# Target, Capture, Treat

## Peptide Nucleic Acid (PNA)-Guided Pretargeting for HER2-Targeted Antibody-Mediated Radionuclide Therapy

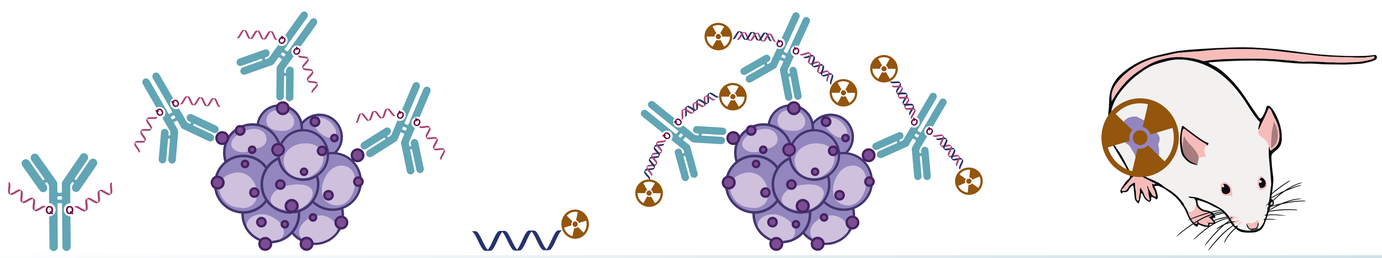
**Westerlund K[1]., Altai M[2]., Zedan W[2]., Clinton J[1]., Geres S[2]., Ulmert D[2]., and Eriksson Karlström A[1].**

1. KTH Royal Institute of Technology, Stockholm, Sweden
2. Lund University, Lund, Sweden

https://doi.org/10.17952/38EPS.2026.P443

In conventional radioimmunotherapy, directly radiolabeled antibodies deliver radiation to tumors but circulate in blood for extended periods, exposing healthy tissue, including the radiosensitive bone marrow, to radiation.

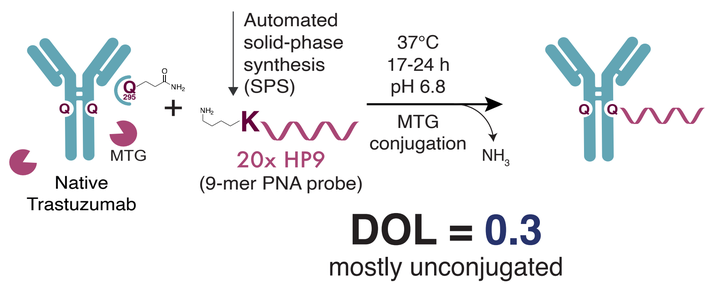
Pretargeted radioimmunotherapy (PRIT) addresses this limitation by administering the antibody and radiolabel separately, allowing the antibody to accumulate at the tumor before the radiolabel is introduced, reducing systemic radiation exposure while maintaining tumor-specific delivery.




## Target

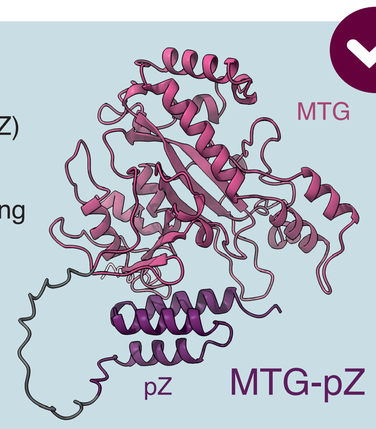
### Arming Trastuzumab with PNA for HER2 Targeting

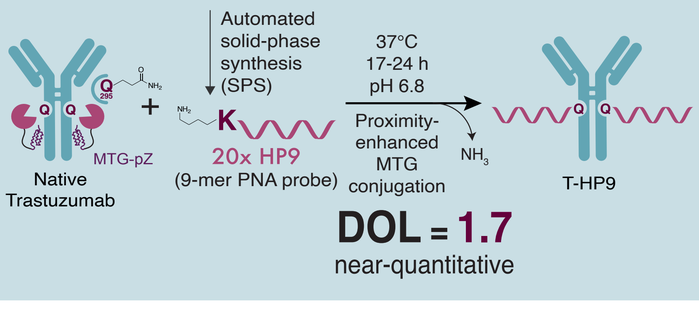
The primary PNA probe HP9 was conjugated to the native anti-HER2 antibody trastuzumab in a site-specific, single-step reaction using the enzyme microbial transglutaminase (MTG). MTG catalyzes an acyl transfer between Q295 in the IgG heavy chain (HC) and a lysine residue on the incoming PNA probe, forming a stable isopeptide bond. Although efficient for small substrates[1,2], soluble MTG achieves only 15% HC labeling (DOL = 0.3) when used for PNA conjugation.




**DOL = 0.3**
mostly unconjugated

Fusion of MTG with an Fc-binding Z-domain (MTG-pZ) enables proximity-enhanced conjugation at Q295, increasing PNA labeling to the HC from 15% to 85% (DOL = 1.7). No PNA purification is required prior to conjugation.




