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Use of an aptamer library based next generation omics platform for the development of a novel trastuzumab response predictive assay

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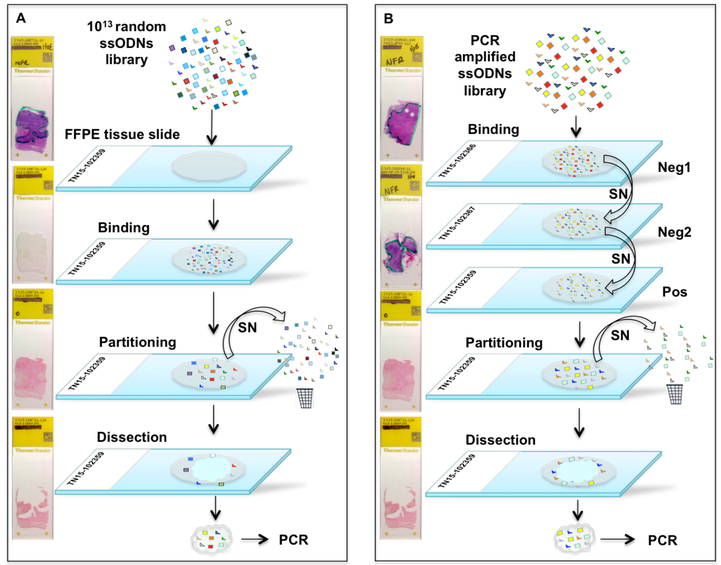
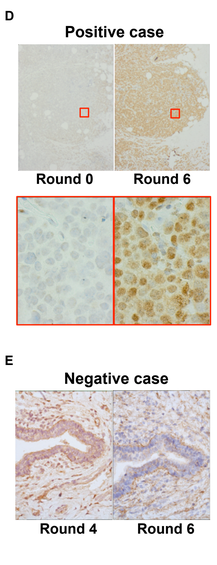
Introduction: Previous attempts to use individual aptamers as diagnostic reagents have failed to consistently achieve performance comparable to antibodies. Here we report a novel systems biology approach, called Adaptive Dynamic Artificial Poly-ligand Targeting (ADAPT) using poly-ligand aptamer libraries to identify responders and non-responders to trastuzumab-based regimens in metastatic breast cancer. To overcome the fundamental limitation of individual aptamer binding affinities, large libraries (10^6 species) were created so that potentially thousands of aptamers could bind to each of a multitude of targets related to trastuzumab response or non-response.

Training of ssDNA library on FFPE tissue





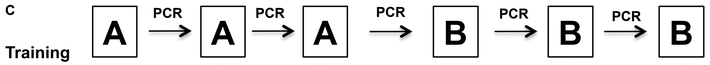
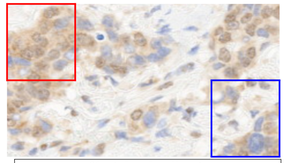


Figure 1. Training of single-stranded oligodeoxynucleotides (ssODNs) library. A. Training toward positive case. Slide images show tissue appearance at different steps: 1 – Hematoxylin&Eosin (H&E) staining of FFPE tissue (cancer is outlined); 2 – unstained tissue during incubation with the ssODN library; 3 – Nuclear Fast Red (NFR) stained tissue after partitioning before dissection; 4 – remaining normal tissue after dissection of cancer tissue with bound ssODNs. One training round consists of binding of the ssODN library to the positive tissue, removal of unbound sequences, dissection of tumor tissue, and recovery of the subset of sequences, specific to the positive cancer tissue. SN: supernatant. **B**. Training against negative cases. Unlike in **A**, supernatant is collected after binding to the tissue of both negative cases, then applied to the same positive as in **A**. **C**. Entire training workflow, letters A and B corresponds to the panels in the scheme above. **D**. Staining of the tissue from the Non-responder (NR) case that was not used during the training process with untrained library (round 0), compared to the NR trained library (round 6; upper panel: 4×, lower panel: 20×); **E**. Staining of the tissue from negative case (responder, R) using the input ssODNs for round 4 (left), compared to the input ssODNs for round 6 (right).

A

Compartment	AHC staining intensity score 0	1	2	3
Cytoplasm				
Nucleus				



B

	Compartment	% stained cells at: 0	1	2	3	Total	Histological score
Red area	Cytoplasm	0	25	75	0		
	Nucleus	15	10	75	0		
Blue area	Cytoplasm	18	46	36	0		
	Nucleus	27	27	46	0		
Total	Cytoplasm	7	32	61	0	154	300
	Nucleus	19	16	65	0	146	

C

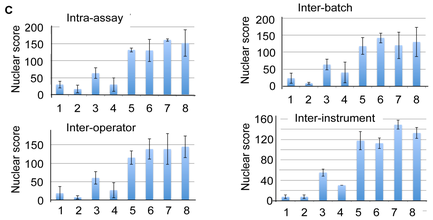


Figure 2. Histological score calculation based on the intensities of Aptahistochemistry (AHC) staining and its reproducibility. **A**. Examples of the different staining intensity levels in the cytoplasm and the nucleus of breast cancer tissue. **B**. Examples of the histological score calculations in two areas of the breast cancer tissue. **C**. Reproducibility of the AHC staining and scoring using trained library, specific to NR (TL-NR), on eight different cases between technical replicates (intra-assay), operators (inter-operator), different batches of the library preparation (inter-batch), and auto-staining robots (Dako; inter-instrument). Cases 1-4 were chosen as examples of relatively low intensity staining, cases 5-8 are examples of the relatively high intensity staining.

