Progressing Engineered Avibody™ protein; ‘PEG-AVP0458’ for Prostate & Ovarian Cancer from the Lab to Clinic

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(Preclinical Manager, Victorian Cancer Biologics Consortium)
Avipep:
- In vivo optimised Avibody Products
- Clinical payloading capability

LICR:
- Ludwig Institute for Cancer Research.
- In vivo preclinical validation of Leads.

PMCC:
- Peter MacCallum Cancer Centre.
- In vivo preclinical validation of Leads.

The VCB leverages a $2m VIC govt. VSA grant

Avipep’s Avibody™ Products

Generation and validation of in vivo optimised Avibody Products for human clinical imaging and Therapy.

GMP Manufacturing:
- Regulatory compliant manufacturing of clinical Lead.

RDDT (VivoPharm):
- Regulatory compliant toxicity assessment.

CTA:
- Cancer Trials Australia.
- Victorian Clinical Trial Sites, incl; Peter MacCallum Cancer Centre, Austin Hospital, Mercy Hospital for Women, Royal Melbourne Hospital.
Avipep’s technology enables a range of formats and payload options. The lead Avibody™ product is a diabody conjugated to a cytotoxic drug for cancer therapy (Antibody-Drug Conjugate: ADC).

Key Competitive Advantages:
- Multivalent binding of antigen
- High tumour localisation
- Bacterial Production
- Specific Conjugation of Payload
- Customisable half life extension

Avibody™ Products

Avibody™ Technology

Addition of Payload

Therapy

Cytotoxic Drug

Radioisotope

Contrast Agent

Imaging
Novel IP for Site-specific conjugation

Exemplified pairs of Cysteine substitutions in framework regions able to form disulphide bond.
• Precisely 4 payload molecules loaded per AviBody—highly efficient!
  – is a defined conjugate product; less complicated biology/Pk etc
  – CMC/Regulatory benefits: reproducible batch-to-batch & analysis

• The conjugated AviBody remains fully functional:
  – in vitro binding affinity & in vivo Pk/stability unaffected

• Genentech has also published an improvement to their Herceptin-DM1 ADC using single thiols for conjugation and shown this to be more effective than Herceptin-DM1 with an improved therapeutic index*
AVP0450: Optimising *in vivo* Biodistribution Gives High Tumour : Blood Ratios

*Discrete PEGs to Engineered Surface Thiols MAINTAINS fine control*

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**No PEGylation**

- ‘Naked’ Diabody beneath renal clearance threshold - kidney accumulation

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**Non Site-Specific PEGylation**

- Random PEGylation improves average drug retention & tumour localisation

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**Site-specific PEGylation**

- Specific conjugation allows tailoring of Pk & resulting tumour localisation to >70% ID/g!

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Lead Avibody™ Product: Clinical Development Program for AVP04

**Phase I Biodistribution trial (Q2 2012)**
- Patient population: ovarian and prostate cancer
- Tracer: $^{124}$I
- Imaging: PET/CT
- IP: PEG-AVP0458 (no drug load)
- 1y Objective: Safety
- 2y Objectives: Biodistribution (Pk, immunogenicity)

**Preclinical Development - AviBody™ Therapeutic**
- Selection of PEG-Drug linker
- in vitro Cell Cytotoxicity
- Conjugate Formulation & stability
- Xenograft Tumour Reduction Efficacy

**Biodistribution profile (validation of AviBody Platform)**

**Phase I Therapeutic Trial**
- Ovarian cancer
- 4-5 escalating doses
- PEG-AVP04-Drug
- Primary objective: safety
- Secondary objective: efficacy

**Preclinical and toxicity studies of PEG-AVP04-Drug**
**Overview**

**Upstream Processing**
- 10L Fermentation of working culture
- Harvest & Isolation of Inclusion Bodies

**Downstream Processing**
- Dissolution of IBs & refolding
- Chromatographic Purification

**Further Processing**
- Conjugation with Maleimide-PEG\(_{24}\)
- Chromatographic Purification

**Fill/Finish**
- Sterile filtration
- Dispensing into 1ml Vials

**Radiolabelling**
- Iodination with 124-I
- Preparation for Clinical dispensing

\[^{124}\text{I}]\text{PEG-AVP0458}
Feasibility
  • Workup: shake flask, 2L, 10L fermenters
  • Initial PoP refolding & conjugation

Upstream Processing
  • 10L Fermentation of working culture
  • Harvest & Isolation of Inclusion Bodies

Downstream Processing
  • Dissolution of IBs & refolding
  • Chromatographic Purification

Further Processing
  • Conjugation with Maleimide-PEG_{24}
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Fill/Finish
  • Sterile filtration
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Radiolabelling
  • Iodination with 124-I
  • Preparation for Clinical dispensing

...in more Detail!

