Use Of Herceptin-Based IHC As a Potential CDx for Herceptin Treatment

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Background

Current ASCO and CAP guidelines require using FDA approved Her2 IHC or Her2 ISH tests to select Her2 positive patients for Herceptin therapy and that all Her2 IHC 2+ results must be confirmed by a Her2 ISH test. Combination of the two tests has reduced the false positive rate dramatically from about 20% by using Her2 IHC alone to about 6% which still represents a significant number of patients who are potentially treated with the expensive drug without therapeutic effect. With more Herceptin based therapies (e.g., ADC, CAR-T, immunotherapy) are available or will be available soon, a more reliable Her2 test is becoming critically important in selection of eligible Her2 positive patients for these therapies. An ideal companion test for Herceptin therapy should be a test that can directly detect Herceptin binding activity to patient's cancer tissues. Novodiax has developed a simple Her2 IHC by directly labelling Herceptin with a unique polymerized HRP, namely ihcDirect-Herceptin, which can directly detect Herceptin binding activity on patient's cancer tissues. This Her2 IHC does not require any secondary antibody or other detection steps that is needed in a "traditional IHC". We report here a superior result by ihcDirect-Herceptin than two conventional Her2 IHC tests based on a large scale evaluation of 119 archived breast cancer tissues.

Results & Discussion

As shown in Tables 1 and 2, among the 22 "3+" tissues by other Her2 IHC, 55% (12/22) were positive by ihcDirect-Herceptin as depicted in Figure 1, and 45% (10/22) of them were tested negative by ihcDirect-Herceptin. These tissues have been repeatedly tested with ihcDirect-Herceptin and remained to be negative (Fig. 2). Since these tissues are "Herceptin-IHC negative", it is highly questionable these patients would respond to Herceptin therapy even though they are 3+ by other Her2 IHC tests. Ninety six percent of the Her2-negative tissues (45/47) by traditional Her2 IHC were consistently negative by ihcDirect-Herceptin. However, two of these negative tissues were positive by ihcDirect-Herceptin test (Table 2).

On the 2+ cancer tissues defined by traditional Her2 IHC tests, the overall agreement of Novodiax ihcDirect-Herceptin with Her2 FISH tests are much higher than that with traditional Her2 IHC (Table 3), 100% with PharmDxTM FISH and 84% with PathVysion FISH versus 68.9% with HercepTest and only 39.2% with a Lab Developed IHC using Her2 antibody clone CB11. It is well documented that majority of current Her2 IHC 2+ tissues would be negative by FISH tests.

When compared with combinations of a Her2 IHC and Her2 FISH, we observed a higher overall agreement of 93.3% with Dako HercepTest and PharmDx™ FISH than with the combination of the CB11 IHC plus PathVysion FISH (79.9%) (Table 3). Many past studies have demonstrated that current Her2 tests have significant discrepancy rates between testing labs.

Table 1. A comparison with Dako Her2 IHC and FISH (45 cases)

HercepTestIHC (No of tissue)	PharmDx™ FISH (No of tissue)	ihcDirect-Herceptin (No of tissue)
3+ (8)	Not Tested	Positive (5), Negative (3)
2+ (12)	Positive (1)	Positive (1)
	Negative (11)	Negative (11)
1+ (21)	Not Tested	Positive (0), Negative (21)
0 (4)	Not Tested	Positive (0), Negative (4)

Table 2. A comparison with CB11 IHC and Abbott FISH (74 cases)

CB11 IHC (No. Tissue)	PathVysion FISH (No. Tissue)	ihcDirect-Herceptin (No. Tissue)
3+ (14)	Not Tested (14)	Positive (7), Negative (7)
2+ (38)	Positive (8)	Positive (2), Negative (6)
	Negative (30)	Negative (30)
0 (22)	Not Tested (22)	Positive (2), Negative (20)

Materials & Methods

A total of 119 archived FFPE breast cancer tissues (Her2 IHC negative: 47, 2+: 50 and 3+: 22) were collected from Brown University and St. Mary Hospitals for this study. Forty five of them had been previously tested with Dako HercepTest and HER2 FISH PharmDx™ (Table 1), and the rest 74 with a Lab Developed IHC Test using a Her2 antibody clone CB11 plus PathVysion FISH from Abbott (Table 2).

Novodiax ihcDirect-Herceptin was produced by directly conjugating Herceptin with PolyHRPs by following a Direct IHC antibody labelling protocol (Patent pending). A simpler IHC protocol was used on all tissues in this study in which the ihcDirect-Herceptin was applied directly onto the tissue sections after a standard dewaxing, antigen retrieval and blocking procedure and then incubated for 30 min at room temperature. An optimal working concentration was obtained by tittering it on multiple HercepTest positive and negative tissues. A simple positive or negative scoring system was used for grading Herceptin binding to breast cancer tissues in this study. Any cancer tissue section with complete or incomplete membrane staining in more than 1% cancer cells was scored positive and otherwise negative. All of the 3+ tissues by traditional Her2 IHC methods that were scored negative by ihcDirect-Herceptin test were retested for more than two times by using both the same protocol and a protocol with longer incubation time.

Conclusion

1. A simple and reproducible one-step Her2 test with Novodiax ihcDirect-Herceptin has been developed and it has been shown to be a reliable Her2 IHC test for detection of Her2 over-expression in formalin fixed breast cancer tissues.
2. Novodiax ihcDirect-Herceptin test uses a simple two-tier scoring system that may potentially reduce the discrepancy rate that has been plagued all current FDA approved Her2 tests for a long time.
3. For the equivocal cases (Her2 IHC 2+) by traditional Her2 IHC tests, Novodiax ihcDirect-Herceptin test results highly correlate with Her2 FISH tests, in particularly with PharmDxTM FISH from Dako, indicating that Novodiax ihcDirect-Herceptin test is potentially a more reliable Her2 IHC test than the traditional Her2 IHC tests in selection of Her2 positive patients for Herceptin therapy.
4. Novodiax ihcDirect-Herceptin IHC directly detects Herceptin binding activity of the therapeutic antibody on cancer tissues. Theoretically, it should be a gold standard CDx for Herceptin therapies.

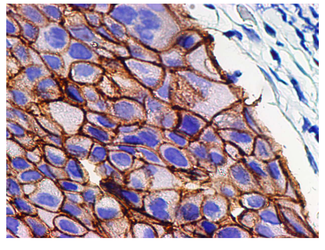

Fig 1: 100% of cancer cells showed positive membrane staining by ihcDirect-Herceptin in this HercepTest 3+ tissue.

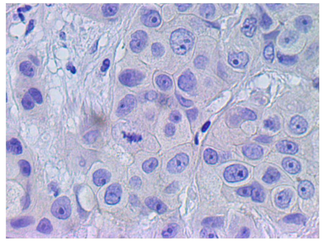

Fig 2. None of the cancer cells showed positive staining by ihcDirect-Herceptin in this HercepTest 3+ tissue. A total of 10 tissues showed the same pattern.

Table 3. Overall agreement of ihcDirect-Herceptin IHC with other Her2 tests

Other Her2 tests	Overall agreement rate
HercepTest IHC plus PharmDx FISH	93.3%
CB11 IHC plus PathVysion FISH	79.9%
HercepTest IHC	68.9%
CB11 IHC	39.2%
PharmDx FISH (HercepTest "2+" tissues)	100%
PathVysion FISH (CB11 "2+") tissues)	84%