Development of ^{212}Pb-based Radio-DARPin therapy (RDT) for the treatment of mesothelin (MSLN)-positive solid tumors

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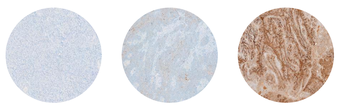
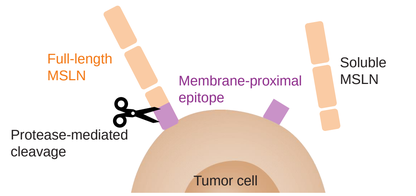
Introduction

MSLN is an attractive target for ovarian cancer

- MSLN is expressed in ovarian cancer with high prevalence (> 80%) and high level of intra-tumoral homogeneity[1]
- Expression is maintained in lymph node and peritoneal metastases[1]
- MSLN expression in healthy tissue is primarily restricted to mesothelial cells

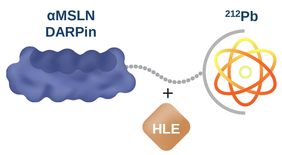


MSLN IHC staining pattern on normal ovarian tissue (left) and ovarian tumors: moderate (middle) and strong (right) expression.



- MSLN is a GPI-anchored surface protein
- MSLN is cleaved by multiple proteases in its membrane-proximal region
- Systemic soluble form (sMSLN) up to 160 ng/ml measured in ovarian cancer patients[2, 3]
- sMSLN can sequester anti-MSLN therapeutics and potentially diminish their efficacy

^{212}Pb x MSLN RDT



- Designed Ankyrin Repeat Protein (DARPin) that targets a membrane-proximal epitope of MSLN was selected to prevent interaction with sMSLN (HLE: half-life extending moiety)
- The radioactive payload is ^{212}Pb, an alpha particle-emitting radionuclide with a short decay half-life which allows high energy deposition on tumor in a short time frame

DARPins bind to MSLN with high affinity

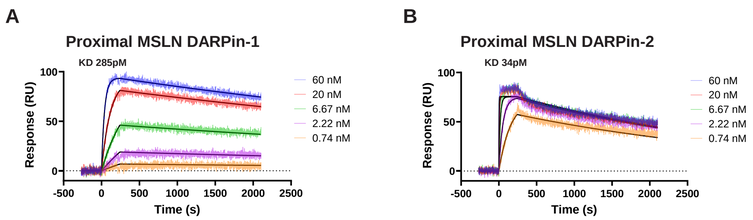


Figure 1: DARPin binding by Surface Plasmon Resonance (SPR)
DARPin binding to MSLN was assessed by SPR. A) SPR sensorgram of Proximal MSLN DARPin-1 x HLE (KD= 285 pM). B) SPR sensorgram of Proximal MSLN DARPin-2 x HLE (KD= 34 pM).

Proximal MSLN DARPins bind to membrane proximal domain of MSLN

DARPin epitope mapping by ELISA

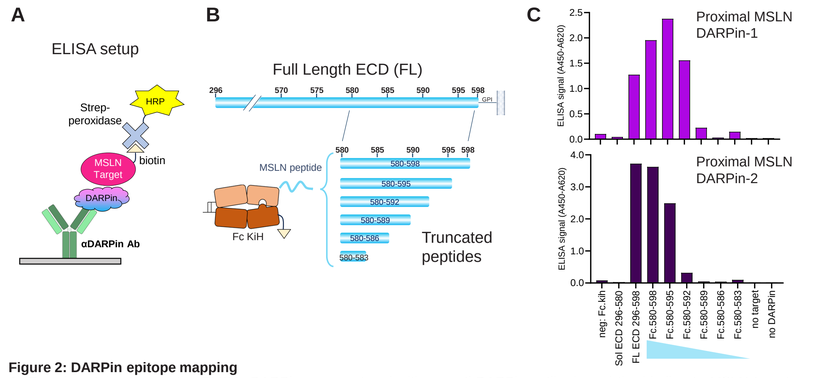


Figure 2: DARPin epitope mapping
A) Experimental set up of the ELISA. DARPins are captured with an anti-DARPin antibody and the binding to MSLN target material is tested. B) Schematic representation of the MSLN target material, consisting of the full length ectodomain (ECD) or Fc construct containing truncated MSLN peptides. C) MSLN epitope mapping data for two MSLN DARPins. Fc construct only, soluble ECD, no target and no DARPin were used as controls.

