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Poster ID: POS037

Pharmacokinetic and Biodistribution of Formatted Affimer® Biotherapeutics Targeting PD-L1

Estelle Adam, Emma Jenkins, Floriane Laurent, Jennifer Hillman, Maureen West, Emma Stanley, David Sanford*, Min Fang*, Agnieszka Kownacka#, Celine Mothes#, Alexandra Oudot$, Vincent Matthew, David Gilfoyle, Barry Jones*, William Bachovchin*, Amrik Basran, Cyril Berthet#

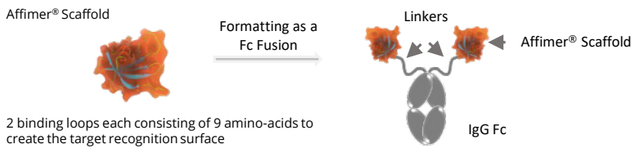
*Tufts University Boston USA, #Oncodesign Dijon France , $CGFL Dijon, France, Avacta Life Sciences, Cambridge, UK.

Introduction

Programmed Cell Death-Ligand 1 (PD-L1) is part of the immune checkpoint system involved in preventing autoimmunity. PD-L1 is upregulated on tumour cells and binds to its receptor, PD-1, expressed by immune cells in the tumour microenvironment. Anti-PD-L1 immunotherapies have shown to be effective treatments both as monotherapies and in combinations with chemotherapies and radiotherapies, providing long term responses in a subset of patients. We have developed a PD-L1 Affimer® antagonist (AVA04) that could differentiate itself from the current clinically approved mAbs due to its smaller size, improved tissue penetration and alternative routes of delivery such as a subcutaneous injection. It also opens the possibility of generating additional PD-L1 antagonist based therapeutics such as targeted radiopharmaceuticals, drug conjugates, bispecifics and cytokine fusions.

The Affimer® Platform Technology

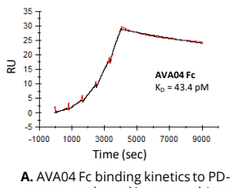
- The Affimer® biotherapeutic protein scaffold is based on human Stefin A, an intracellular protease inhibitor
- Two 9 amino acid surface peptide loops are engineered into the scaffold backbone
- **Phage display compatible:** Large Affimer® phage libraries (1x10^{11}) generated
- **Small size:** 14 kDa, 1/10th the size of an antibody,
- **High expression:** >100 mg/L in flasks (*E. coli*) as a monomer and > 500 mg/L in shake flasks (from transient mammalian cells)
- **No post translational modifications:** ease of manufacturing and improved stability
- **Tissue penetration:** smaller size gives greater potential for tissue penetration and increased efficacy
- **Ease of formatting:** Fc format and in-line fusions, potential to generate multi-specific drugs to blockade multiple disease pathways



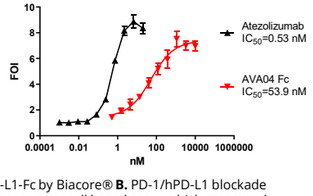
Affimer protein formatting for half-life extension

AVA04 Fc Anti-PD-L1 Potency

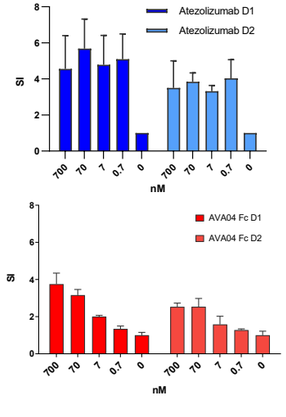
A. Biacore Binding Kinetics



B. Blockade Cell Based Assay



C. T-Cell Exhaustion assay

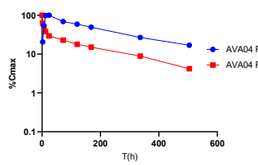


A. AVA04 Fc binding kinetics to PD-L1-Fc by Biacore® **B.** PD-1/hPD-L1 blockade potency evaluated in a recombinant reporter cell based assay which measured TCR -cell signalling through NFAT-mediated luciferase activity (Promega). **C.** T cell exhaustion assay using human PBMCs (2 donors) were incubated for 96h with Staphylococcus enterotoxin B (SEB) with a dilution range of AVA04 Fc or Atezolizumab in two separate experiments (D1,D2). The supernatant was collected and IL-2 measured by HTRF. Atezolizumab (clinical grade) and SEB (data not shown) were used as the positive controls and a non-binding Affimer protein control (data not shown) used as a negative control. Significant IL-2 production was measured with PD-L1 blockade using both AVA04 Fc and Atezolizumab.

