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DARPin-based Pretargeting: Zeroing in on Cancer

PNA-guided delivery of ^{177}Lu enhances tumor uptake and spares vital organs in HER2+ xenograft mice

Westerlund K[1]., Oroujeni M[2]., Van Deventer A[1]., Wang Z[2]., Clinton J[1]., Zelepukin I[2]., Papalanis E[2]., Liu Y[2]., Orlova A[2]., Tolmachev V[2]. and Eriksson Karlström A[1].

1. Department of Protein Science, KTH Royal Institute of Technology, Stockholm, Sweden
2. Department of Immunology, Uppsala University, Uppsala, Sweden

Presenting author: Kristina Westerlund, PhD
krw@kth.se

Pretargeting: A Two-Step Approach to Radiotherapy

Challenge:
Conventional targeted radiotherapy often results in unwanted radiation exposure to healthy tissues.

Our solution:
Two-Step DARPin-Based Pretargeting

Aim:
Improve tumor-targeting while mimizing off-target effects in mice with HER2-expressing xenografts

Key Benefits:
- Enhanced tumor specificity
- Reduced radiation to healthy tissues
- Potential for improved therapeutic window

Step1: Primary agent G3-HP1
- Non-radioactive DARPin-PNA conjugate
- Binds specifically to HER2-expressing tumors
- Carries HP1: a 15-mer PNA-based recognition sequence
- Is allowed to clear from bloodstream

Step 2: Secondary probe ^{177}Lu-HP2
- Radiolabeled with Lutetium-177 for therapy and imaging
- Administred after optimized interval
- Either:
  - Binds specifically to primary agent at tumor site via complementary PNA-sequence, or
- Rapidly clears through the kidneys

Two-Step DARPin-based PNA-guided Pretargeting

Results and Discussion

1. PRODUCTION OF PRETARGETING PROBES

Primary agent: Secondary probe:

I a) Expression in *E. coli*. b) IMAC purification

G3-H$_6$
anti-HER2 DARPin

II Automated solid-phase synthesis (SPS)

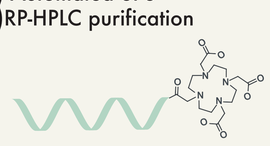
GGG

\+ HP1
15-mer PNA-probe

III a) Sortase A mediated ligation b) IMAC and RP-HPLC purification

G3-HP1 (22 kDa)
DARPin-PNA conjugate

a) Automated SPS b)RP-HPLC purification

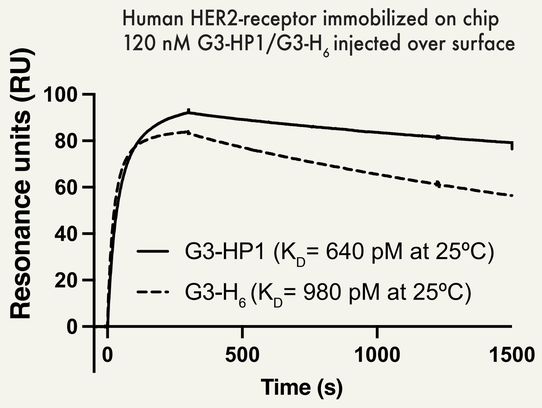


HP2 (5.2 kDa)
Complementary 15-mer PNA-probe, carrying a DOTA-chelator for radiometal-labeling

