OBT076, a clinical-stage ADC, displays synergy with oxaliplatin and cisplatin in preclinical gastric cancer models.

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Abstract

Background: CD205 is a transmembrane glycoprotein, robustly expressed in malignancies from varied histotypes, making it an ideal target for Antibody Drug Conjugate (ADC) therapy. OBT076 is a novel and selective clinical stage ADC with potent activity against CD205-positive liquid and solid tumors. We explored the cellular cytotoxicity profile of combination chemotherapy with OBT076 and two standard-of-care platinum-based compounds, Oxaliplatin (Ox) and Cisplatin (Cis), in preclinical gastric, pancreatic and colorectal cancer models.

Methods: A panel of gastric cancer and other solid tumor cell lines were selected, based on mRNA data, for CD205 expression. This was confirmed using flow cytometry. The cell lines were assessed for susceptibility to monotherapy with OBT076, Ox and Cis. Cells lines displaying positive cytotoxic responses were assessed for susceptibility to combination chemotherapy by first treating cells with a single dose of OBT076 for 72 hours, leading to 20% growth inhibition (IC_{20}), and subsequently with either Ox or Cis for 48 hours (OBT-Ox and OBT-Cis, respectively). The ATP-dependent fluorescence-based cellular viability system cell titer-glo was used as the cytotoxic readout.

Results: In our preclinical gastric cancer model, pre-treatment with OBT076 followed by either Ox or Cis displayed synergistic combination effects, reducing the half-maximal inhibitory concentration (IC_{50}) of Ox and Cis by 40- and 10-fold respectively. The resultant cellular cytotoxicity of OBT-Ox and OBT-Cis combination therapy was higher compared to monotherapy with either agent. Moreover, while monotherapy with Ox was less potent than with Cis, OBT-Ox achieved similar potency to OBT076-Cis. For colorectal and pancreatic cancer models, the effects were less pronounced: OBT076-combination therapy increased Platinum sensitivity by 2 to 5 times. No synergistic effects were observed when the treatment order was reversed. These results and those from ongoing *in vivo* studies in gastric cancer models will be discussed.

Conclusion: OBT076, a clinical stage ADC displays synergistic anti-tumor effects in preclinical gastric cancer models, when used in combination with Ox or Cis. OBT076 is currently being evaluated in the gastric clinical population where Platinum-based therapies remain the mainstay of treatment.

i. CD205 Prevalence in Cancer

(a) Expression of CD205 in wide variety of solid tumors

(b) Expression of CD205 in hematological tumors

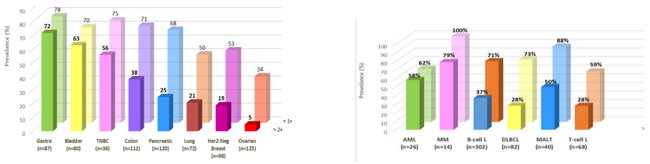


Figure 1. (a) IHC survey of solid tumors indications showing high prevalence of CD205 expression. Gastric, bladder, and triple-negative breast cancer show the highest prevalence by IHC staining 2+ or greater. (b) In liquid tumors CD205 is prevalent in MM, AML, MALT, B-/T-cell lymphoma

ii. CD205 Expression on Cancer Cell lines

(a) FACs binding assay

(b) Quantification of receptor density

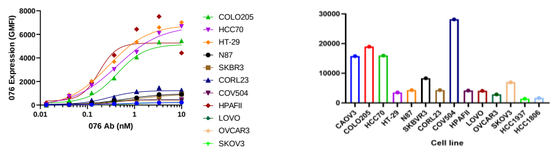


Figure 2. Endogenous cell lines that are sensitive to platinum therapies were screened for CD205 expression, the target for OBT076 ADC, by flow cytometry assay (a) and quantification of receptor density (b) was carried out. Several cell lines with varying degrees of CD205 expression were selected and showed sensitivity to the OBT076 ADC and platinum therapy (Figure 5). Cell lines that have shown cytotoxicity to both agents were selected to be investigated for combination therapy.

iii. Combination of OBT076 and Oxaliplatin

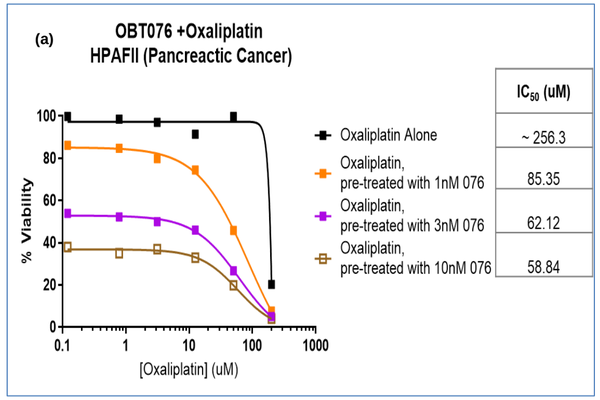


Figure 3(a). A 5-fold enhancement in IC_{50} was observed when cells were treated with OBT076 for 72 hrs followed by oxaliplatin for 48hrs.

