Targeted nano-immune conjugates to melanoma: Pre-clinical testing of bevacizumab targeted nab-paclitaxel.

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Abstract

Background: Phase 3 clinical results demonstrate that nab-paclitaxel (ABX) exhibits single agent activity in patients with metastatic melanoma relative to dacarbazine[1]. Additionally, phase 2 clinical data suggest that ABX combined with bevacizumab (BEV) with/without carboplatin exhibits greater clinical efficacy than ABX alone[2]. We hypothesized that the latter may be due to a pharmacokinetic, non-covalent interaction between bevacizumab (BEV) and nab-paclitaxel (ABX) allowing for greater localization of ABX to sites of VEGF overproduction (metastases). Herein we present pre-clinical data describing the utility of experimentally created ABX/BEV complex nanoparticles used to deliver ABX to VEGF producing cancer targets.

Methods and Results: To test whether BEV binds to ABX we employed flow cytometry and discovered that the BEV does bind to ABX while preserving the ability of BEV to bind its ligand, VEGF. To measure binding affinity we used Biolayer Interferometry (BLItz) to measure association and dissociation constants. Results of BLItz experiments demonstrate that the binding affinity of BEV to ABX is pH and temperature dependent. The size and stability of the nanoparticle formed by binding BEV and ABX was determined using light refraction (Mastersizer) and Brownian motion (Nanosight). Results of these experiments suggest that a range of nanoparticles can be produced and stability of the BEV containing particle is increased relative to ABX particles alone. Finally the tumor efficacy of the BEV/ABX complex relative to ABX and BEV as well as sequential administration of the two drugs was tested in a xenograft model of A375 human melanoma cell line. Pharmacokinetic studies in these mice suggest that more ABX is deposited in the tumor site when treated with the complexes relative to ABX alone.

Conclusions: The therapeutic antibody BEV has the capability to bind to the chemotherapy drug ABX while still binding its ligand, VEGF. The resulting nanoparticle is more stable than the original ABX particle. In a preclinical model of human A375 xenografts in nude mice, AB160 treatment offers significantly improved antitumor activity beyond that of ABX, BEV or ABX+BEV with no additional toxicity. Initial results indicate that the improved efficacy of the ABX/BEV nanoparticle relative to ABX alone is primarily due to the ability of the anti-VEGF antibody (BEV) to target the chemotherapy agent to the tumor where local VEGF concentration is very high. Clinical translation of the safety of a 160nm AB particle (AB[160]) in patients with metastatic melanoma is in progress.

Objectives

Objective 1: Objective one was to determine whether bevacizumab has the ability to bind to nab-paclitaxel and characterize the type and affinity of that bond. Additionally, we wanted to confirm that, once bound to nab-paclitaxel, bevacizumab would retain its ability to bind its ligand, VEGF and nab-paclitaxel retained its toxicity. To do this we employed flow cytometry to determine binding, ELISA for VEGF binding, proliferation assay for A375 toxicity, Mastersizer and Nanosight to measure size and stability of the particles, and BLItz to measure the dissociation constants of the protein:protein interaction.

Objective 2: Objective two was to determine tumor efficacy of nab-paclitaxel, bevacizumab, and the nab-paclitaxel bevacizumab nanoparticle (AB160). To do this we employed an A375 human melanoma xenograft model using female athymic nude mice.

Bevacizumab nab-paclitaxel interaction

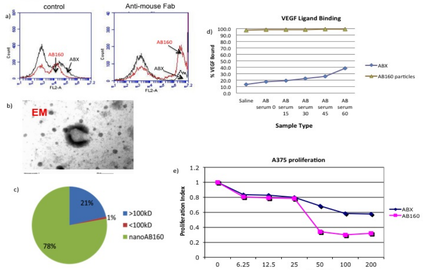
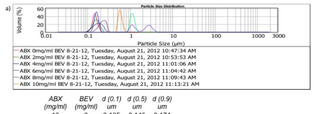


Fig 1: Bevacizumab binding of nab-paclitaxel was determined by flow cytometery using a PE conjugated goat anti-mouse IgG Fab (a) and electron microscopy with gold conjugated anti-human IgG (b). Paclitaxel distribution was determined using HPLC of AB160 after running sample over size exclusion columns (c). Ligand binding of VEGF to ABX bound bevacizumab was confirmed with a standard VEGF ELISA (d). Paclitaxel toxicity was confirmed by proliferation of A375 after in vitro exposure to ABX and AB160 (e).

Particle Size and Stability



ABX (mg/ml)	BEV (mg/ml)	d (0.1) um	d (0.5) um	d (0.9) um
10	0	0.125	0.146	0.174
10	2	0.122	0.157	0.196
10	4	0.138	0.159	0.182
10	6	0.124	0.174	0.235
10	8	0.171	0.226	0.278
10	10	0.516	0.577	0.67
10	15	0.981	1.129	1.31
10	25	1.036	2.166	3.233

b)

AB160m, Kd	← conditions "X" →		
↑ conditions "Y" ↓	8.2 x 10(-10)	1.513 x 10(-11)	< 1 x 10(-12)
	1.45 x10(-10)	6.17 x 10(-9)	1.749 x 10(-11) (AB160/IGG2)
	2.2 x 10(-8) (AB160/IGG)	1.512 x 10(-8)	8.205 x 10(-10) (AB160/P2B)
	1.3 x 10(-9)	1.389 x 10(-5)	1.999 x 10(-9)

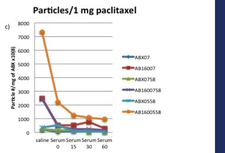
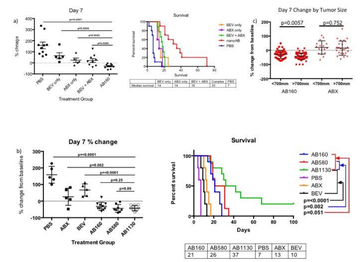


Fig 2: The size of ABX and AB160 particles were determined by Mastersizer (a). Dissociation constants between ABX and bevacizumab were measured under various conditions using Biolayer Interferotomotery (b). The number of particles was ascertained using Nanosight technology under the conditions in (b), added to AB serum and calculated as number of particles/1 mg of paclitaxel (c).

