AFP-Maytansine Conjugate – a Novel Targeted Cancer Immunotherapy

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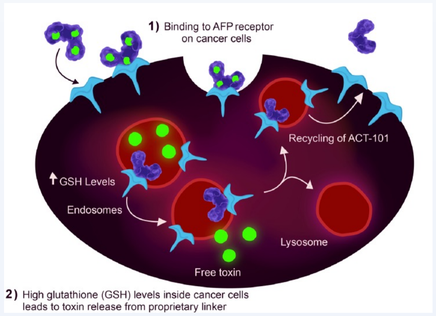
Background

- The alpha fetoprotein receptor (AFPR) is an oncofetal antigen expressed on the surface of many common cancers, including solid tumors such as breast, lung, ovarian, colorectal, prostate and hematologic tumors as well as on myeloid derived suppressor cells (MDSC). The AFPR is not expressed on normal adult cells, and therefore is a highly attractive target for novel cancer therapeutics.
- Alpha fetoprotein (AFP) is the natural ligand for the AFPR. ACT-101 is a non-glycosylated form of human AFP produced by recombinant DNA technology. It differs from naturally occurring AFP by one amino acid substitution (glutamine for asparagine at aa 233)
- ACT-101 binds to the receptor for AFP and after binding is internalized by the cell. By covalently conjugating a novel maytansine toxin (ABZ-981) to ACT-101 we can selectively deliver the toxin payload to cancer cells and MDSCs while sparing normal cells. Toxin is released through a proprietary linker which is glutathione (GSH) sensitive to the much higher concentrations of GSH that exist in tumor cells relative to the bloodstream (Figure 1).

Figure 1. ACT-101-maytansine conjugate mechanism of action



Methods

Prior to conducting *in vivo* tumor models, cell-binding assays employing flow cytometry were conducted on various cell lines to confirm expression of the AFPR. In these studies U-937 (human lymphoma) cells were used for comparison since this cell line is routinely used to measure biological activity of ACT-101. Cytotoxicity of conjugates *in vitro* was also tested against these cell lines.

The first study was a biodistribution study of ACT-101 conjugated to 3 different maytansines (DM1, DM4 and ABZ981). A single intravenous dose (25 mg/kg) was administered to COLO-205 tumor-bearing NCr-*nu/nu* mice. Concentration in blood, tissues and tumor was assessed by measuring both ACT-101 levels (by ELISA) and free maytansine plus metabolites (by LC-MS/MS) at 4, 8 and 24 hours post dose.

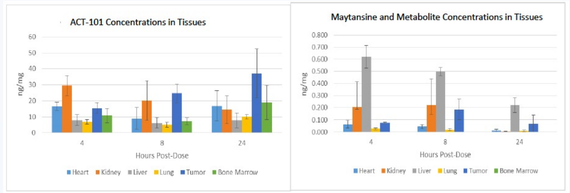
In a follow-up study in non-tumor bearing NCr-*nu/nu* mice, the maximum tolerated dose (MTD) of four optimized ACT-101 maytansine conjugates incorporating ABZ981 as the toxin and with different linker chemistries and protein:toxin ratios was determined. Each conjugate was administered at a dose of either 5, 10 , 20 or 40 mg/kg/day for a total of 10 days (n=3 per dose group). Each mouse was treated once a day by IV tail injection for 5 consecutive days, with 2 days off and then 5 more days of treatment. Daily clinical observation (mortality and morbidity and changes in body weight (bw) and liver physical abnormalities and enzyme measurements taken at the end of the dosing period, were used to assess toxicity.

An *in vivo* efficacy study was conducted using the dose determined from the MTD study for each ACT-101-maytansine conjugate. Ten million COLO-205 human colon tumor cells were implanted subcutaneously into 6-8 week-old male athymic nude (NCr-*nu/nu*) mice on Day 0. Fifty (50) mice were randomized on the day prior to treatment (n=10 per group). Treatment was initiated when tumors reached 100 to 200 mm^3 (Day 8 after implant). Groups 1-4 were treated with one of the ACT-101 maytansine conjugates and Group 5 received vehicle control. Clinical signs (mortality, morbidity and toxicity) were assessed daily. Tumor measurements and bw were assessed on the day prior to treatment, daily during treatment and 2X weekly thereafter for 60 days following implantation. Tumor response was calculated as the % difference at each time point between mean tumor weight in each treatment group and the mean tumor weight of the control group (Group 5) at the same time point.

