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

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Abstract

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 to VEGF.

Objective 2: Objective two was to determine tumor efficacy of nab-paclitaxel, bevacizumab and the nab-paclitaxel-bevacizumab nanoparticles (AB100).

Methods and Results: To test whether BEV binds to ADX we employed their capacity and discovered that the BEV does bind to ADX while preserving the ability of BEV to bind to VEGF. To determine the binding affinity we used Biacore Biacore T100 (GE) in measure association and dissociation constants. Results of BLI experiments demonstrate that the binding affinity of BEV to ADX is pH and temperature dependent. The rate and stability of the nanoparticles formed by binding BEV and ADX was determined using light refraction (Masterzoner) 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 related to the antibody alone.

Pharmacokinetics and biodistribution:

• BEV binds to ADX while maintaining its ability to bind the ligand VEGF and preserving the toxicity of the paclitaxel. The paclitaxel component of the nanoparticle is as active as the free drug.

• 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 solution for 24 hour period. The particles become more stable in human AB serum depending on the mixing conditions.

• AB100 increases 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 increases when treated with larger AB particles. Tumor response also correlated to tumor size possibly due to the antibody concentrations.

• Higher levels of isolated BEV relative to site control were seen in mouse tumors treated with BEV alone or BEV in the context of the AB100 directly 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 AB100 relative to ABX alone. Pharmacokinetics of paclitaxel concentrations in blood plasma were analyzed in data. In data it was noticed that pharmacokinetics were altered with AB100 relative to ABX alone.

• Other therapeutic IgG antibodies (nabumetone) with similar Fc fragments also bind to ADX and demonstrate increased tumor efficacy in vivo and in vitro. Also other chemotherapy drugs (laspide) bind to ADX increasing tumor efficacy while limiting toxic side effects.

References


Conclusions

Fig. 4. Pharmacokinetics of paclitaxel in blood plasma from two mouse experiments (A) and at baseline (1) in melanoma mice (B). A graph of both experiments (1, 2) in mouse tumors (C) and paclitaxel concentrations of 4 tissue masses in mouse tumors (D).

Mouse Tumor Efficacy

Fig. 5: Other nano-immune conjugates were prepared and tested in vitro cultures systems and mouse models. ABX/BEV particles were prepared and tested on C57Bl/6 melanoma cells and tested on 201R5 melanoma cells. The conjugates were tested alone and combined with chemotherapy (DTIC) on melanoma cells in vitro. The conjugates were also tested on human melanoma xenograft tumors in vivo and in vitro.

Bevacizumab nab-paclitaxel interaction

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 to VEGF. VEGF and nab-paclitaxel, showing for greater localization of VEGF in the presence of EGF than in the absence of VEGF. 

Objective 2: Objective two was to determine tumor efficacy of nab-paclitaxel, bevacizumab and the nab-paclitaxel-bevacizumab nanoparticles (AB100). To do this we employed an ABX+BEV antigen model using feraline tyrosine-nude mice.