Affibody-mediated Peptide Nucleic Acid Pretargeting Using Dual Hybridization Sequences for Increased Therapeutic Potential

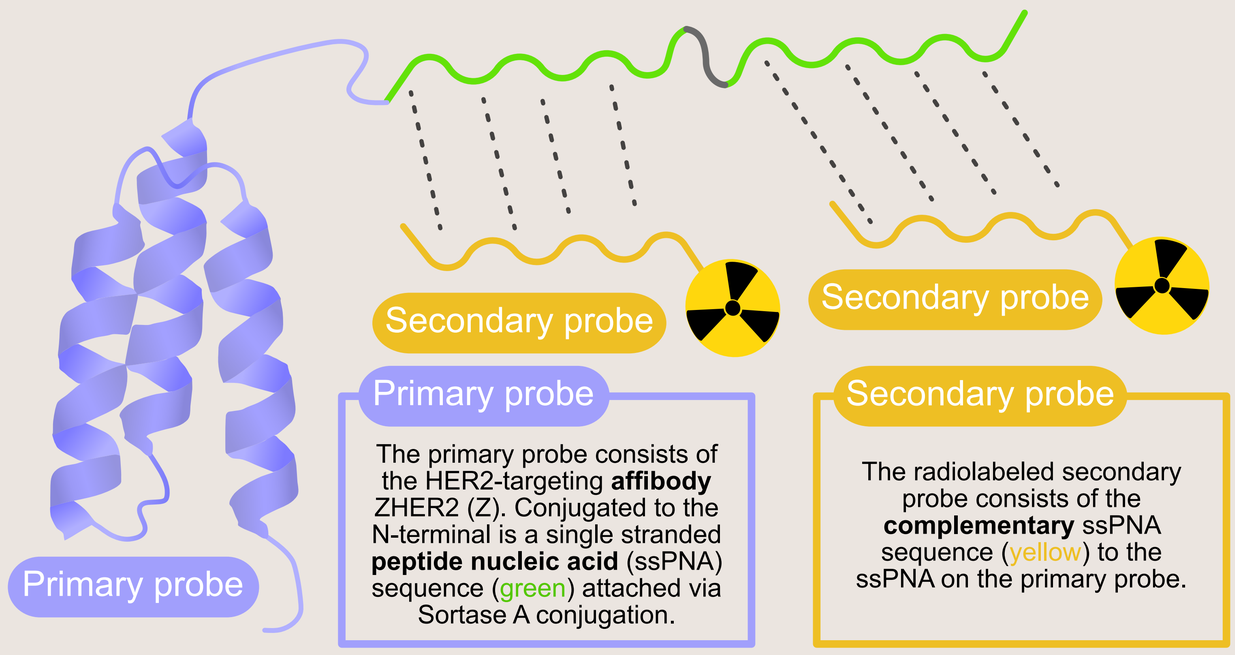
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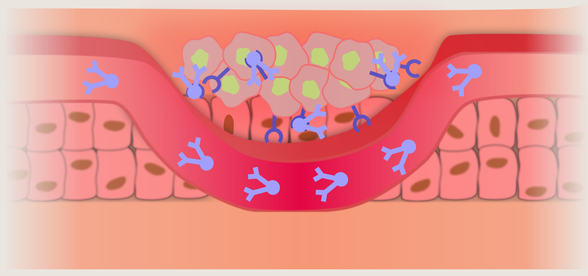
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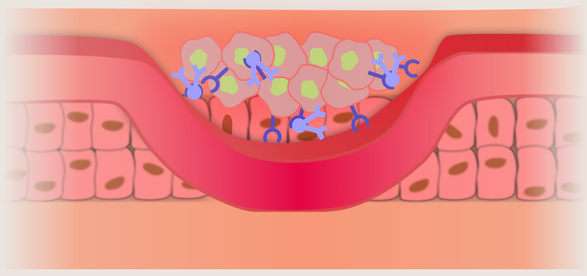
Key concepts

- **Pretargeted radioimmunotherapy** is the concept of separating the tumor targeting agent from the radioactive cell-killing agent in order to reduce toxic radiation exposure to healthy tissues.
- First, a **primary tumor-targeting probe** is injected followed by a waiting time where any unbound probe is **cleared** from circulation and healthy organs.
- Secondly, a **fast clearing radiolabeled probe** is injected with high affinity and specificity to the primary probe.
- This results in higher tumor-to-healthy tissue ratios and opens up for potential treatments with higher doses of radioactivity.