Results

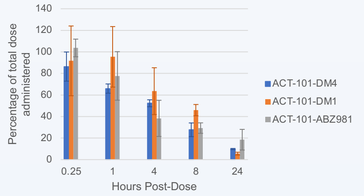
Binding of the ACT-101 to COLO-205 and U937 cell lines was concentration-dependent, with maximum of close to 90% positive binding, confirming high expression of the AFPR. Potency of all conjugates was in the low nM range. In the biodistribution study of ACT-101 conjugated to DM1, DM4 and ABZ981, delivery of toxin to the tumor was highest with the ACT-101-ABZ981 conjugate, with uptake evident at 4 h post-dose and peak concentration at the 8 h timepoint. Concentration in the tumor and off-target tissues with the ACT-101-ABZ981 conjugate is shown in Figure 2. There were no detectable toxin levels in the bone marrow over the 24 h observation period, a positive finding for avoidance of bone marrow toxicities usually associated with chemotherapy. Peak toxin levels in heart and lung were detectable but low, only 0.03% of the total dose administered, but higher in kidney and liver, ~0.36% and ~2.5%, respectively. However, the latter may represent primarily metabolism and clearance rather than tissue uptake.

Figure 2. Biodistribution of ACT-101 (A) and free maytansine (plus metabolites) (B) in tumor and off-target tissues for ACT-101-ABZ981



All conjugates showed good stability in blood with negligible plasma levels of free toxin detected. For the ACT-101-ABZ981 conjugate mean peak levels were 0.006 ng/mL, representing only 0.0004% of the total dose administered. ACT-101 concentrations decreased over the 24h time period in a similar manner with all conjugates (Figure 3), indicating that the half-life of the conjugate is driven primarily by the ACT-101 protein (approx. 7h in mice). Based on these results, ACT-101-ABZ981 was selected as the conjugate for lead optimization.

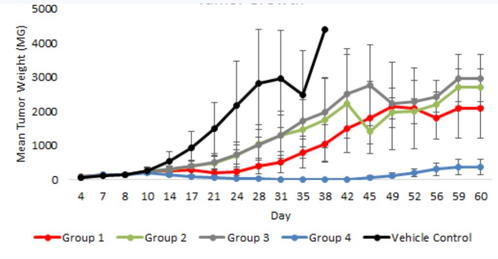
Figure 3. Serum levels of ACT-101 following a single 25 mg/kg IV dose of ACT-101-maytansine conjugates



An MTD study of 4 different conjugates was conducted. Group 1 was given the ACT-101-ABZ981 conjugate used in the biodistribution study and the 3 additional conjugates were optimized to improve toxin release kinetics.(Groups 2-4). The MTD was determined to be 40 mg/kg/day for 10 days (total dose of 400 mg/kg). Two conjugates exhibited toxicities at doses of 20 mg/kg/day (elevated liver enzymes, bw decline) and therefore for the subsequent efficacy study the 10 mg/kg/day dose was selected for Groups 2 and 3 while the 20 mg/kg/day dose was selected for Groups 1 and 4, with the same regimen of two 5-day treatment cycles being used.

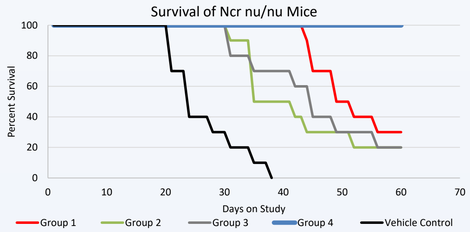
As shown in Figure 4, tumor growth was slower in all conjugate groups compared to vehicle, with a significant reduction ($p < 0.05$) in tumor growth relative to Group 5 (control) for all treatment groups by Day 14 which continued through Day 24, five days after completion of treatment. In Group 4 tumor regression continued post-treatment, with 9 of 10 mice having non-palpable tumors by Day 38. In this group, tumor regression was statistically different from Groups 2 and 3 on Day 24 ($p<0.0001$) and from Group 1 on Day 35 ($p=0.001$).

Figure 4. Tumor growth following treatment with ACT-101-maytansine conjugates in COLO-205 xenograft model (mean +/- SD)



With respect to survival, all animals in Group 4 were alive through to Day 60 with only minimal tumor growth during the last week of the study (Figure 5), compared to Group 5 (vehicle) where all animals were dead due to excessive tumor growth by Day 38. There were no treatment-related toxicities or deaths observed in this study.

Figure 5. Survival following treatment with ACT-101-maytansine conjugates in COLO-205 xenograft model



Conclusions

- ACT-101 targets the AFPR expressed on tumor cells and preferentially deliver toxin to tumor with minimal off-target distribution
- ACT-101-maytansine conjugates demonstrated positive effects on tumor regression and survival in a COLO-205 xenograft mouse model
- Alpha Cancer Technologies Inc. is continuing the development of the Group 4 optimized ACT-101-maytansine conjugate (designated ACT-903) from this study, with potential to be a highly effective, safe and targeted cancer therapy
- Further preclinical proof of concept studies are currently underway to support an IND submission for investigating ACT-903 in a clinical trial

Study sponsored by Alpha Cancer Technologies Inc. Questions to be addressed to: isherman@alpha-cancer.com . Igor Sherman is CEO of Alpha Cancer and has no other conflicts of interest to declare.