DARPins as Powerful Targeting Agents for Radioligand Therapeutics

Poster 5037

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Introduction

The therapeutic window of radioligand therapeutics (RLTs) is often restricted by suboptimal tumor-to-organ ratios. Antibodies can have high affinity and specificity to tumor targets, but their long systemic half-life frequently results in haematological toxicities. Alternatively, low molecular weight ligands are restricted to a limited number of tumor targets and often exhibit insufficient tumor retention and limiting tissue selectivity. Therefore, alternative molecular platforms are urgently needed to exploit the potential of RLTs in a broader field of indications.

DARPins (Designed Ankyrin Repeat Proteins) are small binding proteins that combine short systemic half-life and ideal binding properties. Due to their rigid-body binding mode, DARPins with very high affinity and specificity can be generated against a broad range of tumor targets, and several DARPin-based products are currently investigated in clinical trials. The simple and robust architecture of DARPins further provides high stability, which is beneficial for labelling with radionuclides under harsh conditions, and which enables engineering approaches that are not compatible with other protein scaffolds. A specific surface engineering approach of the constant backbone resulted in a strongly reduced kidney accumulation of optimized DARPins, thereby addressing a general problem of protein-based delivery vectors below 60 kDa in size, which are cleared via the renal pathway.

Affinity-Driven Tumor Uptake of DARPins

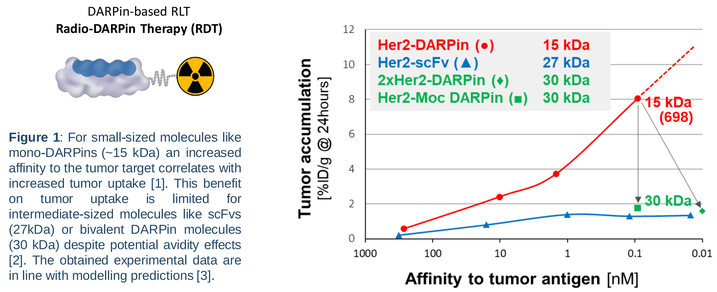


Figure 1: For small-sized molecules like mono-DARPins (~15 kDa) an increased affinity to the tumor target correlates with increased tumor uptake [1]. This benefit on tumor uptake is limited for intermediate-sized molecules like scFvs (27kDa) or bivalent DARPin molecules (30 kDa) despite potential avidity effects [2]. The obtained experimental data are in line with modelling predictions [3].

Ex-vivo Analysis of Tumor Penetration Affinity and Size Matter

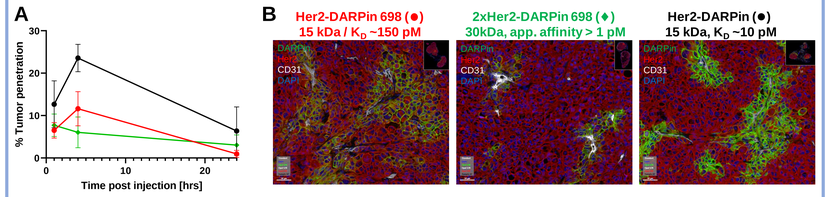
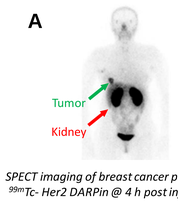
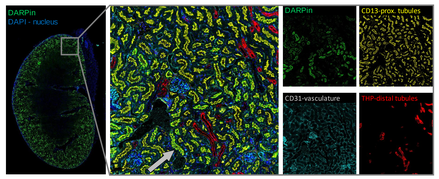


Figure 2: Ex-vivo analysis of DARPin localization in the tumor. SKOV3-tumor bearing mice were administered with different Her2 DARPins: DARPin 698 with K_D ~150pM (identical to DARPin with highest affinity used in Fig.1), the bi-valent version of the same DARPin and a non-related Her2 binding DARPin with even higher affinity of K_D ~10pM), and tumors were subjected to multiplex IF staining for DARPin, Her2 and CD31. **(A)** DARPin tumor penetration of the different DARPin formats 1, 4 and 24 hours after administration, shown as % of DARPin staining area in total tumor area. **(B)** Representative mIF images of tumors treated with the indicated DARPin 4 hours after administration.

Kidney Uptake as a Key Problem of Polypeptide-Based Delivery Vectors



SPECT imaging of breast cancer patient with ^{99m}Tc- Her2 DARPin @ 4 h post injection [4]



Her2 DARPin staining of mouse kidney section by IHC @ 30 min post injection

Figure 3: As any protein-based delivery vectors below the renal filtration cut-off (~60 kDa), DARPins are reabsorbed in the kidney leading to high accumulation of attached residualizing radionuclides (**A**). For this class of delivery vector, classical nephroprotectants such as amino acid cocktails have limited effect [5]. The renal reabsorption of such protein-based delivery vector occurs at the brush border (BB) of proximal tubular cells (green and yellow co-staining example indicated by ⇗)(**B**)

Surface Engineering to Reduce Kidney Accumulation

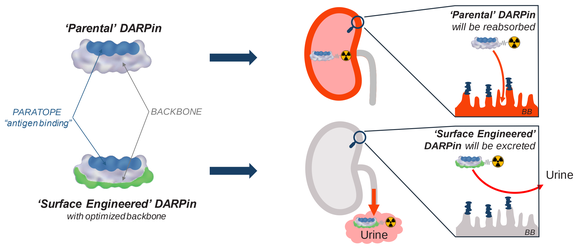


Figure 2: Surface optimization of the DARPin backbone to increase radionuclide excretion over reabsorption in the kidney, enabled by the robust architecture of DARPin scaffold. BB; brush border of proximal tubular cells