**DOL = 1.7**
near-quantitative

**$K_D$ = 30 pM (SPR)**

**HER2 binding is retained after conjugation (Trastuzumab $K_D$ = 20 pM)**

*Single-step, site-specific PNA conjugation yields a well-defined antibody-PNA conjugate ready to target HER2-expressing tumors.*

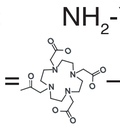
## Capture

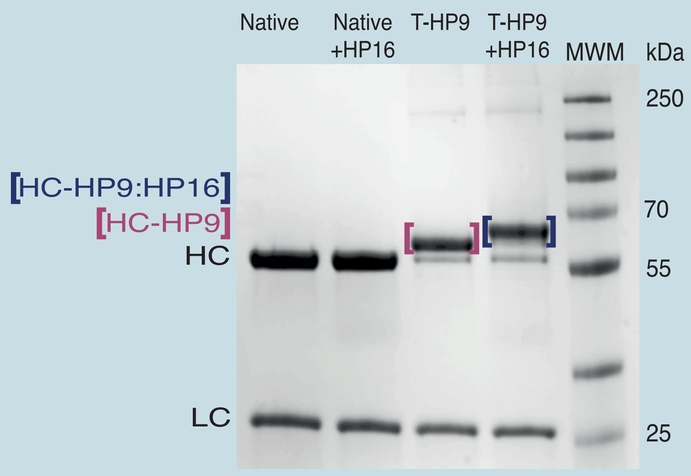
### High-Affinity PNA:PNA Hybridization Enables Selective Probe Capture

Peptide nucleic acid (PNA) is a synthetic DNA analogue with a neutral peptide-like backbone, conferring higher binding affinity and specificity toward complementary sequences than natural nucleic acids. PNA-peptide chimeras are readily synthesized using commercially available building blocks and standard automated solid-phase synthesis (SPS).

Here, two complementary 9-mer PNA strands, HP9 and HP16[3,4], were employed as the primary and secondary probes, respectively: HP9 is conjugated to trastuzumab for tumor targeting, while HP16 serves as the radiolabeled secondary probe.

HP9: RA**K**GGGSS-**cct ggt gtt**-EK(DOTA)-$(PEG_2)$-E-$NH_2$
HP16: $NH_2$-YEE-**gga cca caa**-SS-$(PEG_2)$-DOTA

DOTA =  – a highly versatile radiometal chelator




*Reducing SDS-PAGE of T-HP9 pre-hybridized with HP16 shows a distinct band shift to HC-HP9:HP16, confirming stable PNA-PNA dimer formation.*
*No shift is observed for native trastuzumab + HP16.*

**$K_D$ = 90 pM (SPR)**

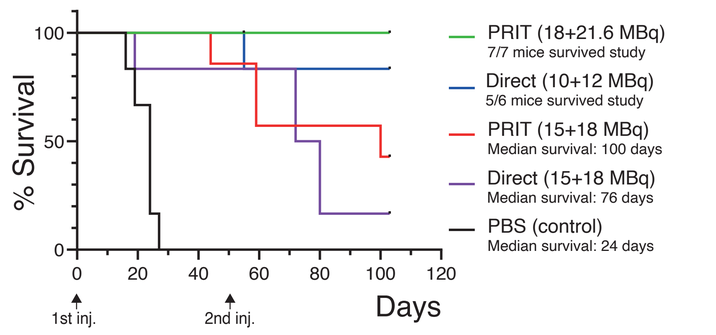
**HP16 binds to T-HP9 with high-affinity**

*HP9:HP16 hybridization is specific, stable even under denaturing conditions, and enables selective capture of $^{161}$Tb-HP16 at the tumor.*

## Treat

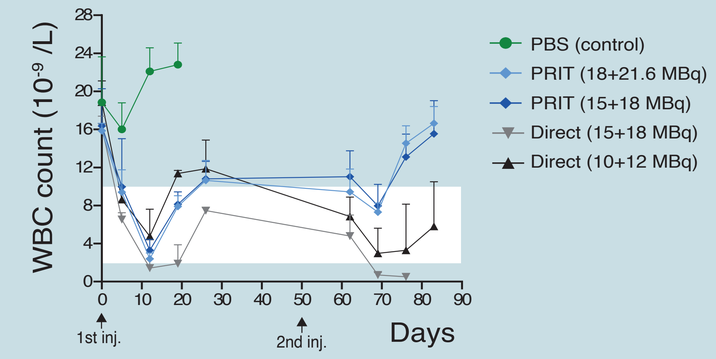
### Delivering Localized $^{161}$Tb Radiation to HER2+ Tumors

The therapeutic efficacy of T-HP9/$^{161}$Tb-HP16 pretargeting was evaluated in SKOV3 HER2+ xenograft-bearing mice and compared to directly $^{161}$Tb-labeled trastuzumab. Two treatment cycles were initiated at day 0 and day 50.




PRIT at 18+21.6 Mbq achieved comparable survival to direct targeting at the lower dose of 10+12 MBq. Direct targeting at a higher dose of 15+18 MBq proved toxic to the mice.

Despite comparable survival, hematological recovery differed markedly. Total white blood cell (WBC) counts recovered toward normal range in PRIT-treated mice following repeated injections, whereas direct targeting groups showed prolonged WBC suppression, even at the lower dose, indicating increased bone marrow toxicity.




**7/7 of pretargeted mice survived the end of the therapy study**

**PRIT (18+21.6 MBq)**

*Pretargeting achieves tumor-selective $^{161}$Tb delivery with 7/7 survival (18+21.6 MBq), while sparing the radiosensitive bone marrow.*

## Conclusions

- **MTG-pZ achieves near-quantitative, site-specific PNA conjugation to native trastuzumab in a single step.**
- **Picomolar HP9:HP16 hybridization confirmed by SPR.**
- **Pretargeting achieves 7/7 survival (18+21.6 MBq) with improved hematological recovery in mice with HER2+ xenografts.**

## Acknowledgements

CANCERFONDEN

## References

1. Dickgiesser et al. *Bioconjug Chem.* 2020
2. Wehrmüller et al. *Chembiochem.* 2025
3. Westerlund et al. *ACS Pharmacol Transl Sci.* 2024
4. Altai et al. *J Control Release.* 2025

Scan to follow our research