Concordance between PTEN protein expression and gene mutations in the large cohort of cancer patients

Anatole Ghazalpour, Wangjuh Sting Chen, Ryan Bender, Wen Wenhsiang, Gargi Basu, Zheng Tan, Zoran Gatalica
Caris Life Sciences

Abstract

Background: PTEN is a tumor suppressor gene in signaling downstream of EGFR. Loss of PTEN protein expression is one of the more common occurrences in human cancers, and its loss potentially reduces the benefit from trastuzumab and EGFR-targeted therapies and mTOR inhibitors. Loss of PTEN is usually assessed with immunohistochemistry (IHC). Mutation analysis of PTEN gene has been recently introduced in clinical use. In this study, we compared the concordance between PTEN IHC and PTEN sequencing technologies using the largest cohort of patients published thus far.

Methods: 6647 patients and 29 tumor types were utilized in this study. TruSeq Amplicon - Cancer Panel on the Illumina's Miseq that employs 7 amplicons to sequence exons 1, 3, 6, 7, and 8 of PTEN gene, and immunohistochemistry using anti-PTEN clone6H2.1(DAKO) were used in this study.

Results: Overall, 5% of the samples contained mutations in PTEN gene. Of the 356 variations identified, 44.9% were frameshift, 23.9% nonsense, 27% missense, 2.5% inframe indels, and 1.7% affecting splicing. When compared to IHC results, significantly larger number (29% or 1859 out of 6431) of patients lacked PTEN protein expression (defined as less than 50% tumor cells staining positive). 25% of the samples that were called wild type by sequencing did not show PTEN expression and 40% of the samples that contained a mutation in PTEN expressed PTEN in IHC. Among PTEN mutations, the largest discrepancy was seen with missense mutation at 52%. In contrast, of the negatively stained samples only 14% were called mutant by sequencing whereas 94% of samples that stained positively by IHC were called wild type by sequencing results.

Conclusion: These observations suggest that there is very little correlation between sequencing and IHC results for PTEN. We conclude that neither the IHC nor the sequencing alone have a full capability to predict PTEN status, but when combined, these two technologies provide a more complete assessment of PTEN status in patients. Additional methods (methylation assays, LOH assays) may be needed to correctly assess PTEN status in tumors.

* We have updated the information for poster presentation therefore the data and the results presented in this poster deviate from the originally submitted abstract. The conclusions drawn from this updated analysis however did not change the conclusions made originally when the abstract was first submitted.

Background

PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that prevents cells proliferation. Loss of PTEN protein is one of the most common occurrences in multiple advanced human cancers. PTEN is an important mediator in signaling downstream of EGFR, and its loss is associated with reduced benefit to trastuzumab and EGFR-targeted therapies. In addition, intra-tumoral PTEN loss has been associated with benefit from mTOR inhibitors (everolimus, temsirolimus) (1).

A common methodology to assess loss of PTEN in tumor samples is immunohistochemistry (IHC). With the recent improvement in sequencing technology, a paradigm shift in loss of functional PTEN assessment has been gaining popularity among clinical labs, that is to sequence the PTEN coding regions and assess its loss of function based on the type and the exonic location of the variations. Latest data however suggests that despite the ability to sequence PTEN, IHC analysis is still superior over sequencing in identifying samples with loss of functional PTEN (2). This study, however, utilized limited sample size (154) and focused only on endometrial cancer and its subtypes. In this study, we compare the results between PTEN IHC and PTEN sequencing technologies using larger cohort and across various lineages.

Methods

A total of 6647 patient samples spanning across 29 lineages were utilized in this study. These samples were sequenced for 48 genes using the TruSeq Amplicon - Cancer Panel (TSACP) on the Illumina's Miseq platform for the Next Generation Sequencing. Included in the TSACP panel are 7 amplicons spanning exons 1, 3, 6, 7, and 8 (exons 7 and 8 are represented by 2 amplicons each) of the PTEN gene (Refseq ID NM_000314.4). These amplicons allow one to detect variants present in the two functional domains of PTEN protein (the catalytic domain and the lipid binding domain). More specifically, these amplicons cover 27% of the nucleotides (141 out of 516 nucleotides) coding for the catalytic domain and 87% of the nucleotides (419 out of 483 nucleotides) coding for the lipid binding domain of the PTEN protein. For immunohistochemistry anti-PTEN clone6H2.1(DAKO) was used in this study.

