Combination of Antibody-Targeted Amanitin Conjugates (ATAC) with Immune checkpoint inhibitors shows a synergistic therapeutic effect

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INTRODUCTION
While cancer therapy has relied mainly on the use of cytostatic cytotoxic antibodies or cytokines, the focus on cancer therapy has shifted recently towards target-specific drugs as well as the manipulation of immune responses. Consequently, the development of the immune check point inhibitors (ICIs) has proven promising in many cancer entities, even tumors which are characterized by immunosuppressive stromal environments. Tumor-infiltrating lymphocytes, such as PD-L1 and CTLA-4. In this study we demonstrate that the combination of ICI with other immune therapies is an attractive way to overcome tumor resistance under several conditions.

Among the class of target-specific therapies, antibody-drug conjugates (ADCs) promise to deliver the target specific payload only to the tumor in a patient-specific and tumor-specific way. We can take advantage of their unique properties by designing them as ICI, which is a well-known target of carcinomas and a promising approach for potential further cancer therapy.

METHODS
Cell lines: IM-1, K74A, and Raji cells were obtained from DSMZ, NOA-1 from ATCC, NMRI and NMRI (C57BL/6) from Charles River. Amanitin-resistant variants of JIMT1 and JIMT2 were engineered using CRISPR/Cas9 technology.

Amanitin: Amanitin-resistant variants of JIMT1 and JIMT2 were engineered using CRISPR/Cas9 technology. Amanitin-resistant molecules were used in all flow cytometric experiments, staining T-, B-, and NK cells as well as NK cell subsets of the observed phenotype, while all NK strains retained NK (Figure 4-A).

1. ATAC treatment in vivo leads to immunity tumor growth.
2. Immune deficient mice were used in all in vivo experiments, eliminating T-, B-, and NK cells as well as NK cell subsets of the observed phenotype, while all NK strains retained NK (Figure 4-A).
3. NF-kB signaling was quantified using real-time PCR analysis (Figure 4). As shown, Amanitin-resistant molecules were used in all flow cytometric experiments, staining T-, B-, and NK cells as well as NK cell subsets of the observed phenotype, while all NK strains retained NK (Figure 4-A).

RESULTS

1. Anti-HER2 ATAC treatment in vivo results in complete tumor remission in Her2/neu transgenic HNMRI FVB mice

A Her2/neu specific (H) monoclonal antibody (MAB) directed against the HER2/neu receptor was conjugated to the cell-killing molecules via a linker. The resulting construct (Figure 1A) resulted in significant tumor reduction and complete tumor regression in Her2/neu transgenic mice (Figure 1B & 1C).

2. Anti-HER2 ATAC treatment in vivo results in complete tumor remission in Her2/neu transgenic HNMRI FVB mice

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3. Treatment of ATACs in vivo leads to immunity tumor growth.

Amanitin-resistant variants of JIMT1 and JIMT2 were engineered using CRISPR/Cas9 technology. Amanitin-resistant molecules were used in all flow cytometric experiments, staining T-, B-, and NK cells as well as NK cell subsets of the observed phenotype, while all NK strains retained NK (Figure 4-A).

4. ATAC treatment induces immunogenic cell death

Amanitin-resistant variants of JIMT1 and JIMT2 were engineered using CRISPR/Cas9 technology. Amanitin-resistant molecules were used in all flow cytometric experiments, staining T-, B-, and NK cells as well as NK cell subsets of the observed phenotype, while all NK strains retained NK (Figure 4-A).

CONCLUSION
ATAC treatment results in a complete and persistent anti-tumor effect in vivo and the combination of ATAC and ICI can overcome tumor resistance to a certain extent. In vitro studies confirmed that ATAC can overcome tumor resistance to a certain extent. The feasibility of the combination of ATAC and ICI resulted in a significant reduction in the tumor burden of the treated mice and a significant increase in the median survival of the treated mice. The combination of ATAC and ICI can be a promising approach for potential drug development.