

Biomarker Expression in Head and Neck Squamous Cell Carcinoma – Implications for Therapy

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Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is an aggressive disease with an unpredictable prognosis. Failure of first-line treatment is common, thus additional therapeutic options are in great need. The purpose of this study was to explore biomarker expression profiles of HNSCC for therapeutic strategies that are not commonly pursued.

Methods: In a cohort of 166 patients with HNSCC, biomarkers that are useful in determining sensitivity or resistance to various chemotherapies were analyzed. Expression of ERCC1 (8F1), MGMT(MT23.3), MRP1 (33A6), PGP (C494), RRM1 (polyclonal), TOPO1 (1D6), TOPO2A (3F6) and TS (TS106) were assayed by immunohistochemistry (IHC) on a Ventana platform. Slides were then evaluated by a pathologist using intensity grades (0-3) and percentage of tumor cells staining (0-100%). Association of potential benefit and lack thereof was determined based on defined thresholds.

Results: The distribution of percentages across all biomarker categories (relative expression) was analyzed by non-parametric Chi Square tests. All differences between relative expression levels attained statistical significance ($p < 0.001$). Our observations demonstrate that the most frequently over expressed biomarkers in HNSCC are MRP1 (90.4%), TOPO2A (79.5%), MGMT (76.7%), ERCC1 (51.9%) and RRM1 (44.1%). Conversely, the biomarkers with greatest frequency of negative expression are PGP (92.2%), TS (58.3%) and TOPO1 (57.6%). Based on these data, a large percentage of patients in this cohort may have a little benefit from cisplatin or carboplatin based on ERCC1, irinotecan or topotecan based on TOPO1, gemcitabine based on RRM1 and temozolomide based on MGMT. Contrarily, based on the frequency of negative expression values for TS and above threshold values for TOPO2A, this cohort may have an increased benefit from 5-fluorouracil or pemetrexed, and doxorubicin or etoposide, respectively. Lastly, the distribution of markers for the classical drug pumps, MRP1 and PGP were in complete opposition. MRP1 was above threshold in 90% of the cohort, indicating resistance to agents like doxorubicin, etoposide and methotrexate, whereas PGP was negative in 92% of cases, suggesting a potential increased benefit from paclitaxel or docetaxel.

Conclusion: To our knowledge, this is the first comprehensive analysis of biomarkers associated with various chemotherapeutic agents in HNSCC. HNSCC is a challenging disease to treat, with few therapeutic options after failure of first-line regimens. Our data suggest that the fluoropyrimidine, 5-fluorouracil, folate analog, pemetrexed, and the taxanes, paclitaxel or docetaxel, are potential treatment options for HNSCC as demonstrated by the preponderance of negative expression values of TS and PGP, respectively, in this cohort.

Background

Head and neck cancer is the fifth most common malignancy, with 780,000 new cases per year worldwide. The incidence rate is twice as high in men as women. Head and neck squamous cell carcinoma (HNSCC) is an aggressive disease with an unpredictable prognosis, where many cases involve regional and distant metastasis on initial presentation. Surgery is the first course of therapy, followed by concurrent radiotherapy and chemotherapy. Unfortunately, failure of first-line treatment is common, leaving the clinician with limited options thereafter. Therefore, additional therapeutic options are in great need, especially in these advanced and metastatic settings.

To advance the field of head and neck cancer management, one consideration involves molecular tumor profiling. By examining the genetic and molecular changes unique to a malignancy, these techniques provide new insights and understanding into HNSCC, with the end-goal of elucidating potential therapeutic targets. One method, specifically, involves immunohistochemistry (IHC), frequently utilized in the clinical setting for its inexpensive, efficient means of obtaining valuable information.

The aim of this study was to explore biomarker expression profiles of HNSCC for therapeutic strategies that are not commonly pursued, regardless of the etiology (viral (HPV) vs. carcinogenic (tobacco and/or alcohol consumption).

Methods

A retrospective cohort study of 166 HNSCC patients were profiled with Caris Target Now™ (Caris Life Sciences, Phoenix, AZ) between July 2009 and July 2011. Four-micron sections were prepared from formalin fixed, paraffin embedded blocks and biomarkers predictive of various chemotherapy responses were analyzed by immunohistochemistry. The expression of ERCC1 (8F1), MGMT (MT23.3), MRP1 (33A6), PGP (C494), RRM1 (polyclonal), TOPO1 (1D6), TOPO2A (3F6) and TS (TS106) were assayed on a Ventana or a Dako platform in a CLIA certified lab, evaluated independently by board certified pathologists and categorized into above threshold, below threshold (intermediate) or negative based on previously defined cutoffs ($\geq 1+$ intensity and $\geq 10\%$ cancer cells population for PGP, MRP1 and TOPO2A, $\geq 1+$ and $\geq 50\%$ for MGMT, $\geq 2+$ and $\geq 30\%$ for TS and TOPO1, $\geq 2+$ and $\geq 50\%$ for RRM1 and $\geq 2+$ and $\geq 10\%$ for ERCC1).

All data were analyzed using SPSS (PASW Statistics 17). In order to ascertain distributional differences, nonparametric chi-square tests of independence were employed. In addition, correspondence analysis was used to explore specific biomarker distributions across identified clusters.

