Development of Drug-Conjugated Monoclonal Antibodies Against MUC16 for Treatment of Epithelial Ovarian Cancers #210

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INTRODUCTION

MUC16 is a well-validated cell surface antigen expressed by ovarian cancer cells; the extracellular domain (ECD) is known as CA125. Drug-conjugated antibodies ("antibody-drug conjugates" or "ADC") directed against MUC16 might therefore have therapeutic value against this disease (1).

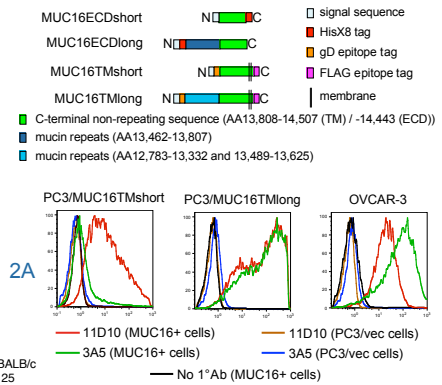
OBJECTIVES

1) Develop and characterize MAb against the ECD of MUC16.
2) Evaluate drug-conjugated MAb for in vitro and in vivo efficacy against MUC16-expressing cell lines. Select lead ADC based on efficacy.
3) Assess toxicity of ADC in rodent and primate models. Determine impact of binding to circulating CA125 on toxicity.

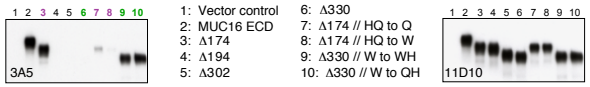
11D10 Binds to a Unique Epitope of MUC16 and 3A5 Binds to a Repeating Epitope

Figure 2: Characterization of anti-MUC16 MAb. MAb 11D10 was generated by immunizing BALB/c mice with MUC16ECDshort. MAb 3A5 was generated by immunization with commercial CA125 (US Biological). Human Fc chimeras were used unless otherwise noted.
(A) Flow cytometry of PC3 cells stably expressing MUC16TMshort or MUC16TMlong and of OVCAR-3 cells (endogenous MUC16 expression) with 11D10 and 3A5; PC3/vector cells and cytometry with cells omitting anti-MUC16 are negative control conditions.
(B) Mouse 11D10 or 3A5 was immobilized onto micro-titer plates for capture of CA125. Captured antigen was detected using chimeric 11D10, ch3A5, or an irrelevant chMAb, followed by HRP-secondary Ab. Irrelevant MAb did not capture any detectable CA125 (not shown).
(C) Lysates from 293S cells expressing MUC16TMshort ("FL") and successive deletions from the N-terminus of the MUC16 sequence ("Δ58" etc.) were blotted and probed with mouse 11D10. Loss of 11D10 binding from Δ110 to Δ192 defines the 11D10 epitope.
(D) Deletions and mutations of MUC16ECDlong define a sequence crucial for 3A5 binding.
(i) Alignment of repeats encoded by MUC16ECDlong. Repeat are consecutive, without gaps. Starting positions of deletion constructs are indicated by red numbers and blue bars. Key region of divergence in Repeat 3 vs. Repeats 1 and 2 is indicated by the blue box.
(ii) Deletion and mutation constructs were expressed transiently in 293S cells. Conditioned media were collected, blotted, and probed with 3A5 or 11D10 (normalizes for protein expression). Lanes 2-6: successive deletions. Substitution of "QH" in Δ174 (lane 3) with "Q" or "W" (lanes 7 and 8) greatly reduces 3A5 binding. Substitution of "W" in Δ330 (lane 6) with "WH" or "QH" (lanes 9 and 10) restores 3A5 binding.
(iii) Close-up of the alignment of repeats around the critical residues for 3A5 binding.

Fig. 1: MUC16 Constructs (Based on NP_078966.2; Ref. 2)



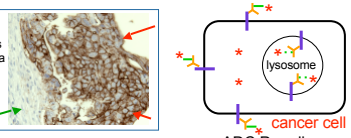
2Di Deletions and Mutations in MUC16 ECD to Define 3A5 Epitope (Numbering Based on MUC16ECDlong)



1: Vector control
2: MUC16 ECD
3: Δ174
4: Δ194
5: Δ302
6: Δ330
7: Δ174 // HQ to Q
8: Δ174 // HQ to W
9: Δ330 // W to WH
10: Δ330 // W to QH

2Diii
rep1 YEENMHHPGSRK
rep2 YEENMQHPGSRK
rep3 YEENMW - PGSRK

Figure 3: IHC staining of a serous ovarian adenocarcinoma by mouse 3A5. Red arrows indicates tumor, green arrow indicates stroma.



