#1033

Multiple Alternative Splicing of HER2 transcripts and Exon Skipping with Potential Consequences on Function and Antibody-Based Therapy.

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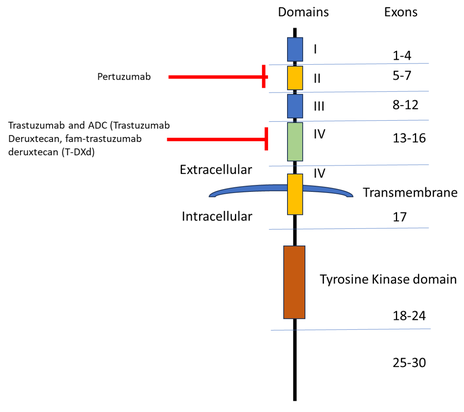
Background:

Multiple studies have demonstrated that ERBB2 (HER2) exon 16 skipping is associated with resistance to antibody-based therapy. However, ERBB2 gene contains 30 exons with potential for alternative splicing involving all exons. With the increasing utilization of antibody-drug conjugates (ADC) in treating patients, it is important to evaluate the various ERBB2 isoforms that are expressed in various tumors. Toward this goal we evaluated the various splicing forms and exons skipping in domains II and III that are involved in dimerization and binding to pertuzumab and in domain IV, which is a juxtamembrane domain and involved in trastuzumab binding.



Methods:

RNA was extracted from 3170 FFPE tissue from various types of tumors including breast, ovary, endometrium, lung, colon. Stomach, esophagus and others. RNA was sequenced using hybrid capture targeted panel. Data analysis is focused on ERBB2 gene.

Results:

Domains II and III				Domain IV			
Skipped Exons	Median % of Transcript	Number of Cases	% of Total	Skipped Exons	Median % of Transcript	Number of Cases	% of Total
6	0.002	2	0.24	13	0.002	9	1.09
6-9	0.006	3	0.36	15	0.007	3	0.36
6-7	0.010	61	7.40	15-16	0.001	1	0.12
6-10	0.010	183	22.21	15-17	0.010	4	0.49
6-14	0.077	1	0.12	15-18	0.001	1	0.12
6-12	0.001	10	1.21	15-19	0.003	1	0.12
6-13	0.012	3	0.36	15-29	0.018	1	0.12
7	0.001	12	1.46	16	0.020	229	27.79
7-8	0.016	3	0.36	16-17	0.003	33	4.00
7-9	0.002	20	2.43	16-20	0.035	1	0.12
7-10	0.003	31	3.76				
7-11	0.006	1	0.12				
7-12	0.001	4	0.49				

Conclusions

1) Low level exon skipping in HER2 is common and involves critical functional domains of the protein
2) Most skipped exons are in membrane anchoring and relevant for dimerization
3) Further studies are needed to correlate with efficacy of antibody-based therapy and development of resistance.

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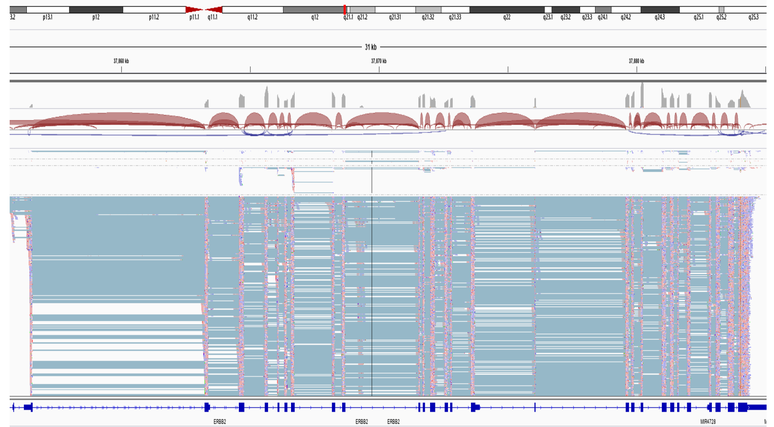
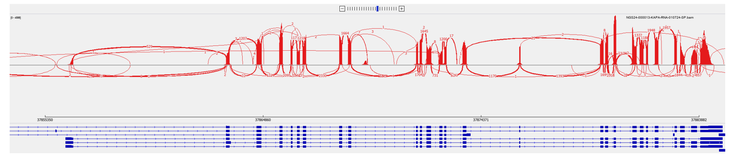
Example of ERBB2 mRNA in breast cancer sample from a patient with ERBB2 overexpression showing the alignment track with significant exonal skipping.



The Sashimi plot showing the various alternative splicing forms in the same sample



Of these cases 199 (24%) samples showed exon skipping in domains II and III ranging from exons 6 to 14. Skipping in domain IV was detected in 236 cases (29%) involving exons from 13 to 29. However, 107 samples of the 824 (13%) showed skipping of exons in both domains II/II and domain IV. The most common exon skipping were in exons 6 to 7 and in exon 16. Most of the cases with exon 16 skipping (1.5% of total) also showed skipping in exons 6 and 7 (Table 1).

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