AVIPEP Therapeutics

Avibodies™ are engineered, bivalent antibody fragments, precisely loaded with imaging or cytotoxic payloads

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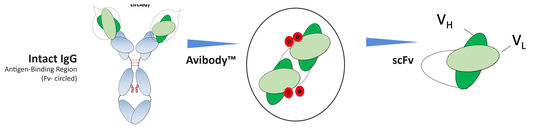
Abstract

While the use of intact antibodies as a platform for ADCs has yielded several marketed products, there have been many failures in clinical development. One factor in the high failure rate may be poor distribution into tumor tissue due to the larger size / slow rate of diffusion of intact IgG antibodies. Avibodies™ comprise only the antigen-binding domains of an intact antibody, engineered into a stable, compact, bivalent format that enables **rapid penetration** into tumor tissue coupled with **good retention** by the tumor. Avibodies can be precisely loaded (site-specifically) with payloads. The specific loading sites are engineered into the protein structure so that resulting conjugates are highly stable, with excellent pharmacokinetic properties *in vivo*. An Avibody, AVP04, that binds to the Tag72 tumor antigen and site-specifically loaded with PEG, was radiolabeled (Iodine-124) for a clinical biodistribution study. AVP04 demonstrates Avibodies are safe, show consistent PK in all patients with fast clearance from normal (non-target) tissues and with excellent retention by antigen-positive tumor tissue (tumor:blood ratios of 22:1 at 7 days post-infusion). AVP04 targeting Tag72 has been developed as an ADC in a pre-targeting format as well. (Rossin, Robillard *et al*., (2018) *Nature Communications* 9:1484-1489.) An Avibody, AVP10, binding to CD30 and site specifically loaded with MMAF, was studied in a mouse xenograft model. AVP10 demonstrates persistent tumor binding and a wider therapeutic window, coupled with fast clearance from normal tissues and the peripheral blood. AVP10 is in preclinical development looking towards a Phase I study.

Technology

Avibodies™ demonstrate superiority over both scFv and IgG formats

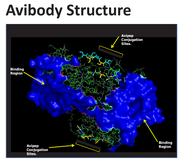


Avibodies: antibody derivatives, engineered fragments comprising antigen-binding Fv domains of intact IgG

Custom engineered, reliable format
- Compact, ~60kD w/ payload
- Bivalent, preserves avidity
- Stable, self-assembling
- Site-specific conjugation; DAR2 or true DAR4

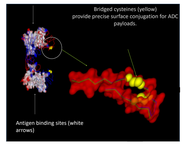
Avibodies™ widen therapeutic index
- Rapid tumor penetration and distribution
- Persistent binding within the tumor
- Fast clearance from circulation

Avibody Structure



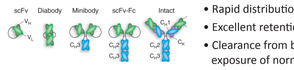
Avibodies provide:
- Generic attachment sites (antibody-independent)
- On any mammalian V-domain framework
- Distant from the antigen binding sites
- Site-specific ADC or TAT/RIT conjugation options (for exact DAR)

Avibody™ Platform allows precise site-specific cysteine conjugation



Avibodies enable exactly two or four payload molecules (drugs/nuclides) per Avibody
- Bridged cysteines (disulphides) are engineered as surface residues
- Distant from either antigen binding site (covered by worldwide patent estate)
- Can be designed into existing framework regions or incorporated into library

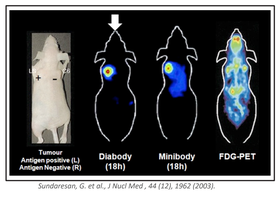
Avibody next-gen ADCs deliver best-in-class Tumour:Blood ratios



- Rapid distribution into tumor (small size)
- Excellent retention by tumor (bivalent)
- Clearance from blood pool (superior tumour:blood ratio & low exposure of normal tissue)

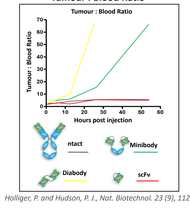
Holliger, P. and Hudson, P. J., Nat. Biotechnol. 23 (9), 1126 (2005)

^{124}I-PET Imaging with an Avibody (a Diabody)



Sundaresan, G. et al., J Nucl Med , 44 (12), 1962 (2003).

Comparative examples of Tumour : Blood Ratio



Holliger, P. and Hudson, P. J., Nat. Biotechnol. 23 (9), 1126 (2005)

In studies, Avibodies show rapid tumor penetration and excellent tumor retention, with fast clearance from normal tissues and the blood.