Results

From the 6647 samples tested 5% (Fig.1b) of the samples contained mutations. When samples and mutations were stratified by the cancer lineage, Female Genital Tract Malignancy had the most frequent PTEN mutation whereas Pancreatic Adenocarcinoma had the least number of samples with PTEN mutation. A few lineages such as Hepatocellular Carcinoma did not have any PTEN mutations (Fig.1a).

Overall, we detected a total of 356 variants representing various mutation classes including frameshift, Indels, mutations at splice junctions, and both missense and nonsense substitution mutations (Fig.1c) . The most frequent mutation detected were frameshift mutations (44.9%) and the least frequent were mutations affecting splicing (1.7%). The majority (over 60%) of the missense mutations detected in the PTEN gene affected the catalytic domain of the PTEN gene (Fig.1d). We also detected mutations both in the lipid binding domain and the non-conserved regions of the PTEN gene (Fig.1d)
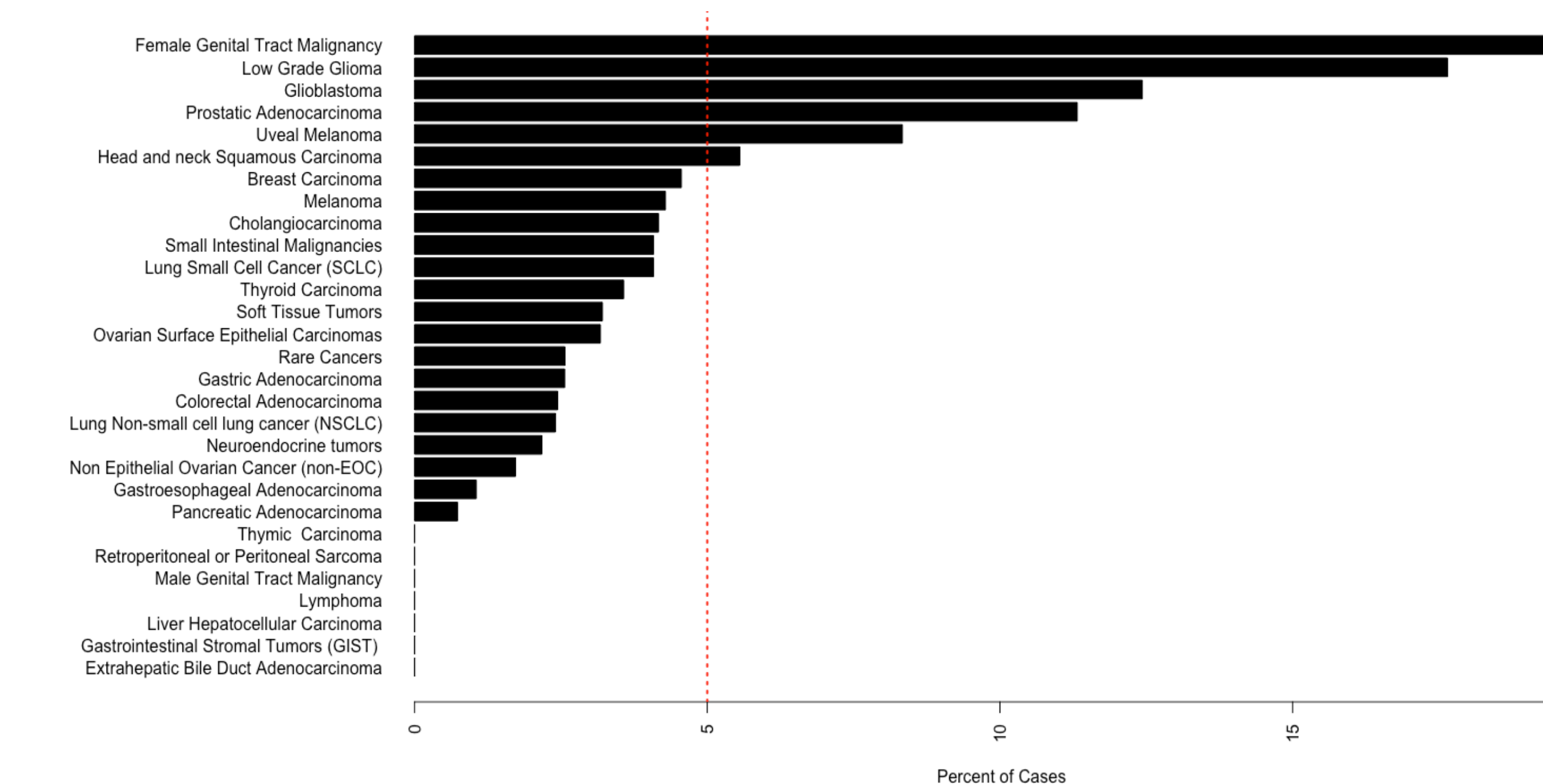

