#B106

Panacea Pharmaceuticals

Antibody-drug conjugates targeted at HAAH

K Malhotra[1], S Walker[1], C Fang[2], K Birthare[2], MS Lebowitz[1], S Fuller[1], M Sesay[2] and HA Ghanbari[1]

[1]Panacea Pharmaceuticals, Inc., Gaithersburg, MD; [2]Goodwin Biotechnology, Inc., Plantation, FL

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ABSTRACT

Antibody-drug conjugates (ADCs) consist of an antibody that is specific for a disease antigen linked to one or more drug molecules. The goal is to utilize the antibody as a means of specific delivery of the drug to diseased cells. Theoretically this should decrease systemic exposure to the drug and allow for use of a lower dose. In cancer, the approach employs an antibody targeted at a tumor associated antigen and a cytotoxic or cytostatic drug. To date there have been three ADCs approved by the US FDA for cancer treatment and many more are now in the development stage. The efficacy of any ADC is dependent on three factors; the antibody, the attached drug and the linker used to connect them. While each of these factors is of import, with the growth of interest in ADCs for cancer therapy, the use of specific drugs and linkers has become more routine. Thus, the specificity of the antibody and target antigen are now perhaps the primary obstacles towards the development of novel ADCs.

Human aspartyl (asparaginyl) β-hydroxylase (HAAH) is an embryonic/developmental protein, which is down-regulated in normal cells after birth but overexpressed on the surface of many malignant cells. It has been demonstrated to be sufficient to induce cancer cell proliferation, motility and invasiveness. The enzyme hydroxylates important residues in the NOTCH protein altering signal transduction pathways that lead to increased growth and metastatic potential. Increased levels of HAAH have been detected by immunohistochemistry in a diverse array of solid and hematological cancers (n > 20), including: liver, bile duct, brain, breast, colon, prostate, ovary, pancreas, and lung cancers as well as various leukemias. HAAH is not found in measurable quantities in normal tissue (n > 500) including normal adjacent tissue within cancer biopsy specimens or in benign proliferative disorders. We have developed a fully human antibody against HAAH, PAN-622, which displays exquisite specificity for cancer. Here we explore PAN-622 drug conjugates for ultimate use in treating both hematological and solid tumors.

PAN-622 was conjugated to three different drugs; a maytansinoid (DM1), monomethyl auristatin E (MMAE) or duocarmycin (DUO). DM1 was conjugated via a non-cleavable thio-ether linker while MMAE and DUO were conjugated using valine-citruline containing linkers that are cleavable by cathepsin B in the endosomal compartment. Conjugation of drugs to the PAN-622 antibody had little effect on the affinity of the antibody for HAAH. Binding affinities were determined by immunoassay on fixed cancer cells (H460, human lung cancer line) and were ~0.1, 0.2 and 0.5nM for PAN-622-DM1, PAN-622-MMAE and PAN-622-DUO, respectively. The affinity of PAN-622 for HAAH as expressed on live cells has previously been shown to be ~1nM. Efficacies of the three ADCs for killing of H460 cells were determined using an MTS assay. The PAN-622-DM1 had an EC50 of ~15nM. The EC50 for PAN-622-MMAE was ~60nM and that for PAN-622-DUO was ~300nM. Importantly, both unconjugated PAN-622 and a non-relevant antibody conjugated to MMAE did not display any killing of the H460 cell line. Efficacy was also measured on a representative hematological cancer line, MOLM-14 (acute myelogenous leukemia) where EC50s were in the 20-50nM range for all three ADCs. This work serves as a proof-of-concept; laying the groundwork for further development of HAAH-targeted ADCs.

Antibody Drug Conjugates (ADCs)

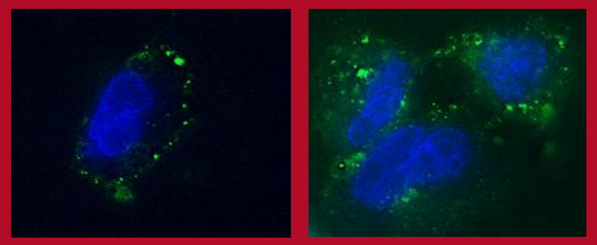
- ADCs consist of a drug or toxin conjugated to an antibody
- The antibody serves to specifically deliver the drug to a diseased cell type, where the conjugate is internalized and the drug may be released from the antibody to effect the target cell
- To date the FDA has approved 3 ADCs for cancer therapy
  - Gemtuzumab ozogamicin – Anti-CD33 conjugated to a calicheamicin for the treatment of AML (withdrawn, but currently back in clinical trials)
  - Brentuximab vedotin – Anti-CD30 conjugated to MMAE with a cathepsin cleavable linker for the treatment of Hodgkin lymphoma and systemic anaplastic large cell lymphoma
  - Trastuzumab emtansine – Anti-Her2 conjugated to DM1 for the treatment of Her2+ breast cancer
- ADC efficacy is highly dependent on the specificity of the antibody/target