• Expression Optimisation
  • Generation of Master Cell Bank
  • Characterisation of Master Cell Bank

AVP0458 ‘Intermediate’

• Process Optimisation
  • Analytical Suite development & Validation
    – AVP0458 (intermediate)
  • Development of reference standards (AVP0458)

PEG-AVP0458

• Conjugation Optimisation
  • Analytical Suite development & Validation
    – PEG-AVP0458
  • Development of reference standards (PEG-AVP0458)

PEG-AVP0458 Vialled Product

• Test Fill & analysis (HOSPIRA)
  • Sterility Validation
  • Microbiological Test Validation
  • Product Stability (HOSPIRA)

[^{124}I] PEG-AVP0458

• Iodination PoP & development (Xenograft imaging)
  • Radio-Analytical suite development & validation
  • Radiolabelling Process Validation
  • Sterility Validation
  • Clinical Assay Development (RDDT)
An improved generation of antibody alternatives

Upstream

- 10 L Fermentation of *E. coli* MCB
- IPTG inducible, fed-batch
- High cell densities
- 5 full-scale production runs (3 under GMP conditions) demonstrates highly reproducible production of IBs
An improved generation of antibody alternatives

**Downstream Processing**

1. **Master Cell Bank**
2. **E. coli Fermentation & Isolation of Inclusion Bodies**
3. **Refolding of Inclusion Bodies**
4. **Purification by Protein Chromatography (SP-BB, QFF, SP-HP)**
5. **AVP04-58 Intermediate**
   - PEGylation
   - Purification by Protein Chromatography (SP-HP & TFF concentration)
6. **PEG-AVP0458 Drug Substance**
   - Sterile Filtration & Vialling
   - PEG-AVP0458 Vialled Drug Product
   - Iodination
   - [124-I]-PEG-AVP0458 -Clinical I.P.
7. **Drug Substance**
   - Cation Exchange Capture
8. **Drug Product**
   - Tangential Flow Filtration
9. **Clinical I.P.**
   - Anion Exchange Chromatography
   - Cation Exchange Chromatography
   - Purified antibody for further processing
Further Processing - PEGylation

- Reduction of conjugation cysteines with TCEP.
- Highly efficient reaction of thiol with maleimide-PEG$_{24}$-MeO
- ‘One-pot’ reaction – conjoined reduction & conjugation step: compatible with scale-up
- Capture & Polishing of PEG-AVP0458 by cation exchange
- Concentration & buffer exchange by TFF

Figure 1: Comparison of PEG-AVP04-58 with AVP04-58. Unreacted AVP04-58 and PEG-AVP04-58 generated using Avipep’s PEGylation protocol were analysed by SDS-PAGE (LP002), SEC (LP024) and WCX (LP045).
Fill/Finish

- Sterile filtration & aliquot as 1 mL vials…x~150-200 units only!
- Bulk material syringe-filtered by hand directly into FlexBoy® bag.
- Validated process feasibility, suitability & product biocompatibility
- Sterility assurance validated by multiple media fill runs.
  - Likely (and unlikely!) possible interventions practiced to demonstrate robustness of sterility – no growth observed!
- Loses & minimal testing requirements are significant in small batch!
- Final CofA issued by Radpharm based on results from AMS (micro) Radpharm and HOSPIRA.
Iodine Radiolabelling - Xenograft biodistribution

\[
\frac{1}{2} \alpha = 6.77 \text{ hr} \\
\frac{1}{2} \beta = 29.72 \text{ hr} \\
\text{AUC} = 16.52 \{\text{hrs}\} \times \{\text{ug/ml}\}
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PEG-AVP0458 Vialled Drug Product

