P2.04: Prevalence of secondary genetic modifiers on cancer drug biomarkers and implications for the clinical utility of gene-based diagnostics.

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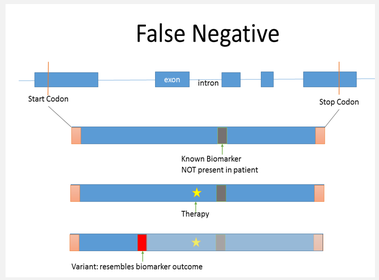
BACKGROUND

Many tests for predictive biomarkers in tumors focus on selected known mutations or regions (eg, hotspot panels with amplicon sequencing) or on some types of mutations (eg, SNPs and CNAs from SNP arrays).

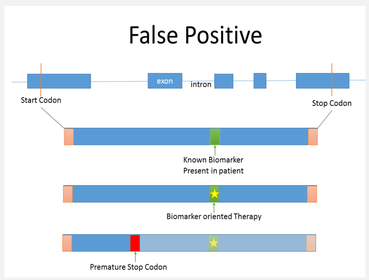
The purpose of this study is to assess the rates and clinical impact of secondary mutations in the same predictive gene, scrutinizing the diagnostic fidelity of specific single-nucleotide variant (SNV) biomarker testing.

METHODS

- We systematically searched cancer cases for both
  - presence or absence of known predictive SNVs and
  - any high-impact mutations (HIMs) in the same gene.
- 1825 predictive SNV biomarkers were used in this study, from an expert-curated set of variant-drug-response relations extracted from the literature.
- For the HIMs, we considered any frameshifts (fs), splice site disruptions, and premature stop codons (PSCs), since they may invalidate biomarkers in their proximity.
- We screened 3 sets of cancer cases:
  - 90 cases of endometrial cancer (EC) from TCGA
  - 30 cases of HER2+ breast cancer (BC)
  - 209 solid tumors (ST) that were given treatment recommendations based on a NGS-based system that considers such HIMs.

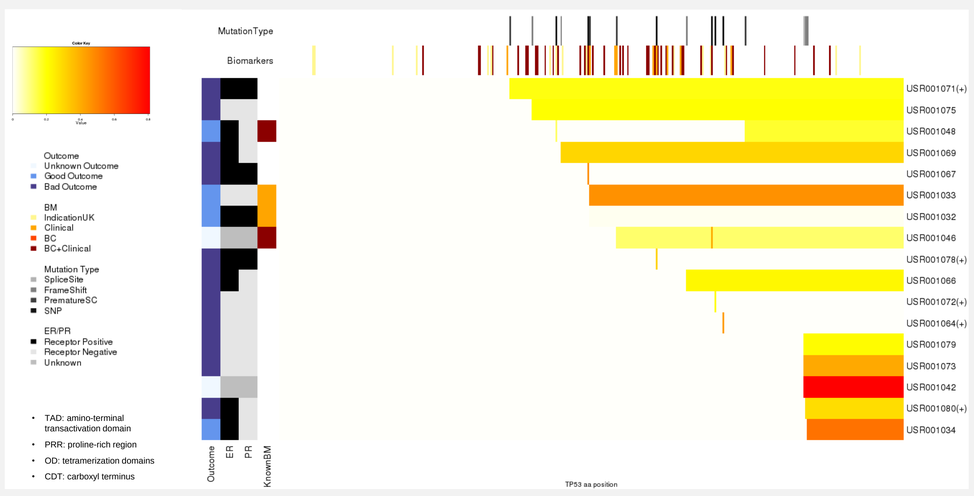


False Negative: When a biomarker mutation is absent, an equivalent effect may be caused by a deleterious upstream mutation.



False Positive: Biomarker mutations found together with a potentially dramatic upstream mutation in the same gene.

RESULTS - False Negative examples (Breast Cancer)



The TP53 mutation map shows a subset of samples that contain either disruptions or SVNs with literature evidence of severe alteration of the protein function. Samples that do not present any potentially disruptive mutations are not included. The color key in the heat map represents the allele frequency of the disruption.

TP53 is a well-known tumor suppressor gene, where several germline variants are known to predispose for several cancers. Somatic mutations in this gene are related to a poor prognosis.

- There is a correlation between additional mutations and outcome.
- Analysis of known biomarkers is not enough to find the correlation.
- There is a clear need to analyze more than AA substitutions.
- Disruption: Mutations that cause a severe alteration in the protein sequence.