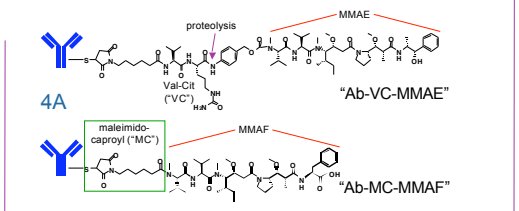


Fig. 4: In vitro comparison of drug-conjugated 11D10 vs. 3A5.
(A) Conjugates (ADC) tested in these studies (Seattle Genetics; Ref. 3). Auristatins MMAE and MMAF are cytotoxic microtubule-destabilizing drugs. "Ab-VC-MMAE" format incorporates a cathepsin-labile dipeptide linker between the MAb and the drug; "Ab-MC-MMAF" uses a more stable linker between the drug and the MAb.
(B) In vitro proliferation. Cells were incubated with ADC for 3-5 days, then cell numbers were determined. PC3/MUC16TMlong cells expressing similar numbers of epitopes for 11D10 and 3A5 were similarly sensitive to each conjugate. OVCAR-3 cells express more epitopes for 3A5 and are more sensitive to 3A5 ADC. [Not shown] PC3/vector and SK-OV-3 cells do not express MUC16 and were not affected by these ADC.

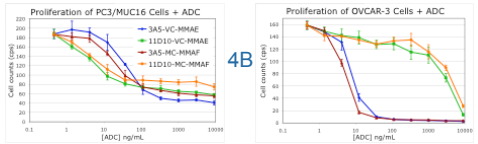
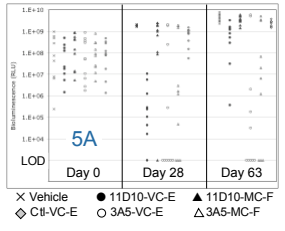


Figure 6: Toxicity of 3A5 ADC. (A) Cynomolgus monkeys were dosed with ADC on Day 1. The only notable signal was transient neutropenia with 3A5-VC-MMAE (target-independent; neutrophils do not express MUC16). (B, C) Nude rats were inoculated with OVCAR-3 cells IP, and tumors were allowed to grow until serum CA125 levels were elevated (16 to 2290 U/mL). Rats were dosed with 3A5-VC-MMAE or a control (non-CA125-binding) VC-MMAE. CA125 binding did not exacerbate toxicity of 3A5-VC-MMAE, and a therapeutic dose level was completely tolerated. [Not shown] Tumor-free rats experienced similar toxicities.



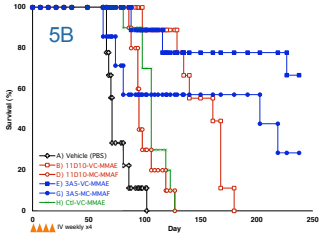
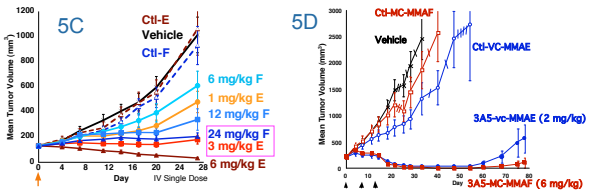


Figure 5: In vivo evaluation of anti-MUC16 MAb-auristatin conjugates.
(A) Mice were inoculated with OVCAR-3/luciferase cells IP. After two weeks, tumor burden (bio-luminescence) stabilized. Mice were then treated weekly x4 with 3 mg/kg of the indicated antibody-drug conjugates. Reduction in bio-luminescence indicates reduced tumor burden; note sustained elimination of tumors in 3A5 but not in 11D10 groups.
(B) Survival of OVCAR-3/luciferase mice (same study as in Panel A).
(C) Single-dose treatment of established OVCAR-3 mammary fat pad tumors demonstrates ≈ 8-fold better efficacy for 3A5-VC-MMAE ("E") vs. 3A5-MC-MMAF ("F").
(D) Weekly dosing in OVCAR-3 mammary fat pad model promotes 3A5-MC-MMAF activity.



CONCLUSIONS

1. MUC16 can be effectively targeted with ADC to inhibit tumor growth.
2. ADC of a MAb (3A5) that binds to multiple epitopes are much more potent than MAb ADC binding to a unique epitope (11D10).
3. Major toxicity in rats and monkeys is transient, MUC16-independent neutropenia. Circulating MUC16 ECD (CA125) does not increase ADC toxicity.

References
1. Polakis, Curr. Opin. Pharmac. 5: 382-387 (2005)
2. Yin and Lloyd, J. Biol. Chem. 276: 27271-27275 (2001); O'Brien et al., Tumor Biol. 23: 154-169 (2002)
3. Doronina et al., Nature Biotech. 21: 778-784 (2003); Doronina et al., Bioconj. Chem. 17: 114-124 (2006)

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