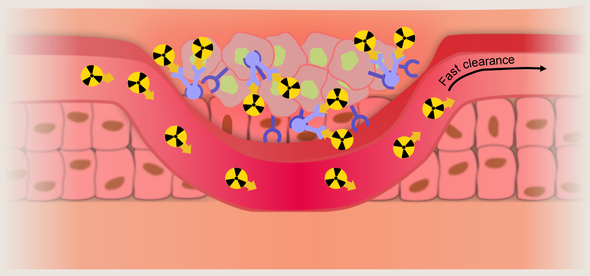




1 Tumor targeting with **primary probe**



2 **Clearance** of unbound primary probe



3 Injection of fast clearing **radiolabeled secondary probe**

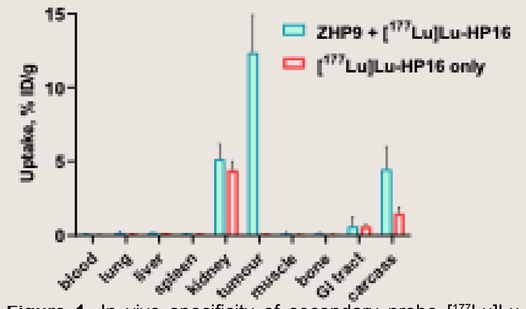


Figure 1. In vivo specificity of secondary probe [^{177}Lu]Lu-HP16 in BALB/C nu/nu mice bearing SKOV3 xenografts at 4h p.i. with and without pre-injection of primary probe, Z-HP9. Data are presented as an average of %ID/g ± SD, n = 4.

Aim

- So far affibody-meditated PNA pretargeting has only been explored in a 1:1 ratio of primary probe (**Z-HP9**) to secondary probe (**HP16**). Results show successful delivery of radioactivity to tumors with favorable tumor to healthy tissue ratio.
- To further develop the **therapeutic potential** of pretargeting we now aim to increase the amount of delivered radioactivity to tumors without increasing radioactivity to healthy tissues.
- By **redesigning the PNA** of the **primary probe** to be able to bind **two secondary probes** simultaneously we hope to further **increase tumor irradiation** without increasing kidney toxicity.
- A new primary probe (**Z-HP9x2**) was produced, consisting of a longer PNA part, namely two **HP9** sequences connected by a short PEG-linker.

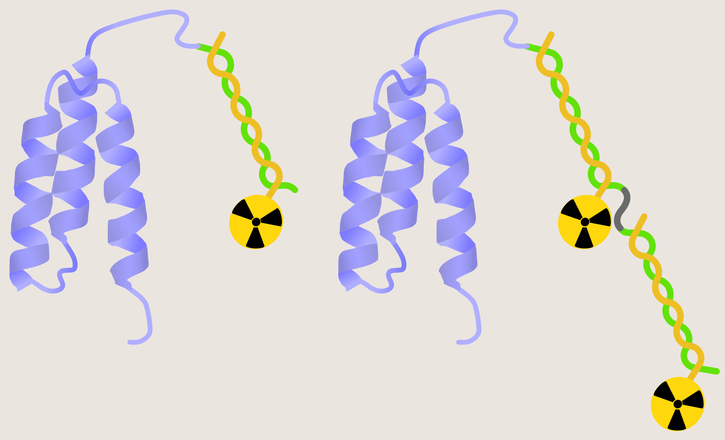


Figure 2. Depiction of primary and secondary probes hybridized through PNA hybridization. **Left**: affibody **ZHER2** (purple) conjugated to the old **HP9** (green) probe with a single secondary probe **HP16** (yellow) hybridized. **Right**: affibody **ZHER2** (purple) conjugated to the new **HP9x2** (green and gray) probe with two **HP16** (yellow) probes hybridized.

