MEDAREX

# Mechanism of Action of a Human Anti-CD70 Antibody-MGBA Conjugate and Efficacy in a Nude Rat Model of Renal Carcinoma

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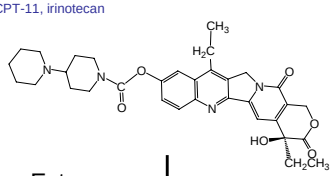
## Introduction

A series of antibody conjugates have been designed using human antibodies from transgenic mice linked to synthetic DNA minor-groove binding alkylating (MGBA) agents. A key feature of these conjugates is that the attached cytotoxic is in prodrug form and requires not only release from the antibody for activity but also cleavage of a 4' carbamate group to release the active moiety. The attachment of such a prodrug may give additional safety advantages over conventional antibody conjugates of cytotoxic drugs. We have established that activation of the prodrug is achieved by several esterases both within tumor cells and in several normal tissues, including plasma. The level of relevant esterase activity in man has been shown to be very similar to that observed in rats and non-human primates, although less than that observed in mice. Therefore we have examined efficacy of anti-CD70 MGBA conjugates in a nude rat model of renal carcinoma. In this model , Caki-1 cells are grown as a subcutaneous tumor xenograft with treatment by ip administration. Efficacy of the antibody conjugate is clearly observed with single low dose therapy, whereas multi-dose therapy leads to complete tumor regression. Safety studies at larger doses in the rat and also in non-human primates have been carried out and demonstrated a substantial potential therapeutic window for the anti-CD70 conjugate. These studies support the role of CD70 as a viable ADC target for renal cell carcinoma.

## Carboxylesterase is also involved in activation of CPT-11 (Irinotecan):

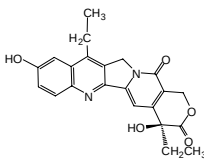
Carboxylesterases are present both in plasma and tissues. Activation can therefore take place either in the circulation or in tissues. For CPT-11 (irinotecan) activation appears to take place in both compartments (Sanghani *et al.* (2003) Clin. Cancer Res. 9, 4983-4991) Human carboxylesterase II is a major enzyme involved in activation of CPT-11.

CPT-11, irinotecan

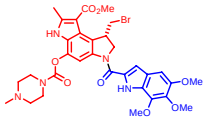


Esterase activity

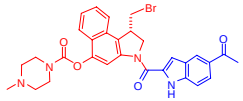
SN-38



## Duocarmycin (KW-2189)

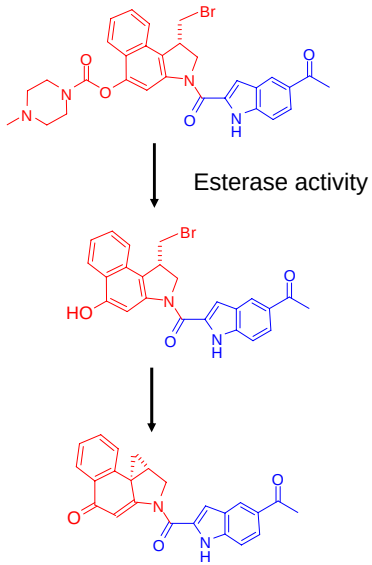


## Synthetic MGBA (MED-2219)

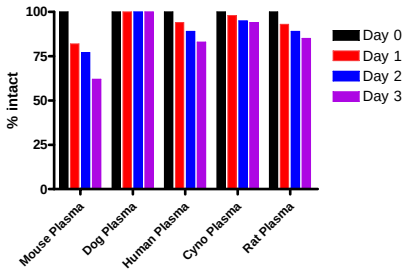


MED-2219 is a synthetic minor-groove binding alkylating agent (MGBA) with a 4' carbamate protecting group. This is the same group used on the compound KW-2189 which is a duocarmycin that has been studied in several phase II clinical trials as a free chemotherapy agent. Cleavage of the 4' carbamate group is required for activation of these compounds as shown below. This is achieved by esterase action, in a similar manner to the activation of the drug irinotecan (CPT-11) which is cleaved to SN-38 *in vivo*.

## Activation of MGBA



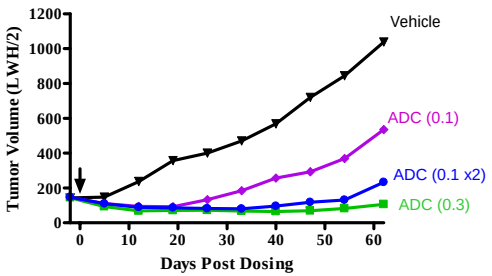

## Plasma Esterase Activation of MGBA




Activation of the MGBA can take place in tumor, tissues or in the circulation. To assess the relative activation rates in plasma, drug was incubated for up to three days in plasma from different species and the % conversion monitored. Mass spec data was used to verify the nature of the conversion. Data was in agreement with the literature in which it has been shown that esterase activity in mouse is very high, whereas activity is lower in the other species tested. Activity in rat plasma was very similar to that observed in human suggesting that the rat is an excellent species in which to assess the role of plasma esterase in activtion of the ADC. Comparative xenograft studies were therefore carried out with the human ccRCC cell line Caki-1 in SCID mice and nude rats

## CD70 ADC Therapy of Established Caki-1 Xenografts in SCID mice

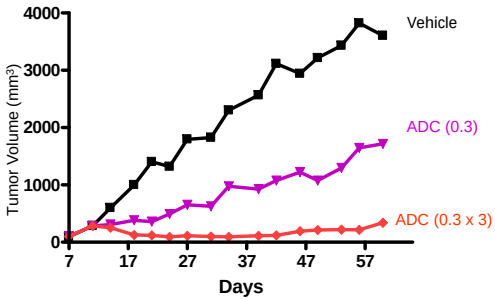
### Single Dose & Multi-Dose Therapy of Established tumors




Efficacy of anti-CD70 ADC on established Caki-1 ccRCC xenografts grown in SCID mice. SCID mice have high plasma esterase activity and therefore activate the ADC rapidly. ADC was dosed either once at 0.1 or 0.3 µmol/kg, or twice at a dose of 0.1 µmol/kg at day 0 and day 14. All dosing IP. Data is presented as the median of groups of 8 mice.

## CD70 ADC Therapy of Established Caki-1 Xenografts in Nude Rat

### Single Dose & Multi-Dose Therapy of Established Tumors




Efficacy of anti-CD70 ADC on established Caki-1 ccRCC xenografts grown in nude rats. Nude rats have low plasma esterase activity and therefore activate the ADC only slowly in the circulation. ADC was dosed either once at 0.3µmol/kg, or three times , weekly, at the same dose. All dosing IP. Data is presented as the median of groups of 8 rats.

## Conclusions

Antibody drug conjugates with DNA minor-groove binding alkylating (MGBA) agents are a novel class of ADCs with attractive properties for development. We have observed that ADCs produced with MGBAs protected with a 4' carbamate group are potent and efficacious in a range of xenograft models as well as being extremely well tolerated (see Poster Number 4061). The wide therapeutic window observed is due to the stability of the antibody conjugate and the nature of the drug itself. In this case the drug attached to the antibody is an esterase activated prodrug, which can be activated by a range of human carboxylesterases. The approved anti-cancer drug CPT-11 is also activated by carboxylesterases. In this study we have demonstrated that activation in rat plasma is similar to the rate of activation in human plasma, and thus the rat is a useful model for the rate of activation of the ADC. Efficacy studies in the nude rat have demonstrated good anti-tumor efficacy at well tolerated doses. These studies support the role of CD70 as a viable ADC target for renal carcinoma and other CD70 expressing malignancies.