Preclinical evaluation of anti-VISTA antibody CI-8993 in a syngeneic huVISTA-KI model

Wichmann CW[1,2*], Burvenich IJG[1,2*], McDonald AF[1,2], Scott FE[1,2], Guo N[1,2], Rigopoulos A[1,2], Soikes R[3], Angelides S[3], von Roemeling R[3], Scott AM[1,2,4,5].

[1]Tumour Targeting Laboratory, Olivia Newton-John Cancer Research Institute, Melbourne, VIC, Australia
[2]School of Cancer Medicine, La Trobe University, Melbourne, VIC, Australia
[3]Curis Inc, Lexington, MA, USA
[4]Department of Medicine, University of Melbourne, Melbourne, VIC, Australia
[5]Department of Molecular Imaging and Therapy, Austin Health, Melbourne, VIC, Australia
*Co-first authors

1. VISTA Targeting

VISTA [**V**-domain **i**mmunoglobulin (Ig) **s**uppressor of **T** cell **a**ctivation] is a type 1 transmembrane protein and member of the PD-1/PD-L1 family of the Ig superfamily. VISTA is expressed in the hematopoietic compartment including on circulating and intratumoral myeloid cells. Within the T lymphocyte compartment, VISTA is most highly expressed on naïve CD4+ and Foxp3+ regulatory T cells [1]. Moreover, tumor types, particularly lung cancer, show VISTA expression primarily in the stromal myeloid infiltrate [2-4].

VISTA acts as a co-inhibitory receptor of resting CD4+ T cells that negatively regulate T cell activation in vitro; and therefore, plays a critical role in enforcing quiescence (inactivity or dormancy) and inhibits anti-tumor immune responses [1].

The Investigational Product CI-8993 is a fully human IgG1κ monoclonal antibody that binds specifically to this immune checkpoint molecule. Phase I safety has been investigated in a prior trials in patients with advanced cancer (NCT02671955) and continues to be explored in Curis' NCT04475523 study. To assist determining the pharmacokinetics and biodistribution of CI-8993 in patients we aimed to develop Zirconium-89 (^{89}Zr)-labeled CI-8993 for PET imaging and quantitation, and validate in preclinical models prior to a planned human trial.

2. Labeling of CI-8993 with Zirconium-89

Conjugation conditions of CI-8993 to the metal ion chelator desferrioxamine B (Df-) were established by optimisation of Df:mAb ratio, reaction temperature, time, and purification method. Conjugates were assessed by iTLC, SE-HPLC, SDS-PAGE, and ELISA. Radiolabeling was performed with ^{89}Zr and the radioconjugate was tested for specific activity, radiochemical purity, antibody integrity and binding to huVISTA.

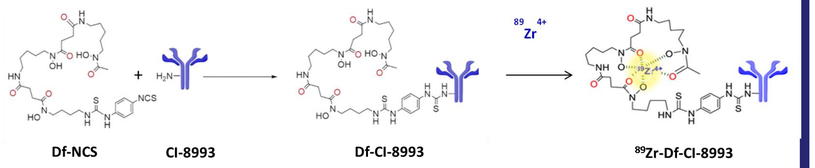
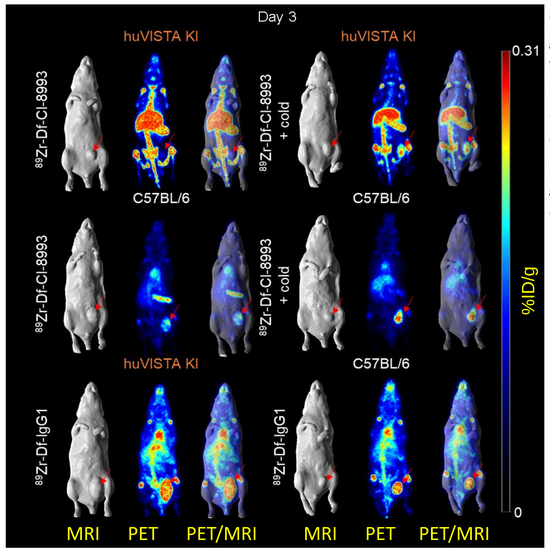


Figure 1. Schematic of radiolabeling reactions.

3. *In Vivo* Biodistribution and Imaging

The *in vivo* biodistribution and properties of ^{89}Zr-Df-CI-8993 and IgG1 isotype control radioconjugates were assessed in female huVISTA knock-in (C57BL/6N-Vsir$^{tm1.1(VSIR)Geno}$) or control C57BL/6 mice bearing syngeneic MB49 bladder cancer tumors. These tumor cells express the male HY antigen, which is a foreign antigen when these tumor cells are implanted into female mice. Under these conditions MB49 is an aggressive, highly immunogenic tumor.