Mouse Tumor Efficacy



AB160	AB580	AB1130	PBS	ABX	BEV
21	26	37	7	13	10

Fig 2: The A375 human melanoma xenograft model was utilized to test tumor efficacy of AB160 relative to the single drugs alone or sequential administration of the 2 drugs. Data is displayed as day 7 % change from baseline and Kaplan Meier curves(a). In another experiment we treated mice with AB160, AB580 and AB1130 (Fig 2) and survival improves when mice were treated with larger particles (b). Day 7 % change was calculated from several mouse experiments and broken down by baseline tumor size (c).

Pharmacokinetics and Biodistribution

a)

	No Tumor		Tumor	
	AB160 ± SD	ABX ± SD	AB160 ± SD	ABX ± SD
C_{max} (ug/mL)	63.3 ± 39.4	65.5 ±14.4	55.7 ± 21.2	63.3 ± 17.3
Half-life (hrs)	4.7	3.8	8.0	4.5
AUC_{0-24h} (ug/ml x h)	129	133	112	128
$AUC_{0-\infty}$ (ug/ml x h)	129	133	112	128

b)

	C_{max} ug/mL ± SD	$AUC_{0-\infty}$ ug/mL x h
AB160, No Tumor	66.1 ± 19.8 (30%)	40.1
ABX, No Tumor	80.4 ± 2.7 (3%)	47.2
AB160, Small Tumor	44.4 ± 12.1 (27%)	26.9
ABX, Small Tumor	48.4 ± 12.3 (25%)	28.9
AB160, Large Tumor	22.8 ± 6.9 (30%)	15.3
ABX, Large Tumor	30.7 ± 5.2 (17%)	19.7

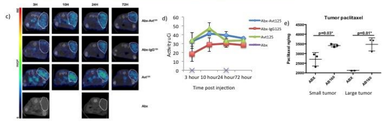


Fig 4: Pharmacokenetics of paclitaxel in blood plasma from two mouse experiments (a and b). Biodistribuion of I-125 labeled BEV relative to AB160 and ABXIgG in mouse tumors (c). A graph on time course of I-125 in mouse tumors (d) and paclitaxel concentrations at 4 hours in mouse tumors.

Other particles

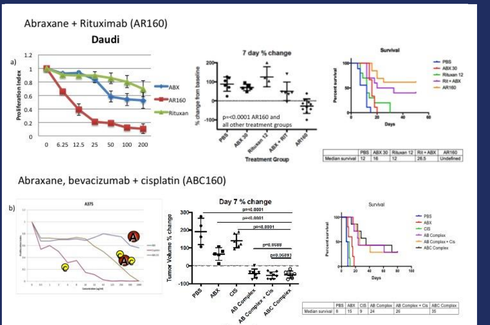


Fig 5: Other nano-immune conjugates were prepared and tested in in vitro culture systems and mouse models. AR160 particles (a) were prepared and tested on CD20+ Daudi lymphoma cells in vitro and in vivo. ABC160 particles (b) were prepared and tested on A375 melanoma cell line in vitro and in vivo.

Conclusions

- BEV binds to ABX while maintaining its ability to bind the ligand VEGF and preserving the toxicity of the paclltaxel. The paclitaxel component of the particles as they dissociate seems to be among protein(s) that are >100Kd.
- The size of the ABX:BEV particles produced is antibody concentration dependent with larger particles formed with higher antibody concentrations. The particles are very stable in saline over a 24 hour period. The particles become more stable in human AB serum depending on the mixing conditions.
- AB160 shows increased tumor efficacy in a human mouse model of melanoma as demonstrated by tumor response 7 days post treatment and progression free survival (PFS). Tumor response increased when treated with larger AB particles. Tumor response also directly correlated to tumor size possibly due to higher VEGF concentrations.
- Higher levels of iodinated BEV relative to isotype control were seen in mouse tumors treated with BEV alone or BEV in the context of the AB160 particles suggesting that locally high levels of VEGF in the tumor attract the antibody containing drug. Tumor paclitaxel concentrations determined by LC-MS also indicate higher levels in mice treated with AB160 relative to ABX alone. Pharmacokentics of paclitaxel concentrations in blood plasma were analyzed in. Data in mice suggest that pharmacokinetics were altered with AB160 relative to ABX alone.
- Other therapeutic IgG antibodies (rituximab) with similar Fc fragments also bind to ABX and demonstrate increased tumor efficacy in vitro and in vivo. Also other chemotherapy drugs (cisplatin) bind to ABX increasing tumor efficacy while limiting toxic side effects.

References

1. Hersh et. al Final overall survival from a phase 3 trial of nab-paclitaxel versus dacarbazine (DTIC) in chemotherapy naïve patients with metastatic melnoma. J Clin Oncol 32:5s, 2014 (suppl; abstr 9045).
2. Kottschade et. al. A randomized phase 2 study of temozolomide and bevacizumab or nab-paclitaxel, carboplatin, and bevacizumab in patients with unresectable stage IV melanoma; a North central Cancer Treatment Group study, N0775. Cancer. 2013 Feb 1:119(3) 586-92.

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