oncodesign Vector of innovation

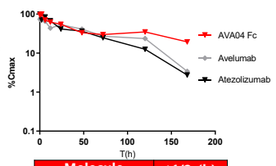
Pharmacokinetics

A. Mouse Single dose PK IV in C57Bl/6



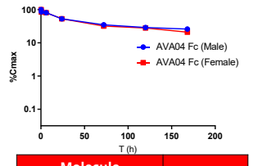
Molecule	$t^{1/2}$ (h)
AVA04 Fc SC	184.2
AVA04 Fc IV	172.6

B. FcRn/HSA double KI Mouse Single dose PK IV



Molecule	t1/2 (h)
AVA04 Fc	128.6
Atezolizumab	37
Avelumab	39

C. Cynomolgus Single dose PK IV



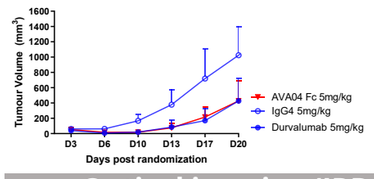
Molecule (Cynomolgus gender)	T1/2 (h)
AVA04 Fc (Male)	239.8
AVA04 Fc (Female)	163.3

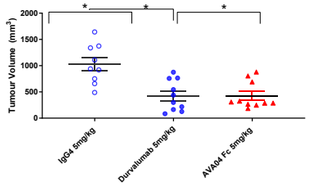
A. C57Bl/ 6 Mice were injected with AVA04 Fc or Avelumab at 10 mg/kg via IV or SC route. **B.** FcRn / HSA double KI mice (Genoway) were injected with AVA04 Fc or control antibodies at 5 mg/kg **C.** Cynomolgus Monkey were injected with 5mg/kg of AVA04 Fc. In all cases, in life sampling were taken from saphenous vein. Serum samples were analysed by ELISA. Results are expressed as % of the Cmax (Maximum concentration observed).

Human Melanoma Xenograft Mouse Model

NCG mice were randomized based on their body weight. A375 cells and activated T cells were inoculated subcutaneously in NCG mice and treatment was initiated 1h post the cell inoculation. In summary, the test article Durvalumab demonstrated significant anti-tumor efficacy compared to Isotype control group in A375 Xenograft model. At D20 the tumour size were compared and AVA04 Fc at 10mg/kg showed similar effect to Durvalumab. (*Dunn's test,p<0.05)

Xenograft Model purified CD3+

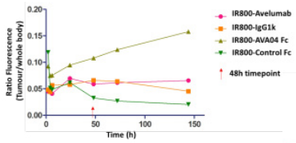




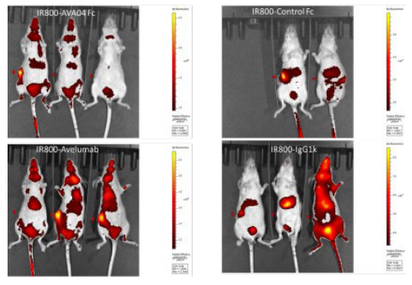
Optical imaging (IRDye 800CW) Biodistribution

AVA04 Fc and antibody control were labelled with a fluorescent IRDye-800. Conjugation dye/protein ratios ranged from 1 to 3.1. A375 tumours were subcutaneously induced in Swiss nude mice. Animals were randomized into four groups of three animals when individual tumor volumes ranged from 400 mm³ to 600 mm³. Conjugates were given via IV route and the animals then imaged. Imaging involved detection of light emitted from the animals with a cryogenically cooled CCD camera. **A.**The ratio Tumour / whole body fluorescence over time. **B.** Images shown are 48h timepoint. Tumours on the right flank of the animal indicated with a red arrow.

