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Affibody-mediated peptide nucleic acid pretargeting for delivery of cytotoxic payloads to HER2 positive carcinoma

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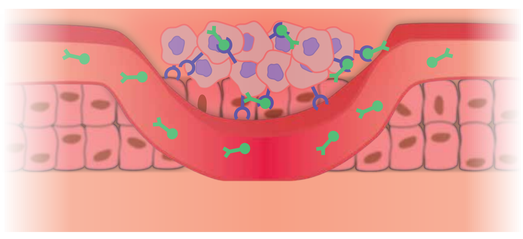
Key concepts

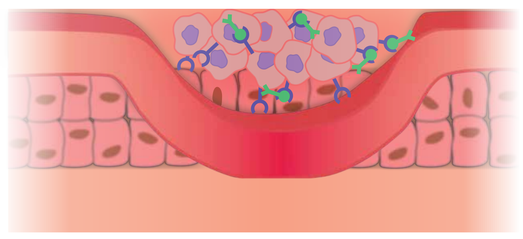
Pretargeted cytotoxic therapy is the concept of separating the tumor targeting agent from the cell-killing agent in order to reduce toxic exposure to healthy tissues.

1. First, a **tumor-targeting primary probe** is injected and allowed to bind the highly expressed tumor associated receptor



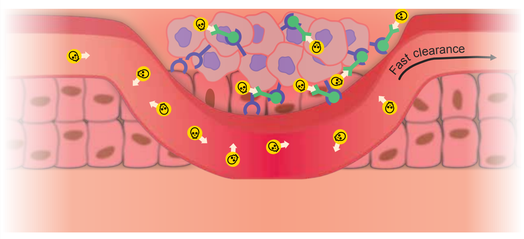
Tumor targeting with **primary probe**

2. A waiting time follows the first injection where any residual accumulated unbound primary probe is **cleared** from circulation and from healthy organs



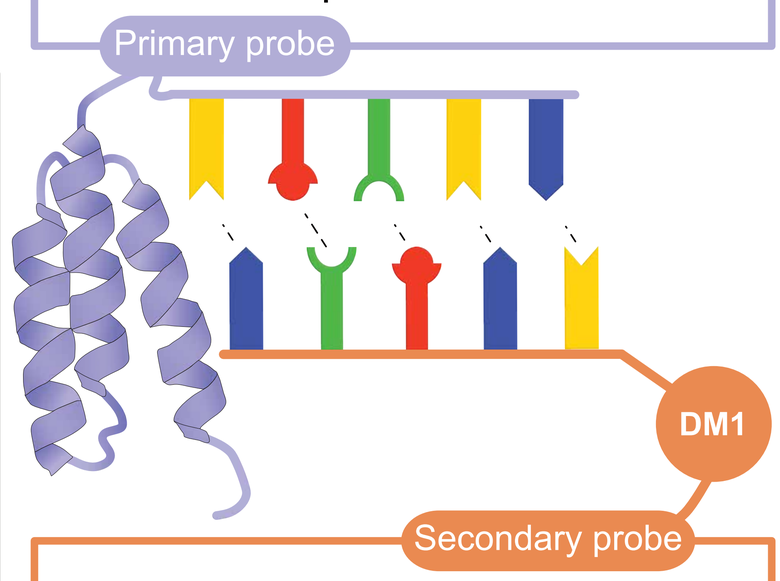
Clearance of unbound primary probe

3. After the waiting time, a fast clearing **cytotoxic secondary probe** is injected with high affinity and specificity to the primary probe, minimizing toxic exposure to healthy tissues



Injection of fast clearing **cytotoxic secondary probe**

The **primary probe** consists of the HER2-targeting **Affibody** ZHER2 conjugated to the single stranded peptide nucleic acid (**PNA**) sequence **HP9**



The cytotoxic **secondary probe** consists of the toxic molecule **DM1** coupled to the **complementary PNA** sequence to that of the HP9 probe

Aim and method

- Previously pretargeting has been successful in a system of targeted radiotherapy with a radioactive metal conjugated to the secondary probe.
- We explore the possibility of changing the radioactive metal on the secondary probe to the toxic molecule DM1.
- Six different 8-mer PNA base long secondary probes were produced. Their binding characteristics and cellular toxicity was analyzed with SPR and cytotoxicity assays.