	Aditional Mutation+			Aditional Mutation-		
Outcome	BM+	BM-	**Total**	BM+	BM-	**Total**
Good	1	1	**2**	2	8	**10**
Bad	0	11	**11**	0	3	**3**
N/A	1	1	**2**	0	2	**2**
Total	2	13	15	2	13	15

Outcome	Disruption +	Disruption -
Good	4	8
Bad	9	5

RESULTS

	Number of detected biomarkers	Number of HIMs around present biomarkers Potential false positives, FP	Number of HIMs around absent biomarkers Potential false negatives, FN
BC- Breast Cancer	5.61	0.55	7.55
EC- Endometrial Carcinoma	6.04	0.37	9.27
ST- Solid Tumors	7.00	0.46	12.52

- For the three cohorts we show the averages per sample. This counts include low allele frequency variants
- In the EC cohort, false positives occur at least 5 times with AF > 40% in cancer related genes.
- As for FNs, we found 13 BC cases with potentially deleterious TP53 mutations with AFs >10%.
- From the 209 ST cases, we have treatment recommendation information for 150. Of those, 12 patients received a recommendation based on a PSC, and one based on an frame shifting indel.

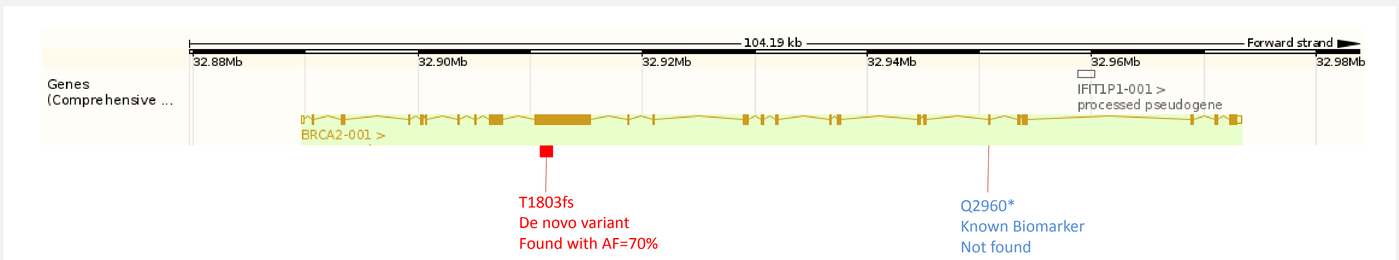
RESULTS - False Positive examples (Endometrial Cancer)

	Biomarker		Disruption		
Sample	Mutation	Allele Frequency	Mutation	Allele Frequency	Type
S229	PTEN.P246L	0.44	R233*	0.34	PSC_U
S320	PIK3CA.R88Q	0.11	R832*	0.52	PSC_D
S304	PTCH1.P1315L	0.49	G725*	0.66	PSC_U
S228	EGFR.R521K	0.50	P20fs	0.66	FS_U

- The four clinical biomarkers shown in the table have been found to have allele frequency (AF) > 10% in EC samples.
- The disruptions found have AF > 30%, indicating that they might have an effect on the biomarker.
- PTEN, PIK3CA and PTCH1 mutations play a role in tumor development. When the affected genes are drug targets for cancer therapies, alterations to them could affect the efficacy of the therapy.

RESULTS - False Negative example (Prostatic Neoplasm)

- Male patient with prostatic adenocarcinoma. 64 years old.
- One of the treatment options recommended (PARP inhibitors) is based on a frameshifting indel.
- BRCA2.T1803fs is predicted to result in loss of BRCA2 function due to frame-shift.
- The indel does not coincide with any known biomarker in our databases nor in public databases. Clinical trials are currently ongoing.
- Known Biomarker: BRCA2.Q2960* - premature stop codon. Not Found.
- Frame shifting indel behavior can be compared to that of the premature stop codon: loss of BRCA2.



Taken from ENSEMBL. http://grch37.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000139618;r=13:32889611-32973805

PARP inhibitors are investigational drugs selectively targeting tumor cells with BRCA1 or BRCA2 gene mutations. There is clinical evidence that PARP inhibitors in combination with chemotherapy demonstrate synthetic lethal anti-tumor activity against cancer harboring mutations in BRCA1/2 and other DNA repair pathway components.

CONCLUSIONS

- HIMs could invalidate conclusions based on the presence or absence of standard SNV biomarkers: it is clinically important to consider them in biomarker-driven treatment decisions.
- Our results may explain why patients with endorsed treatment biomarkers fail to achieve the predicted clinical response and support the need for more holistic approaches to the analysis of predictive biomarkers.
- In contrast to hotspot sequencing and SNP arrays, NGS-based whole gene sequencing enables general assessment of biomarker genes. It consequently may allow more precise cancer diagnostics and may benefit treatment decisions.
- Reassuringly, there are few false positives. In other words, the conclusions based on biomarker mutations found to be present seem to be largely reliable, at least in the investigated cohorts.
- There appear to be many false negatives, for instance in the gene TP53 in the breast cancer cohort. The amount of false negatives may even be correlated to treatment response.

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The results shown here are in port based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.