On day 0, the day of radioconjugate synthesis, mice with established MB49 tumors received intravenous injections of ^{89}Zr-Df-CI-8993 alone (1mg/kg, 4.6MBq), or in combination with 30mg/kg CI-8993. Controls received ^{89}Zr-Df-IgG1 (1mg/kg, 4.6MBq). For each group, 3 mice were imaged with positron emission tomography (PET) and magnetic resonance (MR) on day 0, 1, and 3 using a dedicated small animal nanoPET/MR camera (nanoScan®, Mediso). For ^{89}Zr-Df-CI-8993 groups of 3-4 mice were culled on day 1 & day 3 and tissues, tumor and blood were collected for biodistribution assessments. ^{89}Zr-Df-IgG1 biodistribution was assessed on day 3 only.



Collected tissues were counted in a dual-channel gamma scintillation counter with standards of the injected material. The tissue distribution data were calculated as the mean ± SD percent injected dose per gram tissue (%ID/g) for each construct per time point.

Figure 2. From left to right, each panel shows representative whole body MR image (MRI, surface rendered), maximum intensity projection PET image, and fused PET/MRI images of MB49 tumor bearing huVISTA knock-in mice or control C57BL/6 mice on day 3 post injection of 1 mg/kg ^{89}Zr-Df-CI-8993 alone or with 30mg/kg CI-8993, or ^{89}Zr-Df-IgG1 control. The red arrows indicate tumor.

4. Quality Control

Conjugation of Df-NCS to CI-8993 for 60 minutes at room temperature followed by purification via gel filtration resulted in stable constructs with an average chelator-to-antibody ratio of 1.81. SDS-PAGE showed integrity of CI-8993 was maintained after conjugation, iTLC determined radiochemical purity, SE-HPLC demonstrated radiochemical purity and radiolabeled antibody integrity. ELISA indicated no impact of conjugation or ^{89}Zr-radiolabeling on binding to human VISTA.

QC Parameter	Result
Specific activity [MBq/mg]	182-220
Radiochemical purity **by iTLC** (RCP) [%]	> 99
Antibody integrity by **SE-HPLC** [%]	Main peak: 92.6 Aggregate: 7.3
Stability in human serum after 7 days @ 37°C [%]	RCP = 88

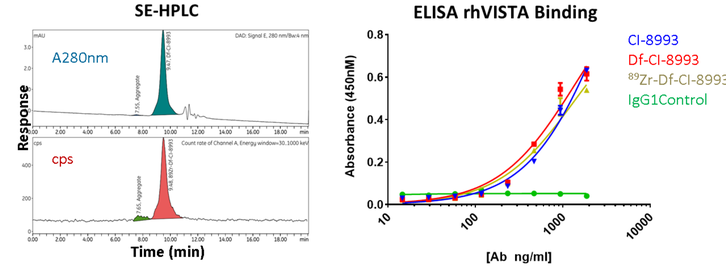


Figure 3. Left: Size exclusion (SE) HPLC A280 and counts per second (CPS) scintillation trace of ^{89}Zr-Df-CI-8993. Right: ELISA analysis binding recombinant human VISTA (R&D Systems).

5. Quantitative Biodistribution

PET imaging and biodistribution in MB49 tumor-bearing huVISTA knock-in female mice showed specific localisation of ^{89}Zr-Df-CI-8993 to VISTA expressing organs (liver: 14.98 ± 0.50 %ID/g; spleen: 292.00 ± 14.51 %ID/g; n = 3) compared to ^{89}Zr-Df-IgG1 control (liver: 4.615 ± 0.15 %ID/g; spleen: 6.37 ± 0.22 %ID/g; n = 4) or in the presence of competing unlabeled CI-8993 (liver: 8.14 ± 0.50 %ID/g; spleen: 41.14 ± 3.00 %ID/g; n = 5). Tumor-to-blood ratios indicated specific tumor targeting of ^{89}Zr-Df-CI-8993 in the presence of unlabeled CI-8993 (20.47 ± 3.09) compared to trace dose ^{89}Zr-Df-CI-8993 (0.97 ± 0.12; P = 0.0001) or ^{89}Zr-Df-IgG1 control (1.75 ± 0.11; P < 0.0001).

6. Conclusion

We have validated ^{89}Zr-Df-CI-8993 for specific binding to huVISTA *in-vivo*. A clinical trial of ^{89}Zr-Df-CI-8993 is planned in solid tumor patients.

Acknowledgements

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