Human Aspartyl (Aspariginyl) β-Hydroxylase (HAAH)

- Embryonic / developmental protein regulating signaling through the NOTCH pathway - Silent in adult cells
- Over-expressed on cancer cells and translocated to the plasma membrane
- Panacea Pharmaceuticals has developed a fully human mono-clonal antibody, PAN-622, targeted at HAAH
- PAN-622 is internalized upon attachment to the cell surface
- HERE WE EXPLORE PAN-622 BASED ADCs

HAAH / PAN-622 IS INTERNALIZED BY CANCER CELLS

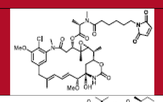
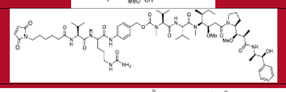
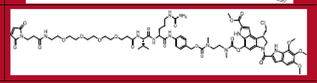
4˚C incubation | 37°C incubation



- PAN-622 was labeled with Alexa-488
- FOCUS (liver cancer) cells were grown on coverslips and incubated with PAN-622-488 at 4°C or 37°C for 4hrs.
- Cells are visualized by confocal microscopy. Nuclei were stained with Hoechst dye.

DRUG TO PAN-622 CONJUGATION

Table 1: Properties of Linker-Payloads

	Liker type / release mechanism	Payload	Structure
MC-DM1	Thiol ether, Noncleavable / lysosomal digestion	Maleimidocaproyl-maytansine	
MC-vc-PAB-MMAE	Val-Cit, Endosomal cathepsin B	Monomethyl auristatin E	
MC-PEG4-vc-PAB-DMEA-DUO	Val-Cit, Endosomal cathepsin B	Duocarmycin SA	

- DM1 is attached to PAN-622 via a noncleavable linker (maleimide-thiol ether)
- MMAE and DUO are attached via a cleavable valine-citruline linker that is subjected to degradation by cathepsin B in the endosomal compartment
- DM1 was conjugated at a 10:1 ratio with PAN-622
- MMAE was conjugated at a 5:1 and a 10:1 ratio with PAN-622
- DUO was conjugated at a 2.2:1 ratio with PAN-622

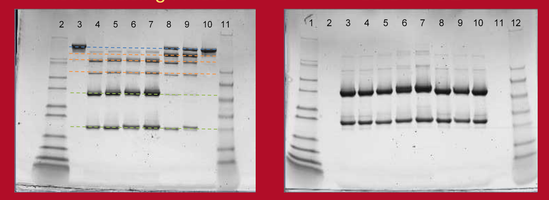
Table 2: Characteristics of PAN-622 ADCs

Testing Items	Unmodified mAb	DM1 Conjugate	MMAE Conjugate (10:1)	DUO Conjugate
Compound	PAN-622	PAN622-DM1	PAN622-MMAE	PAN622-DUO
Lot #	10LBRX 3099-228-003	P-414-CJG-1702-DM1	P-414-CJG-1702-AE2	P-414-CJG-1702-DUO
Appearance	Clear, free of particulate	Clear, free of particulate	Clear, free of particulate	Clear, free of particulate
Protein concentration (mg/mL)	9.55	4.257	5.267	4.594
Recovery[a]	N/A	53.0%	70.2%	71.5%
DAR by UV absorbance	N/A	5.83	9.18	2.46
SDS-PAGE	Single band cluster	Multiple bands	Multiple bands	Multiple bands
SEC-HPLC (% monomer)	99.37	94.78	94.42	93.48
DAR by HIC	N/A	3.47	6.31	1.92

- Conjugates were purified by dialysis using a 30kDa MWCO Dialysis Device overnight in 1X PBS (pH 7.4) for a total of three buffer exchanges.
- ADCs were characterized by via visual appearance, UV-vis, SDS-PAGE, HIC-HPLC and SEC-HPLC.
- Drug-to-antibody ratios were determined by UV-vis & HIC-HPLC.

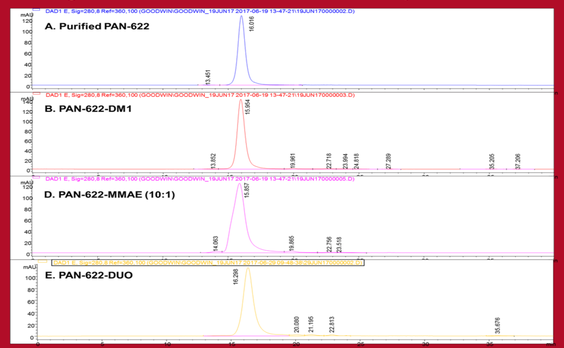
Figure 2: Characterization of ADCs by PAGE

