CD19 – a potential target for Amanitin-based ADCs

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Antitumor activity of monoclonal antibodies can be dramatically enhanced by conjugation to cytotoxic small molecules. By using such antibody-drug conjugates (ADCs) the toxic is delivered to target cells exclusively and thereby kills only those cells. Beside the approval of Kadcyla (T-DM1, 2011) and Adcetris (SGN-35, 2013) nearly 60 ADCs have entered clinical trials, promising to strengthen the therapeutic capabilities for cancer treatment in the next decade. Most ADCs are based on a few cytotoxic compounds only and on an even smaller number of toxicity mechanisms. Most payloads are micellar- or drug targeting toxins (auristatins and maytansines or duocarmycins and PBDs). Accordingly, the use of new drugs that function via alternative toxicity mechanisms could enhance the therapeutic potential of ADCs. Heidelberg Pharma focuses on Amanitin based ADCs or ATACs (antibody targeted Amanitin conjugates) comprising a new class of ADCs with Amanitin as toxic payload. Amanitin is the well-known toxin of the mushroom family and binds specifically to the eukaryotic RNA polymerase II thereby inhibiting the cellular transcription process at very low concentrations. Heidelberg Pharma also pursues the strategy of site-specific conjugation to limit heterogeneity in drug-antibody species, to improve conjugate stability, and to increase the therapeutic window of ADCs. For this purpose, antibodies are engineered at specific locations to incorporate cysteines resulting in so-called Thiomabs.

In the current study, in vitro and in vivo data of Thiomab-based ATACs targeting CD19 (CVID/DL, B-lymphocyte surface antigen B4) are presented. CD19, a classical transmembrane glycoprotein with no significant homology to any known protein, is expressed in B cells and B-cell malignancies like B-cell acute lymphocytic leukemia (B-ALL) and B cell chronic lymphocytic leukemia (B-CLL) but it is not found on hematopoietic stem cells, and other healthy tissues. Additionally, CD19 has a broad expression profile and high internalization efficacy and is thus an ideal target for Amanitin based ADCs.

RESULTS

Antitumor activity of anti-CD19 ATACs were determined in Raji intravenous (i.v.) and subcutaneous (s.c.) xenograft models in vivo.

Mouse efficacy and tolerability experiments

Antitumor activity of anti-CD19 ATACs were determined in Raji intravenous (i.v.) and subcutaneous (s.c.) xenograft models in vivo.

CONCLUSIONS

Leukemia in general ranks as the 6th most common type of cancer and Non-Hodgkin lymphoma as the 8th most common type of cancer in the United States. CD19 (CVID/DL, B-lymphocyte surface antigen B4) is expressed on cells of the B-cell lineage, ranging from the pre-B cells until the terminal differentiation to plasma cells. It is expressed in most acute lymphoblastic leukemias (ALL), chronic lymphocytic leukemias (CLL) and B cell lymphomas. Thus, the CD19 antigen is an ideal target for immunotherapy of B-cell malignancies. In the current study, in vitro and in vivo data of Amanitin-ATACs conjugating CD19 are presented. Amanitin is a toxin withad for ADCs targeting CD19 seems to be a suitable therapeutic option because of the unique mode of action and the molecular characteristics of the toxin. Amanitin is highly active in drug resistant cells, independent of the status of expression of multidrug resistance transporters. By inhibition of RNA pol II, Amanitin binding leads not only to apoptosis of dividing but also of slowly growing and dormant cells. This is a potential advantage for amantitn payloads on certain leukemias, e.g. in slow proliferating CLL cells. HDF has constructed more than 20 different linkers allowing the conjugation of Amanitin to lysine- and cysteine-residues (both, genetically engineered and interchain cysteines) as well as non-natural amino acids of antibodies. In the presented work we characterized Amanitin’s directed against CD19 with synthetic and “natural” Amanitin payloads linked via a cleavable linker to non-natural amino acids of antibodies. In the presented work we characterized Amanitin’s.

Stability of cd19-amanitin ADCs

To test the stability of anti-CD19-ADCs, compounds were incubated in murine plasma for up to 10 days. After the respective incubation period, the compounds were either analyzed using an ELISA (T-DM1) and Anti-CD19 Western blotting of ADCs in B cells (Figure 2a, b) or murine plasma (Figure 2c, d) using specific primary antibodies.

Cytotoxic potency

The CD19 cell lines Raji and Nalm-6 were used to test the cytotoxic potency of anti-CD19 ATACs. Following ATACs were assessed:

Non-human primate tolerability study

Anti-CD19 Thiamob-30.2115 was assessed for a dose-escalating tolerability study in cynomolgus monkeys. The ATAC was assessed separately at doses of 2 mg/kg, 10 mg/kg, 50 mg/kg and 200 mg/kg to the same animals.

REFERENCES