Amatoxin-based antibody-drug conjugates induce immunogenic cell death and improve the anti-tumor efficacy of immune checkpoint inhibitors in humanized mouse models

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INTRODUCTION

Antitumor activity of therapeutic antibodies can be significantly enhanced by conjugation to toxic small molecules. By using amatoxin-based antibody-drug conjugates (ADCs) the toxicity is restricted to cancer cells and thereby kill only those cells. Based on the successful approaches including ENHERTU (Trastuzumab-DTg), Breonamet (Spalverb), and EPOC (Amatera x CD133) promoting to strengthen the therapeutic capabilities for cancer treatment. Most ADCs are based on the successful mechanisms with topoisomerase-, microtubule- or DNA-targeting moieties, as payload.

We here present new data on functional antagonists to inhibit homogenous cell death signals in order to induce a higher tumor cell kill as well as immune activation signals. The novel approach for a complementary tumor anti-ADC compound was evaluated in vitro and in vivo.

METHODS

Cell lines: J-RF 1 and J-RF 2 cells were obtained from DSMZ and NCI-60 Panel.

Synthesis of conjugates: Tumor-reactive antibody-linker construct was developed at Heidelberg Heidelberg Pharma Research GmbH and was conjugated to anti-HER2 (trastuzumab) variable domains. The synthetic antibody fragment was cyclized with the IRD700 and rhodamine and was modified with the AMAT. The AMAT conjugates according to the protocol was approved as an IRD700 N87 (3+). Treatment of the HER2-positive J-RF 1 and J-RF 2 cell lines was determined by FACS analysis with Anti-AMAT (A) and anti-HER2 (B) antibodies. Anti-HER2 expression of HER2 positive J-RF 1 and J-RF 2 cell lines was analyzed by flow cytometry (n=3). The expression of HER2 and HMGB1 was treated with HER2 antibody (C) and HER2 + AMAT (D).

RESULTS

1. Anti-HER2 treatment of homogenous cell line-derived xenografts leads to complete tumor remission and immunity towards tumor re-challenge.

A: Anti-HER2 treatment of homogenous cell line-derived xenografts led to complete tumor remission and immunity towards tumor re-challenge.

B: Anti-HER2 tumor kill and HMGB1 induction in xenograft mouse.

C: Anti-HER2 tumor kills and HMGB1 induction.

D: Anti-HER2 tumor kill and HMGB1 induction.

E: Anti-HER2 tumor kill and HMGB1 induction.

F: Anti-HER2 tumor kill and HMGB1 induction.

G: Anti-HER2 tumor kill and HMGB1 induction.

H: Anti-HER2 tumor kill and HMGB1 induction.

I: Anti-HER2 tumor kill and HMGB1 induction.

J: Anti-HER2 tumor kill and HMGB1 induction.

K: Anti-HER2 tumor kill and HMGB1 induction.

L: Anti-HER2 tumor kill and HMGB1 induction.

M: Anti-HER2 tumor kill and HMGB1 induction.

N: Anti-HER2 tumor kill and HMGB1 induction.

O: Anti-HER2 tumor kill and HMGB1 induction.

P: Anti-HER2 tumor kill and HMGB1 induction.

Q: Anti-HER2 tumor kill and HMGB1 induction.

R: Anti-HER2 tumor kill and HMGB1 induction.

S: Anti-HER2 tumor kill and HMGB1 induction.

T: Anti-HER2 tumor kill and HMGB1 induction.

U: Anti-HER2 tumor kill and HMGB1 induction.

V: Anti-HER2 tumor kill and HMGB1 induction.

W: Anti-HER2 tumor kill and HMGB1 induction.

X: Anti-HER2 tumor kill and HMGB1 induction.

Y: Anti-HER2 tumor kill and HMGB1 induction.

Z: Anti-HER2 tumor kill and HMGB1 induction.

OUTRODUCTION

The treatment with ATAC®-based ADCs led to complete and stable tumor remission in different cell line-derived xenograft models using different target antigens representing a broad spectrum of immunogenicity in mice that achieved complete and stable tumor remission against ATAC® treatment suggest an activation of the immune system.

The complete remission of heterogenous patient-derived xenografts models were treated with ATAC®-based ADCs further underlines the involvement of the immune response because the homogenous xenografts already showed that ATAC®-based ADCs do not have any bystander effect upon extracellular drug-release.

This observation was accompanied by the finding of enhanced IC mediator expression in vitro and in vivo. Especially, the localization of high levels of HMGB1 and HMGB2 in ATAC®-based ADCs highlights the correlation between the treatment with ATAC®-based ADCs and the induction of HMGB1 expression in the scientific rationale for combination treatments in clinical trials.

REFERENCES


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