Non-reducing PAGE | SDS-PAGE



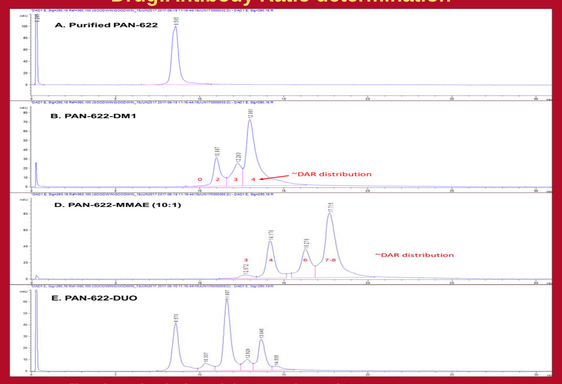
- Non-reducing PAGE. Lane: 1,12-Blank; 2,11-markers; 3,10-PAN-622; 4,8-PAN-622-TCEP; 5-PAN-622-DM1; 6,7-PAN622-MMAE; 9-PAN-622-DUO
- Non-reducing PAGE. Lane: 1,12-markers; 2,11-blank; 3,10-PAN-622; 4,8-PAN-622-TCEP; 5-PAN-622-DM1; 6,7-PAN622-MMAE; 9-PAN-622-DUO

Figure 3: Characterization of ADCs by SEC-HPLC



- SEC-HPLC showed a single primary peak for all conjugates
- Minimal aggregation was observed with PAN-622-MMAE. All other conjugates displayed no detectable aggregation.

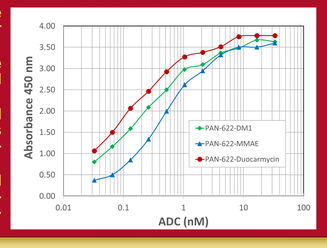
Figure 4: Characterization of ADCs by HIC and Drug:Antibody Ratio determination



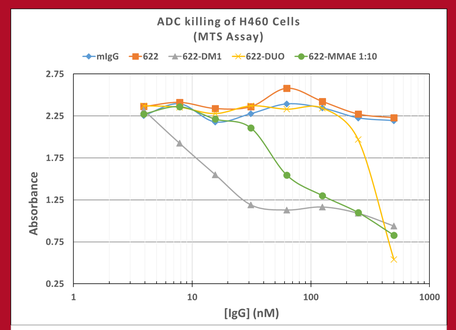
- HIC profiles showed a single peak for un-conjugated PAN-622
- Drug Antibody Ratio distributions were determined and are presented on the chromatograms

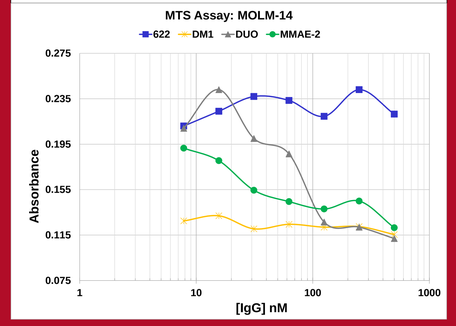
ADCs RETAINED AFFINITIES FOR CANCER CELLS COMPARABLE TO PAN-622

- Binding affinities were determined by a cellular ELISA
- H460 (lung cancer) cells were seeded at $1x10^5$/well and grown overnight
- Cells were fixed and incubated with ADCs followed by a secondary anti-hIgG.
- Predicted were ~0.1, 0.2 and 0.5nM for PAN-622-DM1, PAN-622-MMAE and PAN-622-DUO, respectively



ADC KILLING OF H460 (LUNG CANCER) AND MOLM-14 LEUKEMIA CELLS WITH HIGH SPECIFIC ACTIVITY





- Cells were seeded at $1x10^4$ per well and incubated for 24 hrs. in the presence of PAN-622 or ADC.
- Assays were developed with MTS reagent for one hour prior to read.

SUMMARY

- THREE DIFFERENT ADCs WERE SYNTHESIZED BASED ON THE ANTI-HAAH ANTIBODY, PAN-622 AND INCORPORATING DM1, MMAE OR DUO AS THE CONJUGATED DRUG.
- ALL PAN-622-BASED ADCs WERE PURIFIED AND CHARACTERIZED FOR PURITY AND DRUG TO ANTIBODY RATIOS.
- ALL PAN-622-BASED ADCs WILL WERE EFFECTIVE WITH EC_{50}'S IN THE 15-300nM RANGE ON BOTH LUNG CANCER AND LEUKEMIA CELL LINES.

CONCLUSION

HAAH IS A NOVEL ONCOGENIC TARGET EXPRESSED IN BOTH SOLID AND HEMATOLOGICAL TUMORS. HAAH CAN BE SELECTIVELY TARGETED WITH ANTIBODY DRUG CONJUGATES (ADCs) BASED ON THE FULLY-HUMAN PAN-622 MAB. FURTHER WORK TO OPTIMIZE THESE NOVEL ADCs IS ONGOING.

REFERENCES

Yeung, *et al.*, "Isolation and characterization of human antibodies targeting human aspartyl (asparaginyl) beta-hydroxylase", Hum Antibodies. 2007;16(3-4):163-76.