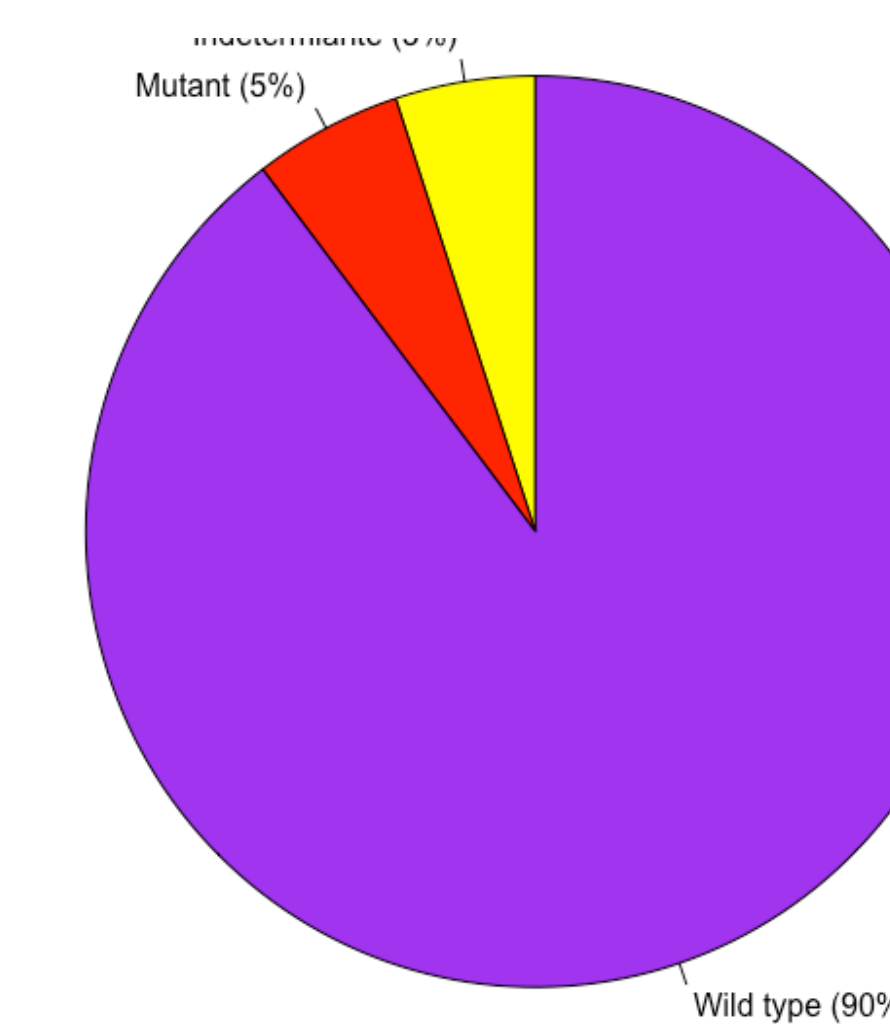**Figure 1a** – Frequency distribution of various cancer types containing PTEN mutation.