[124-I]-PEG-AVP0458 - Clinical I.P.

\[
\text{Blood, Brain, Heart, Lung, Spleen, Liver, Kidney, Colon, Muscle, Bone, Skin, Tail, Tumour}
\]

\[
\% \text{ID/g}
\]

LUDWIG INSTITUTE FOR CANCER RESEARCH

124I-AVP0458 Biodistribution - PET/CT

124I-AVP0458 Biodistribution - PET/CT
Clinical Study Design

Phase I, open-label study of the safety and biodistribution of two escalating doses of PEG-AVP0458, labelled with 3-5 mCi of $^{124}$I for PET imaging

- Relapsed or refractory Ov. Ca or metastatic Pr. Ca.
- TAG72-positive tumours (IHC of archived tumours)
- Detectable metastatic disease
- Single dose infusion of I.P. followed by PET/CT scans
- Follow up PET/CT scans on days 1, 2/3, 4/5, 6/7 after dosing
- Follow up visits up to study end on day 28
- Bloods taken for Pk & immunogenicity evaluation
Site-Specific Loaded AVP04-Drug Conjugates Inhibit Tumour Growth in Xenografts

Figure. Tumour regression study testing efficacy of AVP0450-PEG$_{24}$-DRUG (drug is a well-known ADC cytotoxic agent). Tumour cells were implanted into female Balb/C nu/nu mice and were randomised when mean tumour volume of 100mm$^3$ was reached. AVP0450-PEG$_{24}$-DRUG was administered once at 2mg/kg (day 1), while all other treatments were administered 1q3d x 2 (day 1 and 4). Conjugation of the DRUG payload to a Tumour-specific vehicle (AVP04-50; blue triangles) resulted in the largest reduction in tumour volume throughout the course of the study. As expected, AVP0450-PEG$_{24}$ without a cytotoxic payload had no effect on retarding tumour growth (red square).
Key Messages

• The Avibody™ product platform (multimeric antibody fragments) offers greater control of tumour targeting, clearance and pharmacokinetics resulting in significantly improved delivery of cytotoxic or imaging agents to tumours.

• Novel, specific-conjugation chemistry for attaching either imaging or cytotoxic drug payloads.

• Tumour uptake data (PET images, PK and biodistribution in murine xenograft models) demonstrate key advantages of the AviBody platform.

• Successful demonstration of a cost effective (bacterial) GMP production process from inclusion bodies.

• Successful completion of production pathway using multiple CMOs & partners!

• Clinical Trial initiated; first patient dosed without issues and recruitment ongoing.
Acknowledgements

• Peter Hudson (CSO)
• Anne Nathanielsz (Exec. Asst.)
• Maggie Oh (Clinical Project Mgr)
• Jeremy Wu & Ramya Janarthanaman (Development Team)
• Jenny Carmichael (Protein Modeller)

Wellcome Trust Translational Award

$ Vic. Govt: VSA Award

NCRIS Scheme

HOSPIRA (Adelaide)
Jane Slobedman (Prj.Mgr), Huang Teck-Lee, Ian Milne, Kes Ashworth, Anuja Kumaratilake, Sam Billingham & team

Radpharm Scientific
Majella Clifton, Paula Reynolds, Shane Bourne, Charles Ross

Ludwig Institute Cancer Research
Andrew Scott, Fiona Smyth, FT Lee & team

MedPace (CRO)
Michael Tonso, Steve Saloutis

McCloud Consulting Group (Database management)
Phil McCloud, Jessie Chan

RDDT (Vivopharm) (Clinical Assay Validation)
Peter Tapley, Dean Whelan, Tom Gilbert

Tissupath Pathology (Clinical IHC Antigen Screening)
John Pedersen, John Mills & team

Cancer Trials Australia (Ethics management)
Jenny Han

Global BioSolutions (Preclinical Advice)
Jim Ackland

City-of-Hope Natl Med Centre
Jack Shively, David Colcher, Lin Li

Fox Chase Cancer Centre
Greg Adams, Matt Robinson

Peter MacCallum Cancer Centre
Carleen Cullinane, Rod Hicks

AIBN
David Chin, Linda Lua & team

CSIRO
Olan Dolezal, Charlotte Williams, Greg Coia, Judy Scoble

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