Results indicate the advantages of Avibody™ technology: efficacy combined with reduced off-target toxicities.

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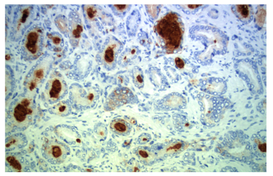
Summary and Conclusions

The results illustrate the advantages of Avibody™ technology in engineering efficacious ADCs:

- Smaller size versus full length IgG allows rapid distribution into tumors from blood & deeper penetration within tumors
- Bivalency allows excellent retention within tumor mass
- Faster clearance relative to full length IgG allows potential for lower exposure of non-target normal tissue

Imaging Study with Avibody

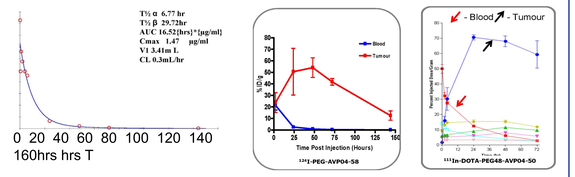
Target: Tag-72 (CA 72-4); Sialyn-Tn (sTn), an onco-fetal O-glycan



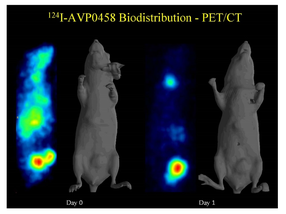
- **Tag-72 is highly expressed on adenocarcinomas, particularly high on ovarian, prostate, gastric, and colon cancers**
- **Over 15 clinical trials (IgG in RIT format) have demonstrated that Tag-72 is a highly selective tumor-specific antigen**

Prostate cancer tissue: positive for TAG72 (dark areas) while benign tissue was negative (light areas)

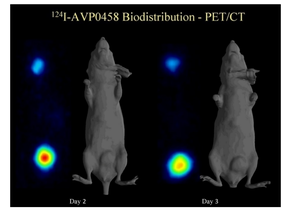
AVP04 Avibody™ targeting Tag-72 has Site Specific conjugation of PEG-payloads with exceptional tumor uptake and no first-pass kidney clearance



^{124}I-AVP0458 Biodistribution - PET/CT



^{124}I-AVP0458 Biodistribution - PET/CT

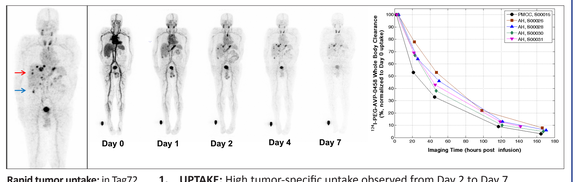


Avibody targeting: ^{124}I PEG-AVP0458 (GMP product) LS174T xenograft biodistribution

Specific conjugation allows tailoring of PK & resulting tumour localisation to >70% ID/g
- Excellent tumour uptake
- Prolonged residence time in tumor
- Low kidney uptake

Lin Li *et al.* (2011) *Bioconjug Chem* **22**:709-716
& (2010) *J Nucl Med* **51**:1139-46.

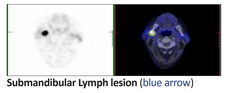
Clinical Biodistribution Trial ^{124}I-PEG-AVP04 in Relapsed Prostate Cancer HIGH and PERSISTENT tumor uptake (PET) and FAST blood/systemic clearance



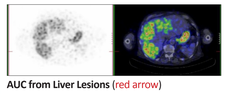
Rapid tumor uptake: in Tag72 positive liver metastases (red arrow) and an unexpected colon metastasis (blue arrow) [Day 4 image]

1. **UPTAKE:** High tumor-specific uptake observed from Day 2 to Day 7
2. **PERSISTENCE:** Avibodies have cleared circulation by Day 2 and remain localised at high levels in tumour (liver and colon metastases)
3. **CLEARANCE:** Similar rapid clearance observed in all patients (five shown; T1/2α 5hr from blood and T1/2β from whole body ~45hr).
4. **HIGH** Tumor:blood ratios- up to 22:1 at 7 days post infusion
5. **REPRODUCIBLE** pharmacokinetics across two dose levels (1 and 10 mg/m^2)

^{124}I-PEG-AVP04 gives higher tumor : blood ratios (AUC) compared to IgG



Submandibular Lymph lesion (blue arrow)



AUC from Liver Lesions (red arrow)

Best-in-class Tumor to Blood Ratio in cancer patients

Antibody	Tumor : Serum AUC
Avibody (AVP-0458)	**3.33**
Typical Intact IgG (CS-1008	0.27
huA33 (Highest Intact IgG	1.28

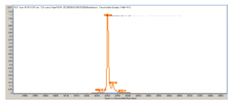
Benchmarking Data provided of Professor Andrew Scott from published RIT trials as Area Under Curve (AUC).

