

Potent antigen-specific anti-tumor activity observed with antibody-drug conjugates (ADCs) made using a new class of DNA-crosslinking agents

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Abstract # B126

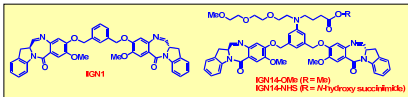
Introduction

Antibody-Drug Conjugates (ADCs) are emerging as potential new modalities for the treatment of cancer. Most of the ADCs currently in clinical testing contain a tubulin-interacting agent (a maytansin or dolastatin derivative). In order to expand the therapeutic potential of ADCs, we have developed a new class of cytotoxic agent with a novel DNA-interacting mechanism of action. Herein, we report the development of our IGN class of effector molecules comprising indolino-benzodiazepine dimers. These IGNs are highly potent by virtue of their ability to alkylate and crosslink DNA. The high potency of these compounds, their novel mechanism of action, and their desired aqueous solubility and stability make them well suited for use as effector molecules in ADCs. We describe the biochemical properties of Antibody-IGN conjugates, their *in vitro* potency and specificity, and *in vivo* anti-tumor activity and pharmacokinetics.

Objectives

- To synthesize IGNs, a novel class of DNA-crosslinking agents, for use in ADCs
- Biochemical characterization of ADCs prepared with an IGN molecule
- Evaluation of *in vitro* potency and specificity
- Evaluation of *in vivo* pharmacokinetics and anti-tumor activity

Structures

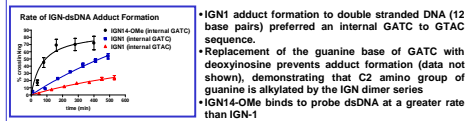


- An IGN dimer consists of two indolino-benzodiazepine units that are linked together with an aromatic spacer having an overall length that enables sequence-selective crosslinking with DNA
- IGN1 was found to be highly potent ($IC_{50} \sim 1 - 40$ pM) against a variety of cancer cell lines
- IGN14-NHS possesses a N-hydroxysuccinimide ester to enable linkage to an antibody

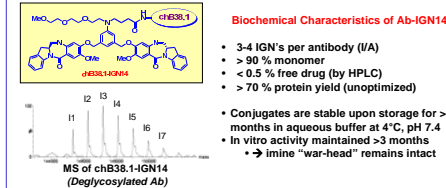
Methods

- dsDNA adduct formation of IGN dimers. dsDNA in 100 mM TRIS-HCl and 1 mM EDTA at pH 8 was mixed with 2 eq of effector dissolved in acetonitrile and incubated at 15 °C. Percent probe DNA crosslinked to effector was monitored by hplc and plotted against time. IGN14-OMe binds to probe dsDNA better than IGN1.
- Conjugation of IGN14-NHS with an antibody. A solution of antibody in an aqueous buffer containing 0.05 M HEPES and 2 mM EDTA, pH 8 was treated with a 10-fold molar excess of a solution of IGN14-NHS in DMA (10% final v/v). After 2 h at RT, the mixture was purified over a Sephadex G25 gel filtration column and the conjugate was further dialyzed to remove unreacted free IGN14.
- In vitro* cytotoxicity assay. Cytotoxic potencies were assessed using a WST-based cell viability assay. Cells were seeded at various densities in 96-well flat bottom plates and surviving cells were measured after 5 days by developing with WST (2-4 hours).
- In vivo* - Plasma PK. CD-1 mice were injected with chB38.1-IGN14 (2 mg/kg Ab dose, 40 µg/kg IGN14 dose) and bled at various time points. [Antibody] and [total conjugate] from mouse plasma at each time point was determined by a sandwich ELISA method using goat-anti-human-IgG-Fc(F2)2 Fragment specific, then detection with either HRP-goat-anti-human-IgG-Fc (F(ab)2) or biotinylated-murine-anti-IGN, and detection with streptavidin-AP (total conjugate).
- In Vivo* Activity - COLO-205 Model. Nude Mice (female, 6 per group) were inoculated subcutaneously in the area over the right shoulder with COLO205 tumor cells (2×10^6 cells/mouse). When tumors were ~ 100 mm³ the animals were treated with the following given i.v.: a) PBS vehicle control, b) chB38.1 (naked antibody control), c) chKTI-IGN14 (non-targeting conjugate control), d) chB38.1-IGN14 at 3 doses.

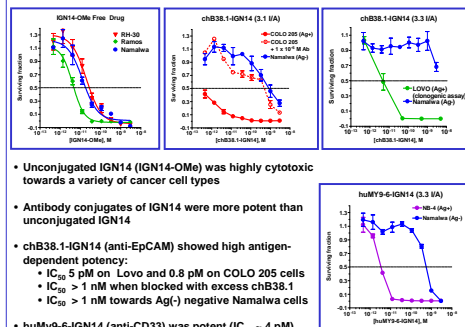
DNA Binding



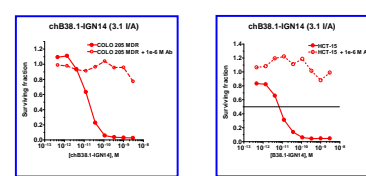
Conjugate Biochemical Characteristics



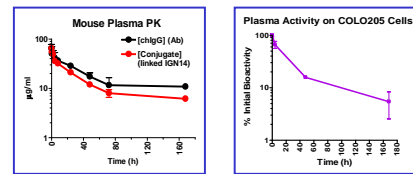
In Vitro Potency



In Vitro Potency Against MDR Cells



In Vivo PK and Tolerability



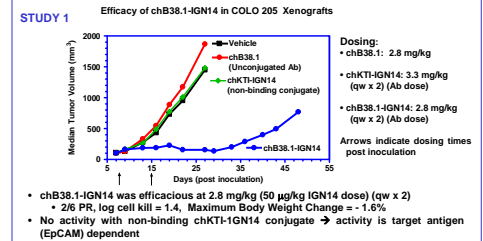
Tolerability of chB38.1-IGN14 in mice

- chB38.1-IGN14 was well tolerated at a single iv dose of 3.75 mg/kg (75 µg/kg IGN14)

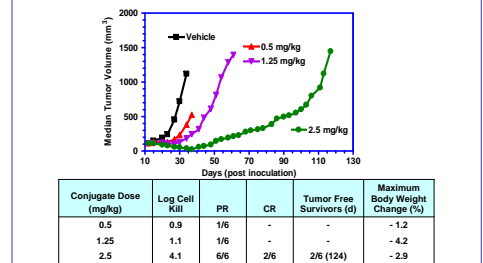
PK Study Results

- Conjugate and Ab have similar plasma clearance
→ demonstrates high stability for Ab-IGN14 link (an amide bond)
- Plasma bioactivity decreases at similar rate to conjugate
→ IGN14 is stable in circulation

In Vivo Efficacy Studies



STUDY 2 Efficacy of huMab-IGN14 (single dose) in KB Tumor Xenografts



Conclusions

- IGN dimers show high *in vitro* potency due to their ability to irreversibly bind and disrupt double-stranded DNA
- Antibody-IGN conjugates are soluble and stable in aqueous solutions
- Antibody-IGN conjugates are highly potent and target specific, even towards multidrug resistant cells and cells expressing low number of target antigens
- PK studies show that Antibody-IGN conjugate is stable in circulation
- Anti-tumor activity observed in two different human tumor xenograft models at non-toxic doses
- IGNs are promising effector molecules with a novel mechanism of action, and are well suited for further development in ADCs