G3-HP1 and HP2 are both:
>95% pure
after final purification

2. AFFINITY DETERMINATION USING SURFACE PLASMON RESONANCE (SPR)

A



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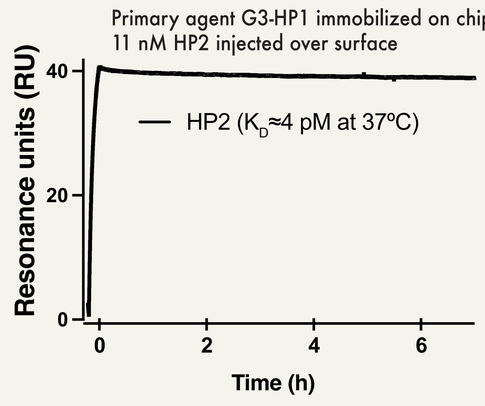
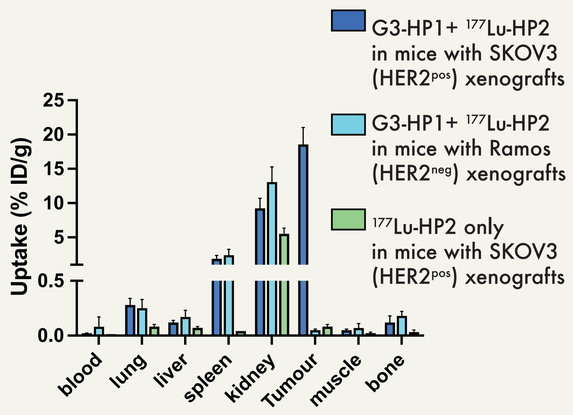


The DARPin-PNA primary agent, G3-HP1, is capable of high affinity binding in vitro to both:
- human HER2 receptor (K_D=640 pM), and
- secondary probe HP2 (K_D≈4 pM at 37°C)

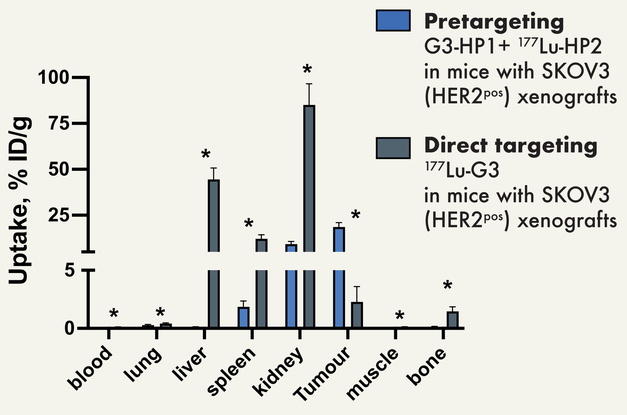
3. BIODISTRIBUTION STUDIES IN BALB/c-nu IMMUNODEFICIENT MICE

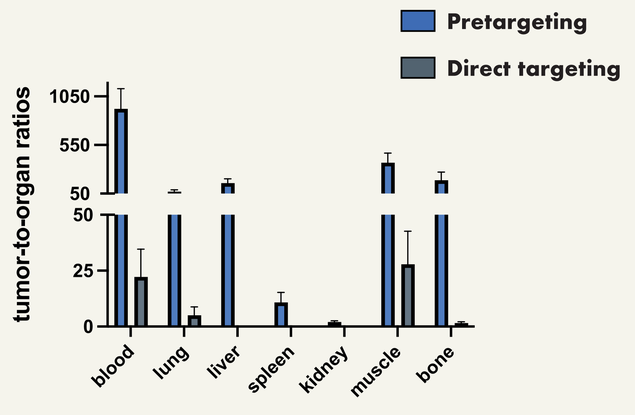
Pretargeting specificity:
4h p.i.



Factors Influencing ^{177}Lu-HP2 Tumor Uptake:
- preinjection of G3-HP1 (PNA-mediated)
- HER2 expression level (HER2-specific)

Influence of pretargeting:
Pretargeting vs. direct DARPin-based tumor-targeting, 4h p.i.





Impact of DARPin-Based and PNA-Guided Delivery of ^{177}Lu-HP2:
- ▲ Tumor: 8-fold higher uptake
- ▼ Kidney: 9-fold lower uptake
- ▼ Liver: 370-fold lower uptake
- ▼ Spleen: 7-fold lower uptake

Key Conclusions:
- Our two-step approch achieves an 8-fold increase in tumor uptake of ^{177}Lu
- Off-target accumulation is markedly reduced, with up to 370-fold decrease in liver uptake
- Results suggest potential for improved efficacy and reduced toxicity in treatment of HER2-positive cancers

Future Implications:
This pretargeting method may pave the way for more effective and safer targeted radionuclide therapies, improving treatment options for HER2-positive cancers.