Cell binding of proximal MSLN DARPins is not affected by sMSLN

Cell binding of DARPins in presence of sMSLN

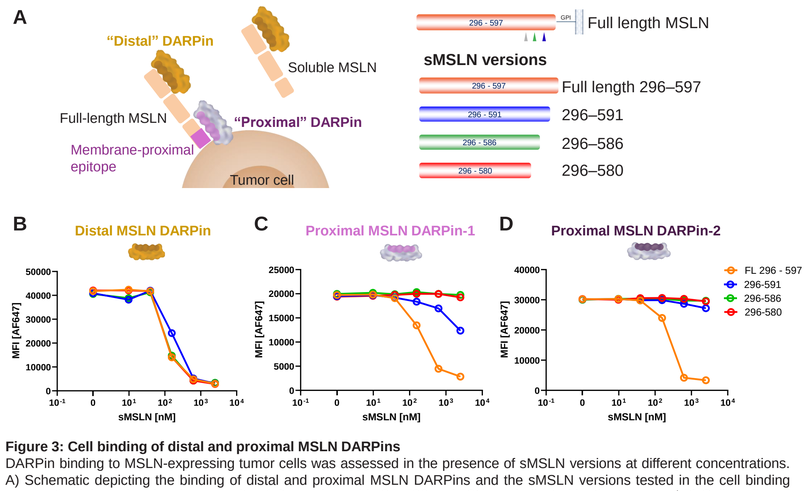


Figure 3: Cell binding of distal and proximal MSLN DARPins
DARPin binding to MSLN-expressing tumor cells was assessed in the presence of sMSLN versions at different concentrations. A) Schematic depicting the binding of distal and proximal MSLN DARPins and the sMSLN versions tested in the cell binding assay. Physiological major cleavage sites (after amino acid 584, 586 and 591) are indicated by the triangles.[4] B) Cell binding of a distal MSLN DARPin and two proximal MSLN DARPins C) and D) in presence of the indicated sMSLN versions. DARPins were detected with an anti-DARPin antibody, and the mean fluorescent intensity (MFI) values are indicated.

Favorable biodistribution of proximal MSLN DARPins

Biodistribution and tumor accumulation in hMSLN-MC38 model

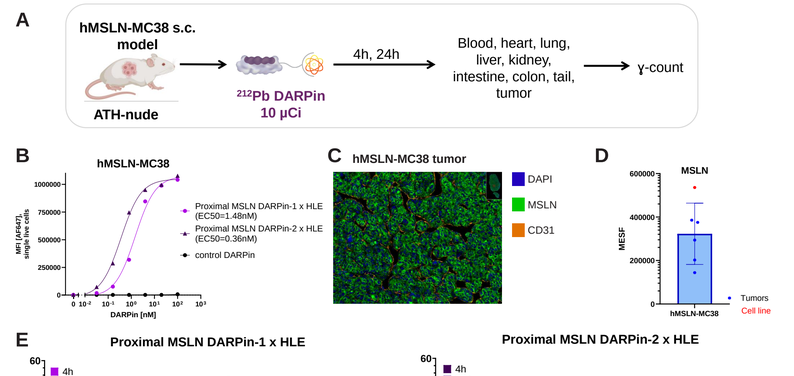


Figure 4: Biodistribution of proximal MSLN DARPins in hMSLN-MC38 tumor model
A) Study design in hMSLN-MC38 model. Tumor bearing mice were administered with ^{212}Pb-labelled DARPins (10 µCi, 0.01 mg/kg) and tissue was collected for analysis 4 and 24 h post injection. B) Cell binding assay of proximal MSLN DARPins and control DARPin on hMSLN-MC38 cells. EC50 values are shown. C) MSLN and CD31 immunofluorescence staining of a hMSLN-MC38 tumor. Nuclei were visualized by DAPI. D) Flow cytometry analysis of MSLN expression in hMSLN-MC38 tumors (blue) and cell line (red). MSLN expression is shown as Molecules of Equivalent Soluble Fluorochrome (MESF). E) Biodistribution of Proximal MSLN DARPin-1 x HLE (left) and Proximal MSLN DARPin-2 x HLE (right) in the corresponding tissue shown as %ID/g at 4 and 24 h (n=5 mice/time point).

Conclusion

- MSLN is a promising target for ovarian cancer but high levels of sMSLN could sequester biotherapeutics and thus reduce their efficacy
- We developed a ^{212}Pb-based RDT with high affinity to membrane-proximal MSLN
- Binding of proximal MSLN DARPins to tumor cells *in vitro* was not affected by sMSLN
- *In vivo* results show a favorable biodistribution with strong tumor accumulation of ^{212}Pb x MSLN RDT in hMSLN-MC38 tumor bearing mice and modest accumulation in other organs
- These initial promising preclinical data provide the basis for further development efforts currently ongoing

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For any questions, please contact: info@molecularpartners.com / attention of S. Wullschleger.

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