Figure 1b – A piechart illustrating the frequency of PTEN mutations detected in 6647 patient samples tested.
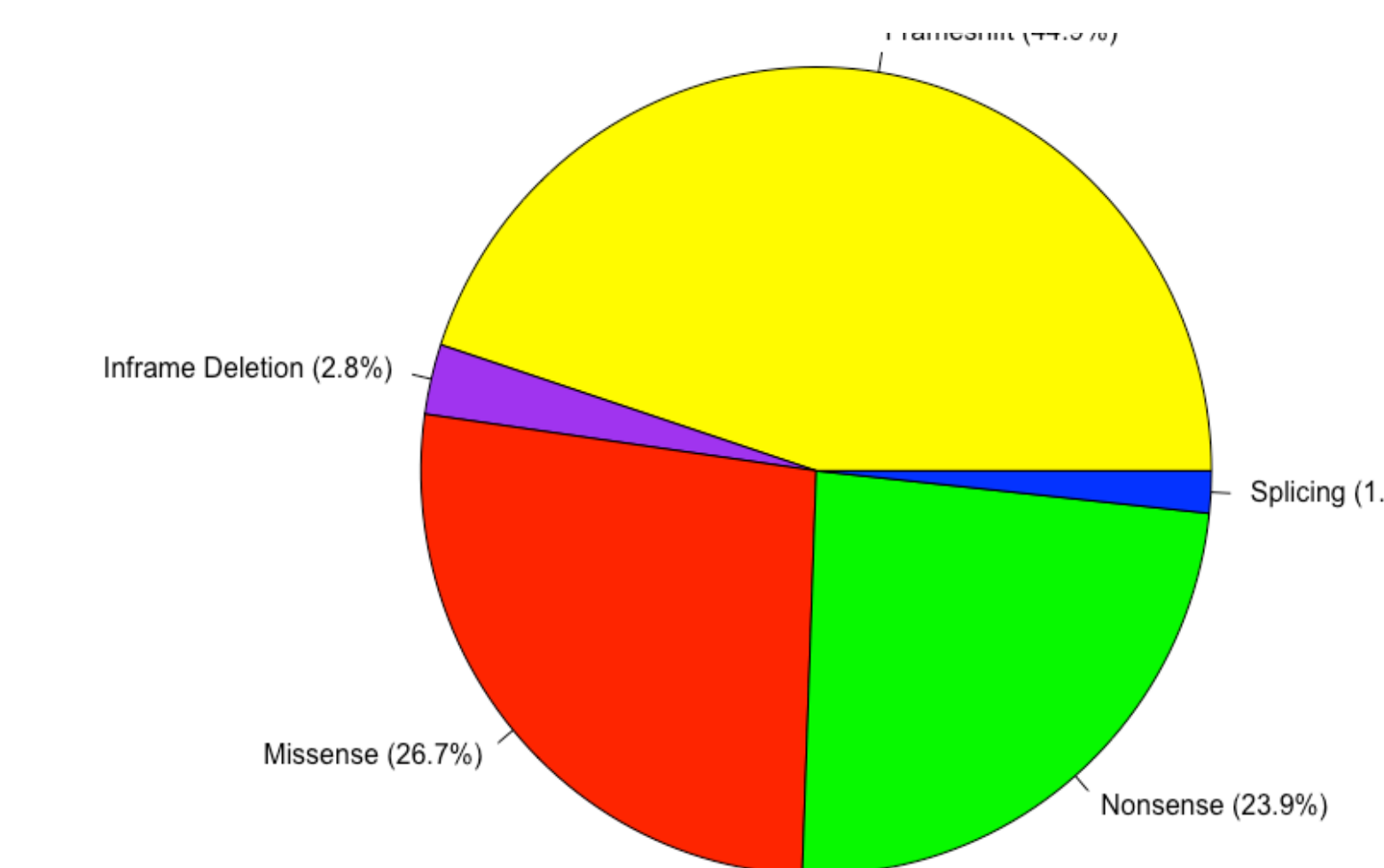