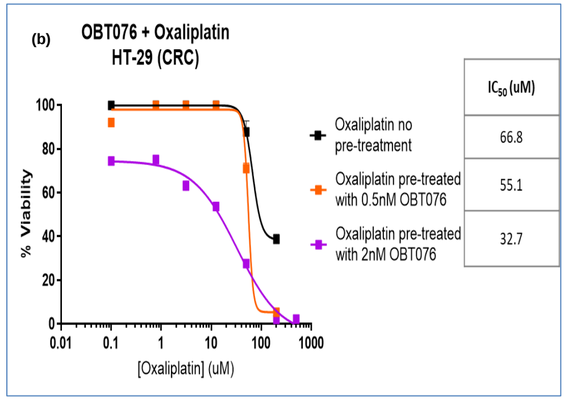


Figure 3(b). HT-29 is sensitive to treatment of OBT076 at a lower concentration. Oxaliplatin and OBT076 each mediate cytotoxicity of HT-29 as monotherapies. However, there is a 2-fold change in the oxaliplatin IC_{50} value when cells are treated with OBT076 for 72 hrs followed by oxaliplatin for 48hrs.

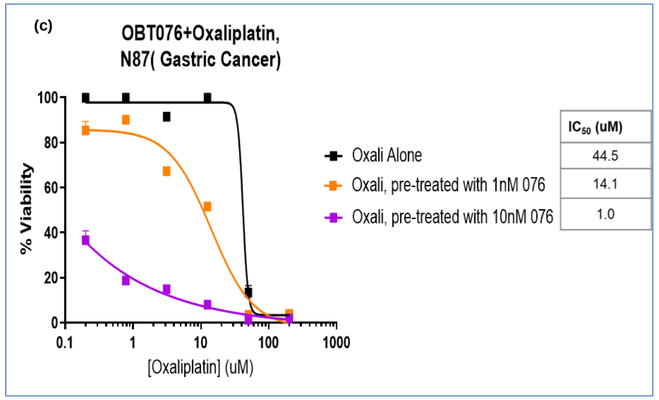
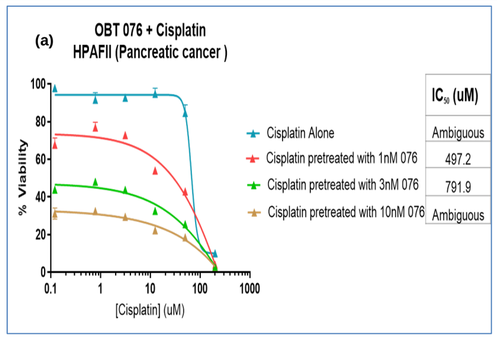
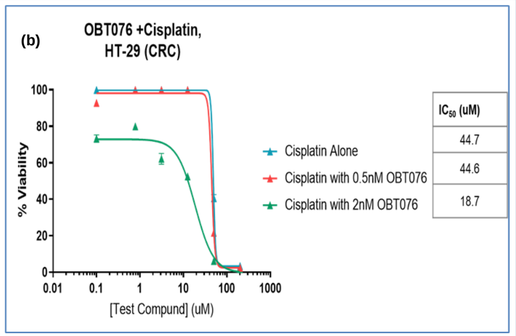


Figure 3(c). N87 is a low CD205 expressing cell line. Significant cytotoxicity was seen with oxaliplatin and OBT076 individually. However, when cells were treated with 10 nM of OBT076 for 72 hrs followed by oxaliplatin for there was a 40-fold enhancement in cytotoxicity as compared to monotherapy.

iv. Combination of OBT076 and Cisplatin





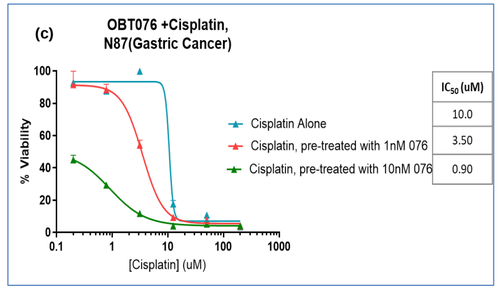


Figure 4.

- Cisplatin and OBT076 mediate the cytotoxicity of HPAF-II(a), HT29(b) and N87(c) cell lines in a dose dependent manner.
- On the HPAFII cells the combination of OBT076+Cis was less effective compared to HT-29 and N87 cell lines. There is 3 to 11-fold enhancement in the cytotoxicity quotient observed in N87(c) and HT-29(b) cell lines.

v. Summary of *In vitro* Combination Study

Indication	Cell Line	Antigen Expression/ Cell	Response to Platinum	Response to OBT076	Combination
CRC	HT29	15,990	Y	Y	Synergistic
	LOVO	4,220	Y	Y	Additive
	COLO205	15,815	Y	Y	Weak Additive
Pancreatic	HPAFII	28,180	Y	Y	Synergistic
Gastric	N87	3,552	Y	Y	Synergistic
	MKN45	2,136	Y	N	N/A

Figure 5. Several cell lines with a wide variety of expression of the target CD205 were sensitive to platinum therapies (Ox and Cis) and OBT076 ADC in the cytotoxicity assay. HT-29, HPAFII and N87 showed synergistic activity for the sequential treatment of OBT076 and Oxaliplatin when compared to monotherapy.

Conclusions

- Several cell lines that are sensitive to platinum therapy displayed low to high expression of CD205, the target for OBT076 ADC.
- OBT076, a clinical stage ADC displays synergistic anti-tumor effects in preclinical gastric cancer models, when used in sequential combination with oxaliplatin and cisplatin.

Pretreatment with suboptimal concentrations of OBT076 enhances the sensitivity to oxaliplatin and cisplatin in gastric tumor, CRC and pancreatic tumor cell lines *in vitro*. Further, *in vivo* investigations are ongoing in tumor models.