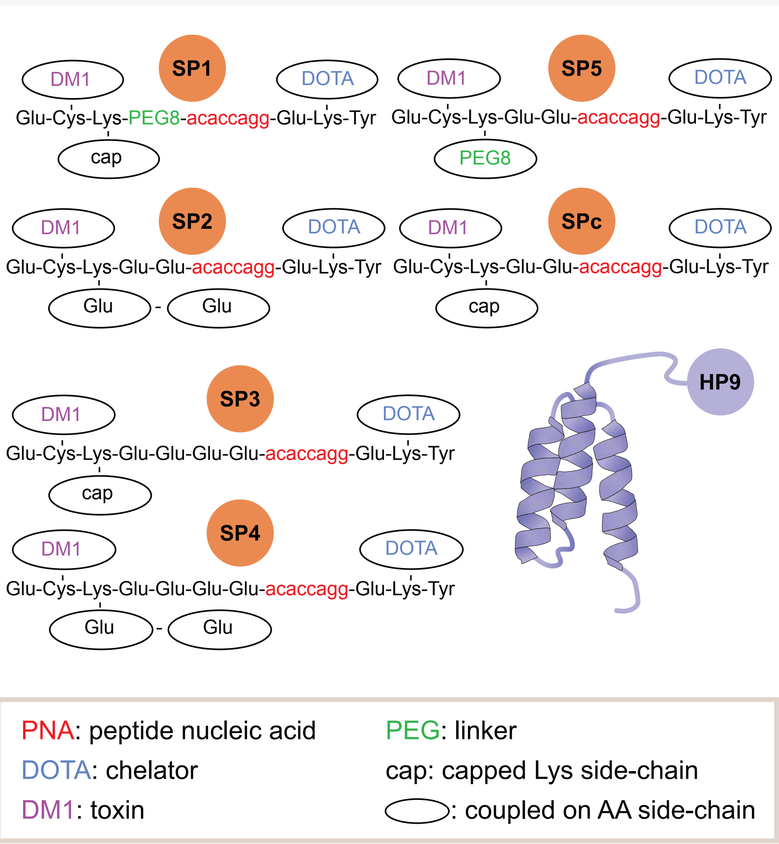


Figure 1: Sequences of the different secondary probes and schematic of the primary probe ZHER2-HP9 (purple).

Results

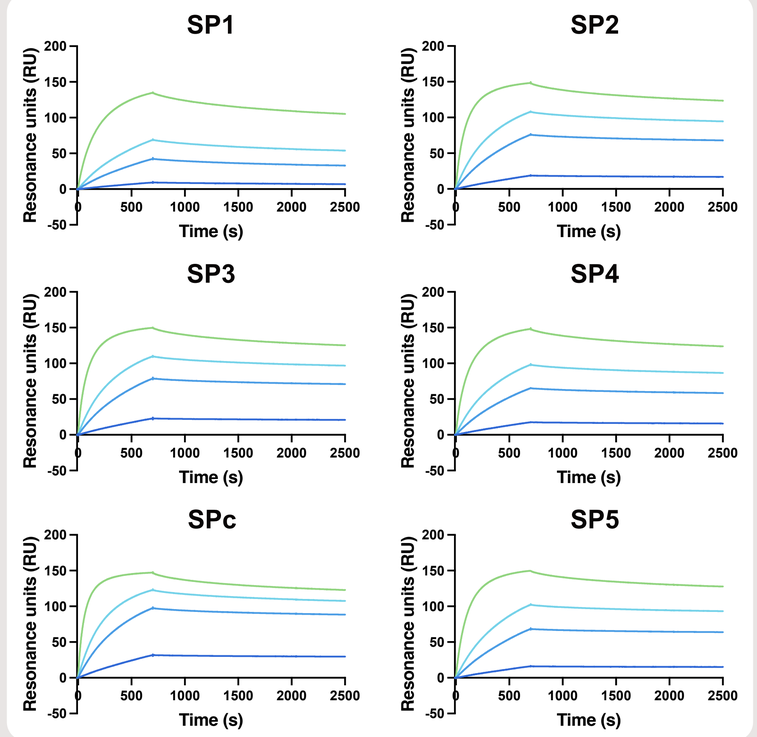


Figure 2: SPR sensorgrams of PNA hybridization between the immobilized ZHER2-HP9 and the six different secondary probes. Four concentrations ranging between 1.4 (dark blue)-70 (green) nM of each probe was used.

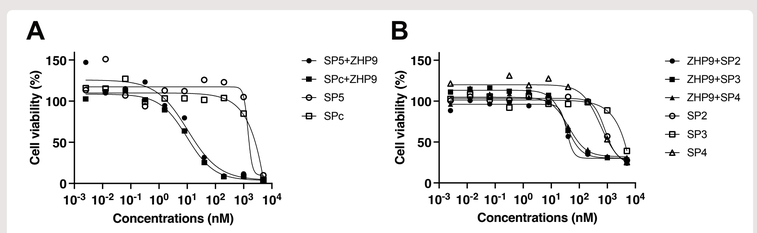


Figure 3: In vitro cellular cytotoxicity assay of secondary probes SP2-SP5 and SPc pre-incubated with and without the primary probe ZHER2-HP9 (ZHP9). Black dots, squares and triangles represent pre-incubated secondary and primary probe, whereas white ones are assays without the primary probe. A; AU565 cells and B; SKBR3 cells.

Conclusions

- Our aim is to utilize **pretargeting** in a new way where cytotoxic payloads are delivered to the tumor site while, at the same time, **minimizing toxic exposure to healthy tissues** and organs.
- We produced a panel of different mertansine (DM1) **cytotoxic secondary probes** with different hydrophilic properties to analyze binding characteristics to the **ZHER2-HP9 primary probe** together with their cell-killing potential.
- **SPR** results show that all six unique secondary probes have **strong picomolar hybridization** characteristics towards the complementary PNA sequence of ZHER2-HP9.
- Cellular **cytotoxicity assays** show **increased cell death** when cells are exposed to secondary probes combined with the ZHER2-HP9 primary probe, in contrast to secondary probes added alone.

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