"...this data supports the use of ^{124}I-PEG-AVP04 (or PEG-avibody constructs) as highly suited to payload delivery of ADC drugs or radioisotopes, and as an accompanying theranostic imaging test."

- Prof. Andrew Scott Director of PET Centre and Dept Nuclear Medicine, Austin Hospital and formerly Director of antibody program at the Ludwig Institute (Melbourne) and a fellow at MSKCC

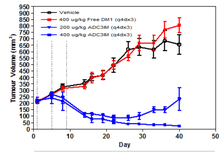
Avibodies as ADCs

AVP-04 as an ADC targeting Tag-72 xenografts

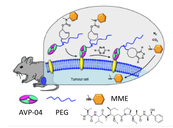


AVP-04-PEG-vc-DM1 (maytansine drug): Homogeneous DAR = 4 confirmed by mass spectroscopy

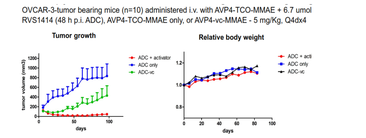
Evaluation in Capan-1 xenograft in female SCID mice demonstrated durable tumor regression:
- Animals with a mean tumour volume of 200 mm^3 were treated on days 1,5,9
- AVP04-PEG without cytotoxic payload (vehicle) and free drug (DM1) had no effect on tumour growth (**black**/**red**)
- AVP04-PEG-Drug resulted in the largest reduction in tumour volume (**blue**)



Two-Step Strategy: Release of Effective payloads within the xenograft tumor



OVCAR-3-tumor bearing mice (n=10) administered i.v. with AVP4-TCO-MMAE + 6.7 umol RVS1414 (48 h p.i. ADC), AVP4-TCO-MMAE only, or AVP4-vc-MMAE - 5 mg/Kg, Q4dx4

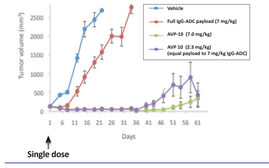


TagWorks NV: a 2-step (pretargeting) approach:
- Step 1) ADC uptake into tumor with rapid blood clearance of the unbound fraction.
- Step 2) systemic administration of an activator that cleaves the ADC linker, leading to MMAE-drug release into surrounding tumor and stromal cells
- Avibodies are retained on tumors for over a week but cleared from the circulation after 2 days – gives optimal PK/PD since the tco-serum deactivation half-life is ~5.5 days
- Pre-targeting relies on high tumor load and rapid systemic clearance
- AVP04 was conjugated with TCO linker and MMAE: (AVP04-TCO-MMAE, "ADC")
- MMAE drug was released with a tetrazine "activator" administered systemically. - precisely within the tumor
- Excellent OVCAR tumor regression was observed, more efficiently than AVP04-vc-MMAE (ADC-vc) - a conventional protease-releasable drug format.

Ref: Rossin *et al.* (2018) *Nature Communications* **9**:1484-1489.

AVP-10: an ADC targeting CD30

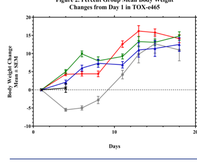
Anti-tumor efficacy of AVP10-MMAF on Karpas-299 (CD30$^+$) xenografts



AVP-10:
- Released active metabolite has no bystander killing
- Superior efficacy to a full length IgG-ADC at an equivalent dose of linked payload (a dose having no body weight loss)
- Improved therapeutic window a function of:
  > Better distribution into tumor and within tumor mass
  > Persistent binding within tumor
  > Rapid peripheral clearance

AVP10 maintained efficacy while avoiding severe life threatening toxicities

Figure 2. Percent Group Mean Body Weight Changes from Day 1 in TOX-cm65



Ref: Charles River Labs on behalf of Avipep

AVP-10:
- Confirmed 2.3 mg/kg (186 nmol/kg MMAF) safety
- No body weight loss
- Dosing Q2dx3; total dose = 6.9 mg/kg
- Well tolerated
- Mice exhibited transient and reversible thrombocytopenia and elevations of liver transaminases (AST and ALT)

Pharmacokinetics and biodistribution of labeled AVP-10 in tumor-bearing SCID mice