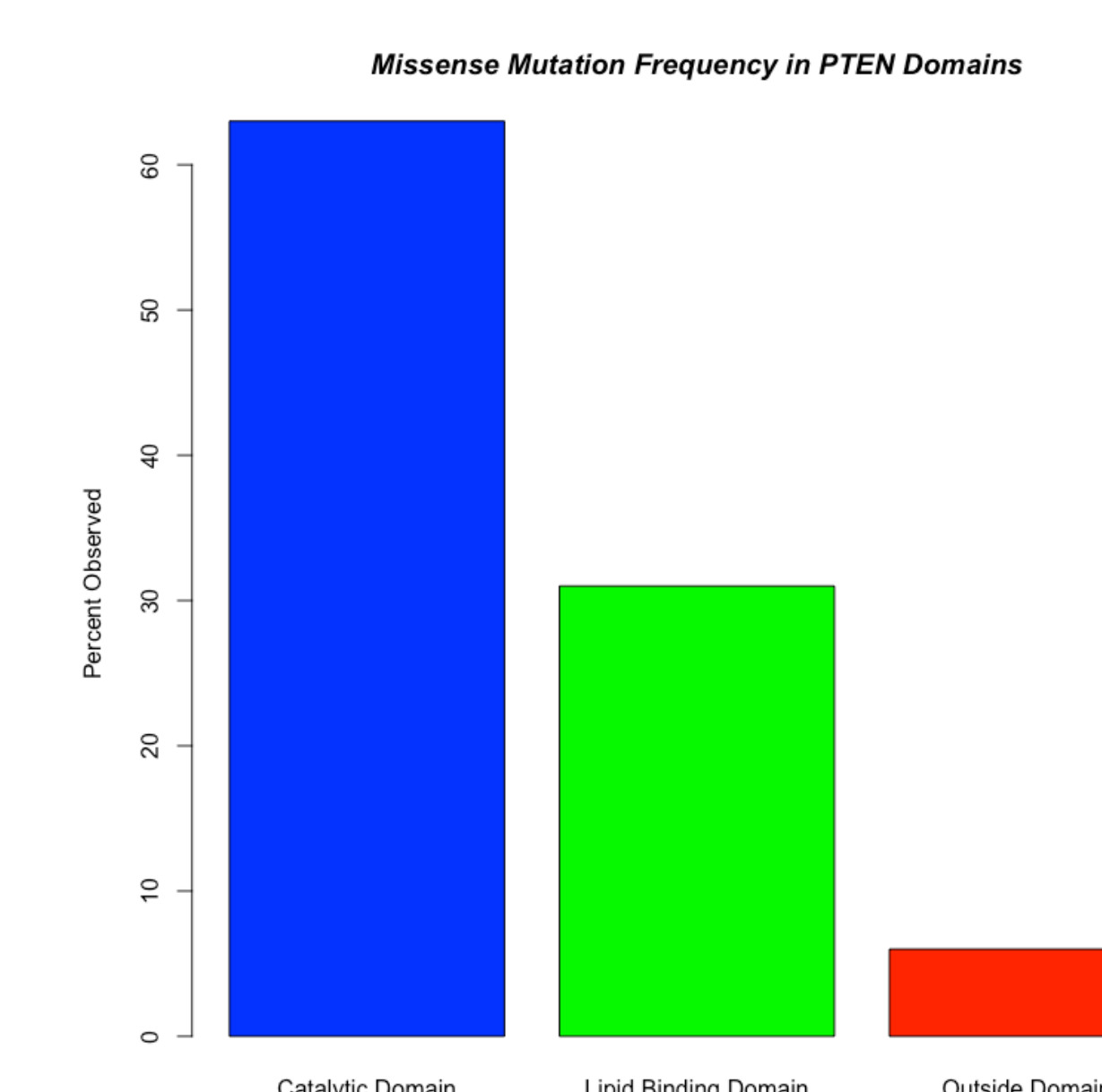**Figure 1c** – A piechart illustrating the frequency of various classes of PTEN mutations.

