Characterization of the mechanism of action, pharmacodynamics and preclinical safety of ADCT-402, a pyrrolobenzodiazepine (PBD) dimer-containing antibody-drug conjugate (ADC) targeting CD19-expressing hematological malignancies

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ADCT-402 is an ADC composed of a humanized antibody directed against human CD19 (stochastically conjugated to the highly cytotoxic PBD-based linker-drug conjugate of the pyrrolobenzodiazepine (PBD) dimer payload). ADCT-402 was administered intravenously to treatment groups of seven mice. The in vitro and ex vivo antitumor activity of ADCT-402 was determined in preclinical models derived from cell lines, xenografts and patient samples. Analysis of these data guided the clinical development of this promising ADC in B-cell malignancies.

Introduction

Human CD19 antigen is a 95 kilodalton type I membrane glycoprotein belonging to the B-cell antigen receptor (BCR) complex. The expression of CD19 is limited to the various stages of B-cell development and B-cell malignancies (MCL, DLBCL). In contrast, the expression of CD20 is more widely expressed in the majority of the B-cell malignancies, including B-ALL leukemia and non-Hodgkin lymphoma (NHL) of B-cell origin. CD20 has high internalization rates and is not destroyed in the circulation (1, 2).

Figure 1: Structure of CD19.

ADCT-402 has potent and targeted cytotoxicity against a panel of human lymphoma and leukemia cell lines (3).

In vivo, ADCT-402 demonstrates dose-dependent antitumor activity against leukemia and lymphoma xenograft models. Moreover, ADCT-402 is highly selective to malignant and non-malignant CD19-expressing B cells in vivo.

AIM of this study

To further define the mechanism of action of ADCT-402 and validate its pharmacology and preclinical safety in vivo.

Materials and Methods

The single cell gel electrophoresis (Comet) assay was performed to quantify the amount of DNA interstrand cross-links (ICLs). This assay is widely used to measure DNA-DNA and DNA-protein cross-links formed in cells following DNA damage.

In vivo analysis of ADCT-402 was performed in a panel of B-cell-derived xenografts and primary cell lines. CD19 expression was determined by immunohistochemistry (IHC) analysis on formalin-fixed paraffin embedded (FFPE) tumors, and PBD conjugation was determined by liquid chromatography-mass spectrometry (LC-MS).

Figure 4: Time course of DNA ICL.

Results

Table 1: CD19 expression in matched NHL clinical samples.

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<tr>
<th>Sample Type</th>
<th>CD19 Expression</th>
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<tr>
<td>Initial Diagnosis</td>
<td>&gt; 80% tumour cells are CD19-positive</td>
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<tr>
<td>Relapsed/Refractory</td>
<td>&gt; 80% tumour cells are CD19-negative</td>
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Figure 5: Immunohistochemistry analysis on FFPE tumors using human CD19 antibody and phospho-histone H2A.X (Ser 139) antibody. The single cell gel electrophoresis (Comet) assay was carried out to quantify the amount of DNA interstrand cross-links (ICLs) formed in cells following DNA damage.

Figure 6: PK analysis in cynomolgus monkey.

Conclusions

1. CD19 high and homogenous expression is maintained in a panel of mature NHL samples taken at initial diagnosis and relapse/refractory confirming CD19 expression is a robust target for ADC development.

2. ADCT-402 was specifically bound, internalized and trafficked to the lysosomes in CD19-positive Ramos cells in co-localization with lysosomal markers observed within 1 hour, while 24 hour non-lysosomal staining was observed, suggesting complete lysosomal degradation of ADCT-402.

3. In vivo, single doses of ADCT-402 resulted in specific, potent and dose-dependent antitumor activity in the Ramos xenograft model. DNA ICLs were readily measured in tumors by 24 hours from administration of ADCT-402, while marked PBD immuno-staining showed evidence of DNA DSBs. At the same time points, detection of phosphorylated histone H2AX immunostaining indicated a DNA-DNA damage response was initiated.

4. ADCT-402 was stable and leukocyte well tolerated in a repeat dose cynomolgus monkey model and PK properties were consistent with normal antibody clearance with a half-life of about 14 days.

5. Together, these data further define the in vitro and in vivo mechanism of action and pre-clinical safety of ADCT-402 and provide relevant pharmacodynamic assays to guide the clinical development of this promising ADC in B-cell malignancies.

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References


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