Antitumor activity of therapeutic antibodies can be significantly enhanced by conjugation to cytotoxic small molecules. By using such bicyclic amatoxins (ATAC) the toxic activity is limited by a cleavable linker resulting in an anti-CD37 antibody targeted Amanitin conjugate (ATAC) in B cell malignancies.

**Methods**

Cell lines and antibody. **B** cells, **Raji** cells (NHL, MEC-2, MEC-20 and Raji) and **Ramos** cells (DLBCL) were obtained from the American Type Culture Collection (ATCC). MEC-2 and MEC-20 lines were obtained from the INRA avian tumor repository of the Institut National de la Recherche Agronomique (INRA), France. Mouse monoclonal anti-CD37 antibody (Clone 37, IgG2a) was produced in-house and purified.

Synthesis of conjugates. Cytotoxic-recombinant antibody constructs were synthesized at Heidelberg Pharma using a linker-specific, recombinant trypsin protease encoded exclusively on the intracellular domain. The strategy was then used to synthesize trypsin-proteolytically cleavable antibodies.

Flow cytometry. Flow cytometry was performed using a FACs Calibur and analyzed using Flowjo software. Antibodies used were: anti-CD37 (clone 37, IgG2a), anti-CD8 (clone 14, IgG1) and anti-CD4 (clone 4, IgG1). After 7 days treatment with ATAC in Raji cells, cells were harvested and analyzed using a FACs Calibur.

**Results**

1. **Conjugation of CD37 Antibody Targeted Amanitin Conjugate (ATAC)**

Nick polymer is linked to the human IgG antibody using the cleavable or non-cleavable linker (orange) conjugated to substituted cysteine residues of anti-CD37 antibody via a thiol-reactive dibenzoylmethane chemistry, resulting in homogeneous ATAC with a DF of 2 toxins per IgG (Figure 1).

2. **Anti-CD37 THIO-MAB® binds selectively to CD37**

The binding property of anti-CD37 THIO-MAB® was analyzed by flow cytometry. Flow cytometry was performed on Raji cells with ATAC or non-cleavable linker. The antibody binds to Raji cells and does not bind to Raji cells with a non-cleavable linker.

3. **Cytotoxic activity of anti-CD37 ATAC on B and CD8**

The CD37**-**cell line, **MEC-2** (CD8**+**CD37**+**Raji and Raji were used to test the cytotoxic properties of ATAC with cleavable linker (Figure 3a) and non-cleavable linker (Figure 3b). Both ATAC showed flow-cytometrically proven viability of the low cytotoxic transfectant target cells.

**Discussion**

Amanitin 1.0 mg/kg po was given in the treatment of B cell malignancies (Raji and Ramos); MEC-20 and Raji cells were obtained from the American Type Culture Collection (ATCC). MEC-2 and MEC-20 lines were obtained from the INRA avian tumor repository of the Institut National de la Recherche Agronomique (INRA), France.

**Conclusion**

This study demonstrates that the transgenic engineered ruminant anti-CD37 antibody (ATAC) with or without a cleavable linker is effective against B cell malignancies. This ATAC with cleavable linker results in an anti-CD37 antibody targeted Amanitin conjugate (ATAC) in B cell malignancies.