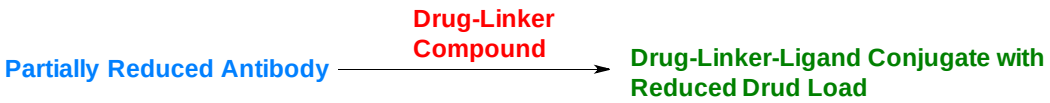
General Antibody Drug Conjugate Protocol



SchemeA

Scheme A illustrates a useful method for preparing each antibody with about 2 to 4 drug-linked ligand conjugates.

General procedure: Prepare conjugates containing 2 to 4 drugs per antibody.

Partial reduction of antibodies

Generally, to prepare conjugates with two drugs per antibody, a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP) (about 1.8) is needed in PBS containing 1 mM DTPA (Equivalent)) Reduce relevant antibodies and adjust pH to 8 with 50 mM borate. The solution was incubated at 37 ° C for 1 hour, and subjected to equilibrium purification in a 50 mL G25 desalting column in PBS / 1 mM DTPA at 4 ° C. The thiol concentration can be determined according to the general method M, the protein concentration can be determined by dividing the A280 value by 1.58 extinction coefficient (mg / ml), and the ratio of the thiol to the antibody can be determined according to the general method N.Conjugates having 4 drugs per antibody can be made using the same methodology, using about 4.2 equivalents of a suitable reducing agent to partially reduce the antibody.

Conjugation of Drug-Linker to Partially Reduced Antibody

Partially reduced antibody samples can be coupled with corresponding drug-linker compounds using about 2.4 and about 4.6 molar equivalent drug-linker compounds per antibody to prepare 2 and 4 drugs per antibody respectively. The coupling reaction was reacted for 1 hour under ice bath conditions, the drug was quenched with about 20 times cysteine, and purified by elution on a G25 desalting column at about 4 ° C. The obtained drug conjugate ligand conjugate was concentrated to about 3 mg / ml, sterile-filtered, aliquoted and stored frozen.

Scheme B

Scheme B depicts the construction of a Drug-Linker-Ligand Conjugate by reacting the Sulfhydryl group of a Ligand with a thiol-acceptor group on the Linker group of a Drug-Linker Compound.

An illustrative method for attaching a ligand antibody to a drug conjugate is outlined in steps L-M.

General Procedure L: Attachment of an Antibody Ligand to a Drug-Linker Compound

All reaction steps are usually carried out at 4 ° C. In the case where the ligand is a monoclonal antibody having one or more disulfide bonds, the monoclonal antibody in phosphate buffered saline at pH 7.2 is reduced with dithiothreitol (final concentration of 10 mM) at 37 ° C. (5-20 mg / mL) solution. The reaction was carried out for 30 minutes (see General Procedure M) and the low molecular weight reagent was separated by size exclusion chromatography on a Sephadex G25 column in PBS containing 1 mM diethylenetriaminepentaacetic acid.

The sulfhydryl content in the Ligand can be determined using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as described in General Procedure M (see Riddles, P. W., Blakeley, R. L., and Zerner, B. (1979) Anal. Biochem. 94, 75-81).

To the PBS solution of the reduced ligand according to the general method L, the drug-linker compound in MeCN was added so that the solution was 20% MeCN / PBS (v / v). The amount of drug linker compound is about 10 % more than the total number of thiol groups on the ligand. After 60 minutes at 4 ° C, cysteine (20 times higher than the concentration of the drug linker compound) was added, the solution was concentrated by ultrafiltration, and any low molecular weight drugs were removed by gel filtration.

The amount of the drug linker compound per antibody was determined by UV / visible spectroscopy molecular formula. The amount of quenched drug linker compound was then determined using reverse phase HPLC. The size-exclusion high performance liquid chromatography can be used to determine the aggregation state of the ligand antibody of the drug-ligand-ligand conjugate. Drug-Linker-Ligand conjugate was used without further purification.

General Procedure M: Reduction of the interchain disulfide bonds of an Antibody

To a solution of 24 mg of an antibody (2.4 mL of 10 mg/mL solution) in suitable buffer is added 300 uL of Borate buffer (500 mM sodium borate/500mM sodium chloride, pH 8.0) followed by 300 uL of Dithiothreitol (DTT, 100 mM solution in HO). The reaction mixture is stirred using a vortex instrument and incubated at 37°C. for 30 min. Three PD10 columns are equilibrated with PBS containing 1 mM DTPA (in PBS) and the reduced antibody is eluted through the three PD10 columns and collected in 4.2mL PBS/DTPA solution (1.4 mL per column). The reduced antibody is then stored on ice. The number of thiols per antibody and the antibody concentration are determined according to General Procedure N.

Reference

[1] Senter, Peter D., Svetlana O. Doronina, and Brian E. Toki. Drug Conjugates and Their

Use for Treating Cancer, an Autoimmune Disease or an Infectious Disease. Seattle Genetics Inc, assignee. Patent US7659241B2.31 July ***2002***. Print.