HP9x2 DOTA
Gly-Gly-Gly-Ser-Ser-cctggtgtt-AEEA-cctggtgtt-Glu-Lys-AEEA-Glu

HP9 DOTA
Gly-Gly-Gly-Ser-Ser-cctggtgtt-Glu-Lys-AEEA-Glu

HP16
DOTA-AEEA-Ser-Ser-aacaccagg-Glu-Glu-Tyr

Figure 3. Sequence of **new** primary PNA probe **HP9x2** (top), **old** primary PNA probe **HP9** (middle) and secondary probe **HP16** (bottom). PNA bases in red, DOTA chelator in blue, amino acids in black and AEEA (PEG_2) molecule in green.

Results

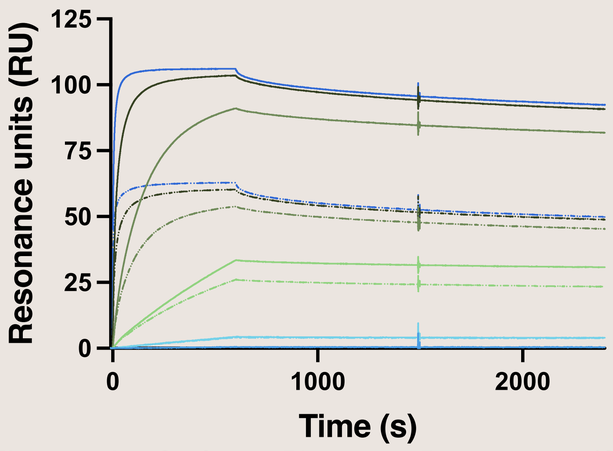


Figure 4. Surface Plasmon Resonance (SPR) sensorgrams of **HP16** as analyte at concentrations ranging from 0.05 nM (light blue) - 5000 nM (dark blue) over surfaces immobilized with **equimolar** amounts of either **Z-HP9** (dotted lines) or **Z-HP9x2** (full lines).

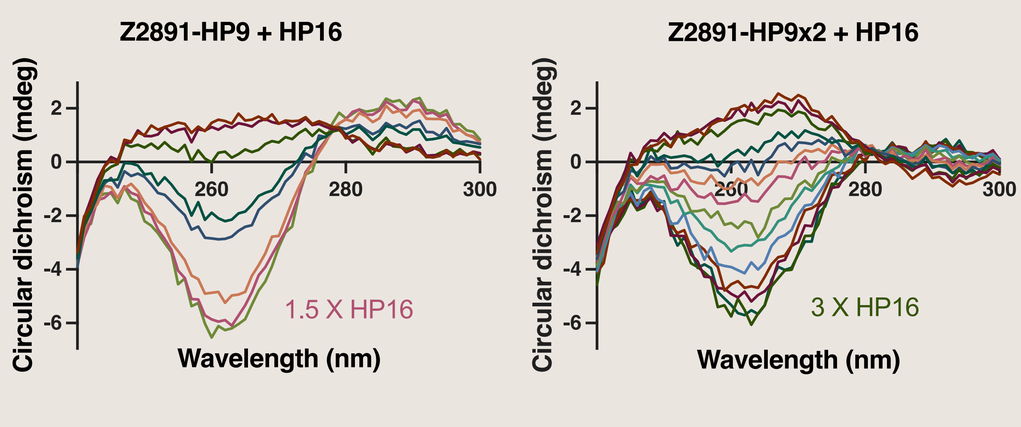


Figure 5. Circular dichroism (CD) data plots showing induced structural changes from PNA-PNA hybridization of Z-HP9 (left) and Z-HP9x2 (right) by adding increasing amounts of HP16. **Left**: Molar ratios of Z-HP9:HP16 ranging from 1:0.25 (brown) to 1:2 (light green). **Right**: Molar ratios of Z-HP9x2:HP16 ranging from 1:0.25 (brown) to 1:3.25 (dark green).

Conclusions

- The aim of this study is to further **increase the tumor-to-healthy tissue ratio** of radioactivity for pretargeted radioimmunotherapy with affibodies.
- By increasing the **length if the primary PNA probe** to have the capacity to hybridize with **two radiolabeled secondary probes**, instead of one, we hope to increase tumor irradiation without increasing irradiation of healthy tissues.
- Initial characterization of the **new, longer Z-HP9x2** primary tumor binding probe shows good potential as both SPR and CD results indicate that hybridization of twice the amount of HP16 secondary probe at higher concentrations is indeed possible compared to the **older primary probe Z-HP9**.

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