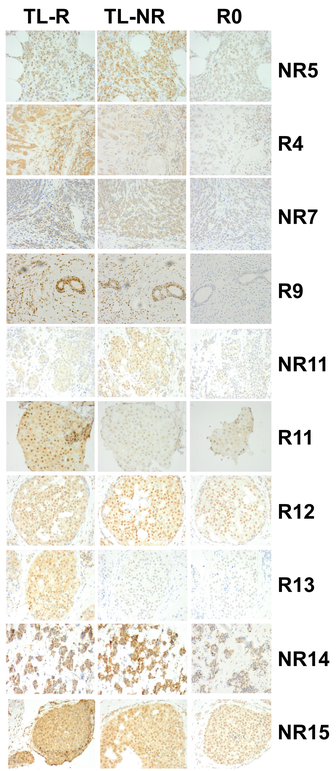


Figure 3. Staining profile comparison of the non-enriched library R0 and trained libraries toward NR (TL-NR) and R (TL-R). View areas are matched in each raw between libraries. The library R0 usually exhibits little to no staining, while the enriched libraries can be scored from 1+ to 3+. Library TL-NR, enriched toward a Trastuzumab non-responder case, mostly exhibits stronger intensity on NR cases. Library TL-R, enriched toward a Trastuzumab responder case, mostly exhibits stronger intensity on both shown R cases. Magnification: 20x.

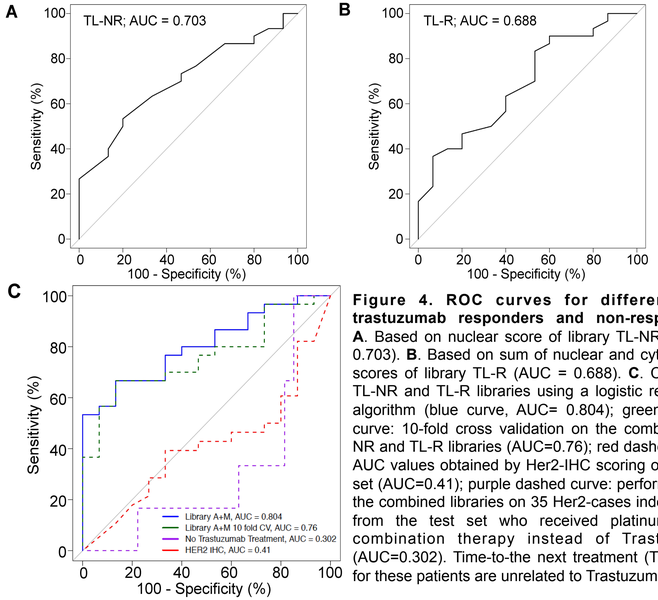


Figure 4. ROC curves for differentiating trastuzumab responders and non-responders. **A**. Based on nuclear score of library TL-NR (AUC = 0.703). **B**. Based on sum of nuclear and cytoplasmic scores of library TL-R (AUC = 0.688). **C**. Combined TL-NR and TL-R libraries using a logistic regression algorithm (blue curve, AUC= 0.804); green dashed curve: 10-fold cross validation on the combined TL-NR and TL-R libraries (AUC=0.76); red dashed curve: AUC values obtained by Her2-IHC scoring of the test set (AUC=0.41); purple dashed curve: performance of the combined libraries on 35 Her2-cases independent from the test set who received platinum/taxane combination therapy instead of Trastuzumab (AUC=0.302). Time-to-the next treatment (TNT) data for these patients are unrelated to Trastuzumab.

Summary:

- ✓ ssODN libraries were trained on FFPE breast cancer tissue of the patients, who were classified as responders and non-responders time-to-the next treatment of 6 months as the measure of response.
- ✓ Standard histological scoring was utilized for analysis of the staining intensities and analyzed by aboard certified pathologist blinded to the patient information.
- ✓ Libraries, trained toward Responder and Non-Responder, were tested on the set of independent cases and differentiated the responders and non-responders with AUC of 0.688 and 0.703 correspondingly.
- ✓ Histological scores of the libraries, trained in opposite directions, were combined using logistic regression model and demonstrated superior differentiation between responders and non-responders (AUC=0.804).
- ✓ The ADAPT assay works independently of Her2 status and offers significantly higher response predictive value (AUC of 0.8 vs 0.4 based on Her2 status).
- ✓ Trained libraries were uninformative for the set of cases with TNT from platinum/taxane treatment, which might indicate their specificity to the molecular network, involved in trastuzumab response.
- ✓ Developed test requires prospective validation to confirm the clinical utility.