A. Tumour/whole body fluorescence over time

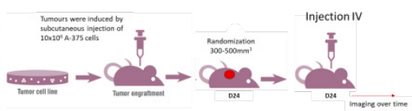


B. Whole body fluorescence image at 48h

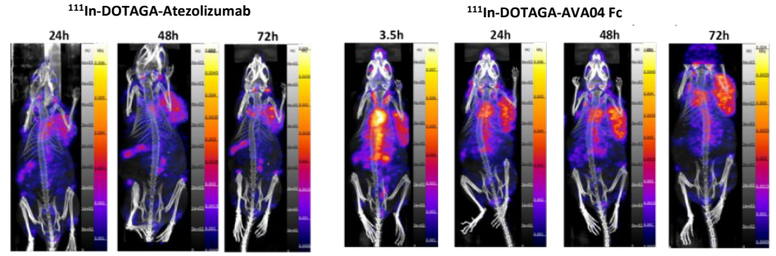


SPECT imaging Biodistribution

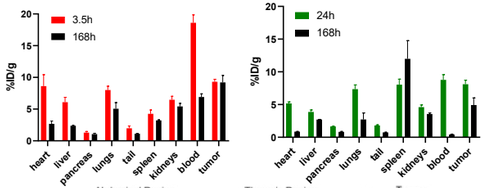
AVA04 Fc and antibody control were conjugated with DOTAGA then radiolabeled with indium-111. The specific activity ranged from 30 to 40 MBq/nmol with a radiochemical purity above 96% for both molecules. A375 tumours were subcutaneously induced in Swiss nude mice. Animals were randomized into two groups of seven animals on D24, when mean tumor volumes were approximately 950 mm³ and individual tumor volumes ranged from 477 mm³ to 1412 mm³. Products were then injected via IV route.



A. Representative SPECT images of Swiss nude mice bearing subcutaneous A375 tumours. Three animals treated with ~10 MBq doses were imaged at each time point (^{111}In-DOTAGA-AVA04 Fc : 3.5, 24, 48, 72h; ^{111}In-DOTAGA-Atezolizumab : 24, 48, 72h). ^{111}In-DOTAGA-AVA04 Fc showed accumulation at 3.5h to 168h in the tumour with the highest %ID/g of 9.3%, and lower quantities in the blood, kidneys and lungs (6.91, 5.42, 5.05%, respectively). In ^{111}In-DOTAGA-Atezolizumab, one week post treatment, the %ID/g increased in the spleen compared to 3.5 h post treatment but decreased in the tumor (4.92%).

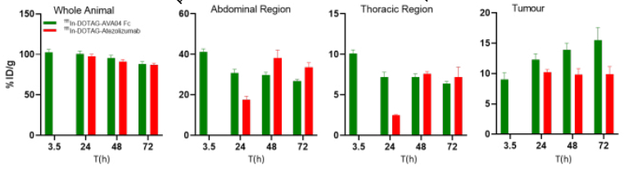


B. Biodistribution analysis showing the summary of the percentage of injected dose per gram (%ID/g) of Swiss nude mice bearing subcutaneous A375 tumours. Three animals were euthanized at two time point 3.5 and 168 h for ^{111}In-DOTAGA-AVA04 Fc (A) and 24 and 168h for ^{111}In-DOTAGA-Atezolizumab (B).



C. SPECT imaging analysis showing the injected dose (%ID) by region of Swiss nude mice bearing subcutaneous A375 tumours.

Three animals treated with ~10 MBq doses were imaged at each time point (^{111}In-DOTAGA-AVA04 Fc : 3.5, 24, 48, 72 and 168h; ^{111}In-DOTAGA-Atezolizumab-: 24, 48, 72 and 168h). Mean and ±SD are indicted on the graphs. Note that the scales are different for each graph.



Conclusions

AVA04 Fc was shown to have a binding affinity of 43 pM to human PD-L1, blockade the PD-L1/PD-1 axis, significantly increase IL-2 production in a T cell exhaustion assay and to have a significant anti-tumour growth effect in a mouse A375 human melanoma xenograft model.

Pharmacokinetic in various mouse models demonstrated AVA04 Fc has a half-life ranging from 128-184h in single dose via IV or SC route. In Cynomolgus monkey the half-life was evaluated between ranging from 163-240h in single bolus injection

Biodistribution in A375 melanoma xenograft model were performed. AVA04 Fc was successfully labelled with IRDye800 or radiolabeled with indium 111 and shown to be still functional. AVA04 Fc-IRDye 800 accumulated in the tumour at 48h. SPECT image analysis showed also good accumulation of AVA04 Fc in the tumour compared to the control antibody and shows great potential as both an *in vivo* imaging agent or a targeted radiophamaceutical