Surface Engineered DARPins Show Strongly Reduced Kidney Accumulation

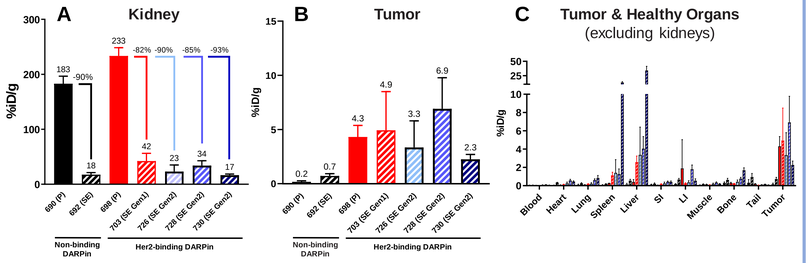


Figure 5: Biodistribution of DARPins labelled with 111-Indium in SKOV3 breast cancer mouse model, 4 hours after injection. Surface engineering (SE) resulted in reduction of kidney accumulation by up to 90% compared to parental DARPins (**A**) but had no impact on tumor uptake for the Her2-binding DARPin (**B**), and no significant effect on accumulation in healthy organs was observed most DARPins (**C**). As a result, the tumor to kidney ratio of 1:35 the 'Parental' Her2 DARPin 698 (P) was reduced to 1:8.5 for the 'Surface-Engineered' DARPin 703 (SE Gen 1), and to 1:5 for the best SE Gen2 DARPins 728.

Kidney Accumulation is Further Reduced by Orthogonal Approaches

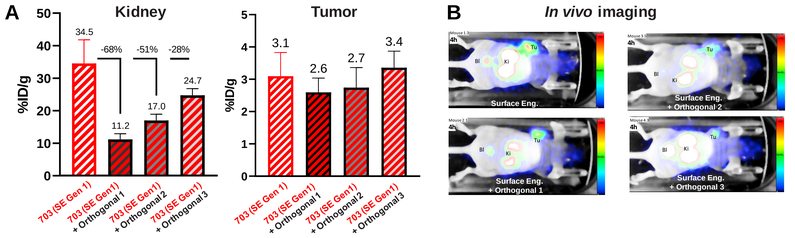


Figure 6: The combination of surface engineering with another orthogonal approach for increased radionuclide excretion resulted in a further reduction of kidney accumulation by up to 68% without affecting tumor uptake, as shown in biodistribution of Her2-binding DARPin Gen1-SE DARPin labelled with 111-Indium in SKOV3 breast cancer mouse model, 4 hours after injection **(A)**. The biodistribution data have been confirmed by imaging analysis at 4hour timepoint **(B).** As a result, the tumor to kidney ratio of 1:11 for the Her2 DARPin 703 (SE Gen 1) was reduced to 1:4 for the best approach (703 (SE Gen 1) + Orthogonal 1), same experimental setup as in Figure 5.

Reduced Kidney Uptake in Biodistribution Time Course

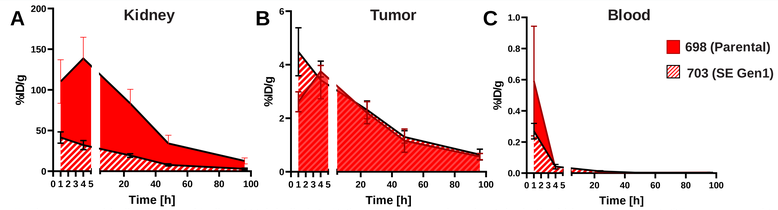


Figure 7: Time course biodistribution of Indium-111 labelled DARPins shows the reduced uptake in the kidney of the Gen1-SE as compared to the parental **(A)** without impacting the tumor uptake **(B)** and the distribution of DARPins in the blood **(C)**. As a result, the AUC in the kidney for the 'Surface-Engineered' 703 (SE Gen 1) is 76% reduced as compared to the 'Parental' 698. The AUCs in the tumor remains comparable for the two DARPins.

Summary and conclusions

- Affinity and DARPin size have an impact on tumor penetration.
- Surface engineering is a promising strategy to strongly reduce the kidney accumulation of DARPins without affecting tumor uptake.
- The combination with other orthogonal strategies results in a further reduction of kidney accumulation.
- Our proprietary "Radio DARPin Therapy" (RDT) platform represents an attractive solution for the development of next-generation targeted radio therapeutics.
- Several DARPin programs in indications with high unmet medical need are currently underway at Molecular Partners AG (DLL3 as the first disclosed target).

1. Bragina et al., J Nucl Med, 2022
2. Altai et al., EJNMMI Research, 2020
3. Zahnd et al., Cancer Res, 2010
4. Adams et al., Cancer Res, 2001
5. Schmidt & Wittrup, Mol Cancer Ther, 2009

Acknowledgment:
Zoya Ziauddin Siddiqui[1], Mirela Matzner[1], Alienor Auge[1], Norbert Fic[1], Waleed Ali[1], Tanja Chiorazzo[2]