Figure 1d – A barplot representing the frequency of mutations found in conserved and non-conserved regions of the PTEN gene.

Comparison of IHC results with next generation sequencing results revealed very little correlation between the two technologies. Overall, significantly larger number of samples exhibited lack of PTEN protein expression compared to samples with mutation in the PTEN gene (29% vs 5%). One fourth of the samples that were called Wild Type by sequencing stained negatively by IHC and 40% of samples that contained mutations in the PTEN gene expressed the PTEN protein as assessed by the IHC. When stratified by mutation type, the largest discrepancy was seen for missense mutation (52%) (Fig.2). We also analyzed the data to see whether IHC data can predict the sequencing results. Of the negatively stained samples by IHC only 14% were called mutant by sequencing, whereas 94% of samples that stained positively by IHC were called wild type by sequencing results.
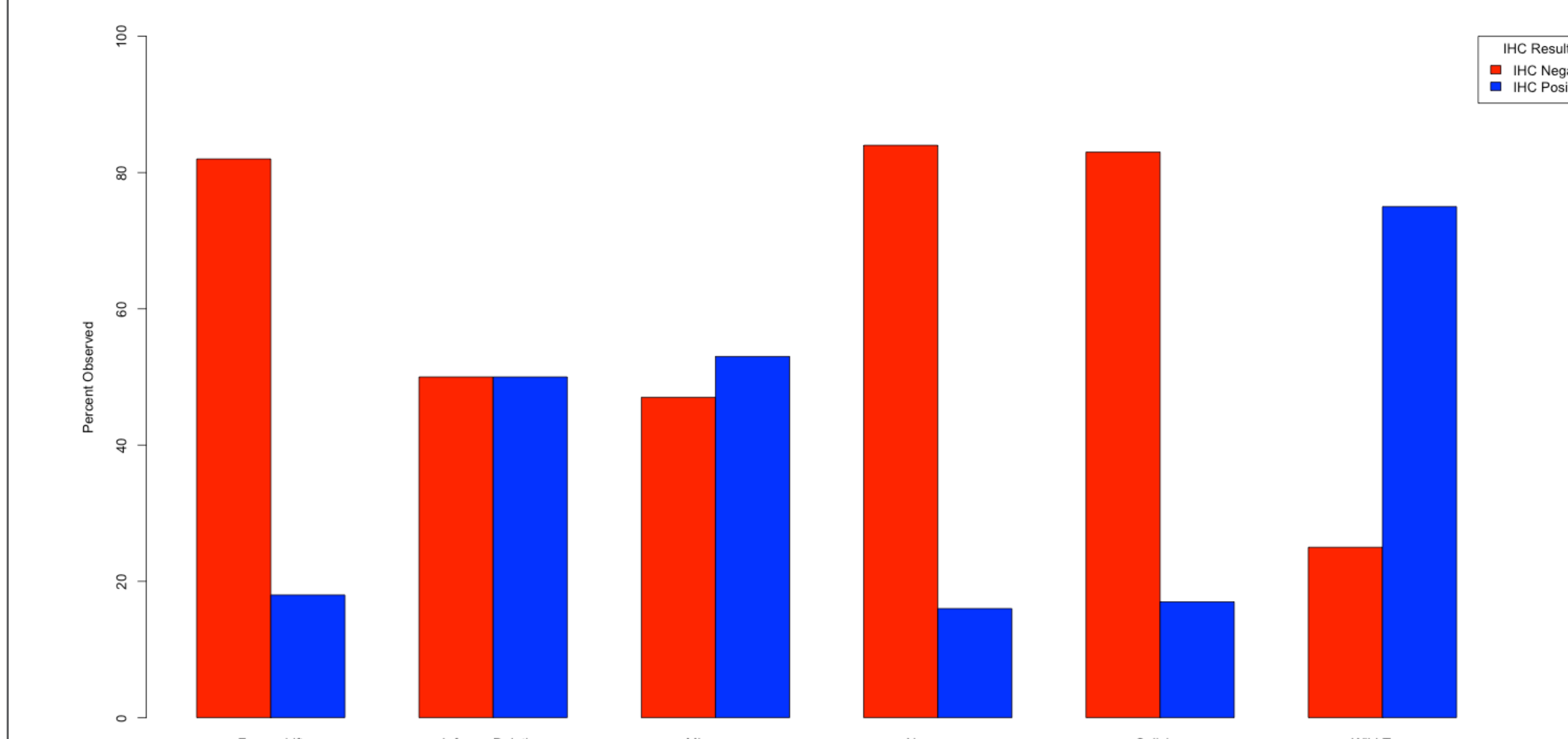

Figure 2 – Comparison of IHC results with the mutation types detected by sequencing . Samples in red bar are those stained negatively by IHC and blue bar represents samples stained positive.

Conclusions

- There is very little correlation between sequencing and IHC results for PTEN.

- Neither the IHC nor the sequencing alone have a full capability to predict PTEN status, but when combined, these two technologies provide a more complete assessment of PTEN status in patients.

- Majority of PTEN mutations affect the catalytic domain of PTEN

References

1. Meric-Bernstam et al, *PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosterin mTOR inhibitors.* Clin Cancer Res. 2012, March; 18(6):1777-89

2. Djordjevic et al, *Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing.* Mod. Path. 2012, May; 25(5):699-708