Results

Figure 1a displays the molecular profile of the total cohort (N=166) and depicts the relative distribution of “above threshold” (grey), “below threshold” (pattern fill), or “negative” (red) IHCs, including cases that yielded no result (black). Biomarkers are listed in order of decreasing number of available IHC stains. Non-parametric Chi Square tests identified significant differences in the relative distribution for ERCC1, MGMT, MRP1, PGP, RRM1, TOPO2A, TOPO1 and TS ($p < 0.001$). The most frequently over-expressed biomarkers (Figure 1b.) were MRP1 (90.4%), TOPO2A (79.5%), MGMT (76.7%), ERCC1 (51.9%) and RRM1 (44.1%) which are therapeutically correlated to potential resistance to temozolomide (MGMT), platinum agents (ERCC1) and gemcitabine (RRM1), while the above threshold values for TOPO2A would suggest potential benefit to anthracyclines or etoposide. The biomarkers with the highest relative underexpression (Figure 1c.) included PGP (92.2%), TS (58.3%) and TOPO1 (57.6%). In terms of therapeutic targets, TS could likely contribute to benefit from fluoropyrimidines and folate analogs, while TOPO1 relates to potential resistance to irinotecan/topotecan.

Figure 1a

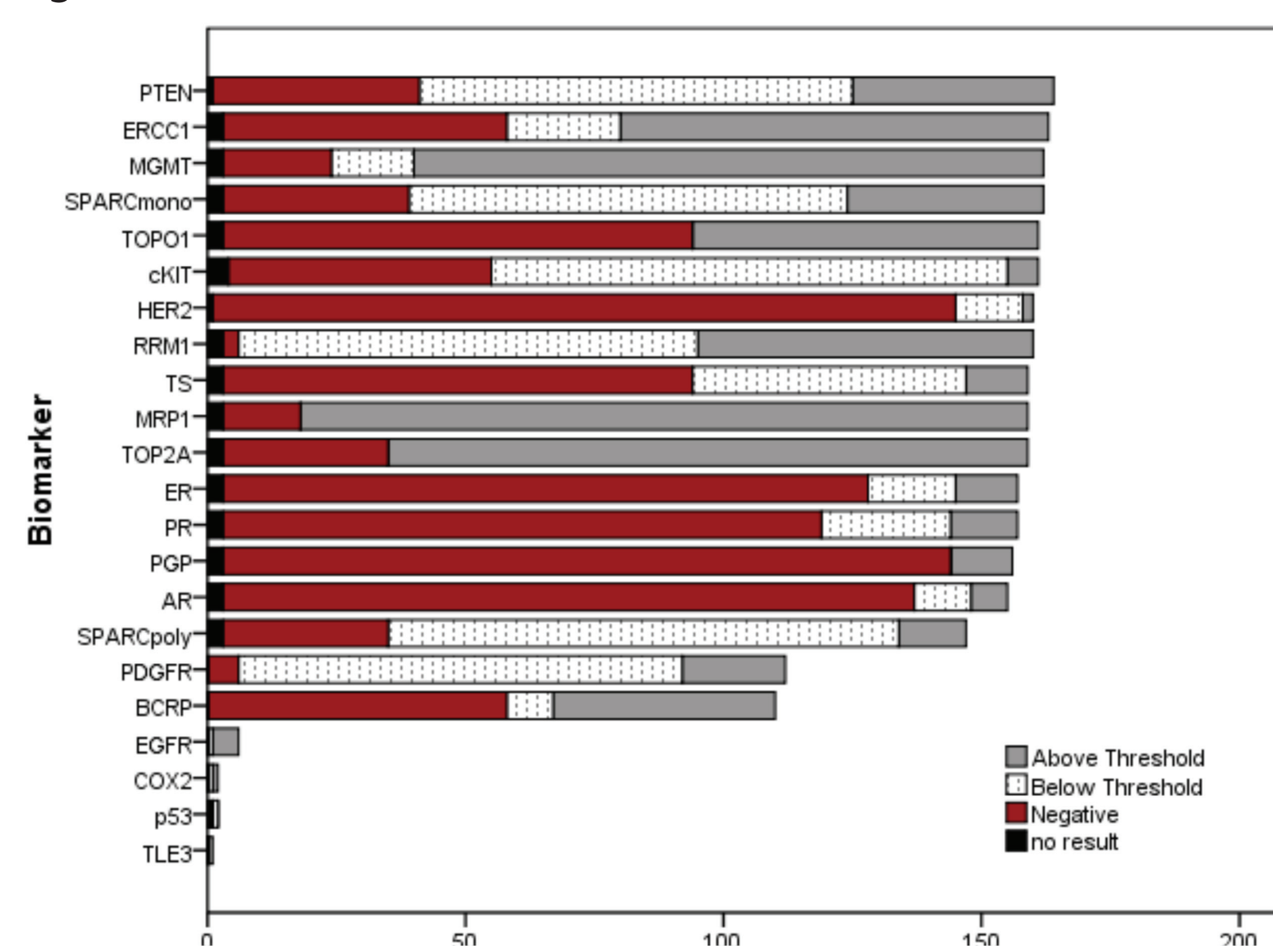


Figure 1b

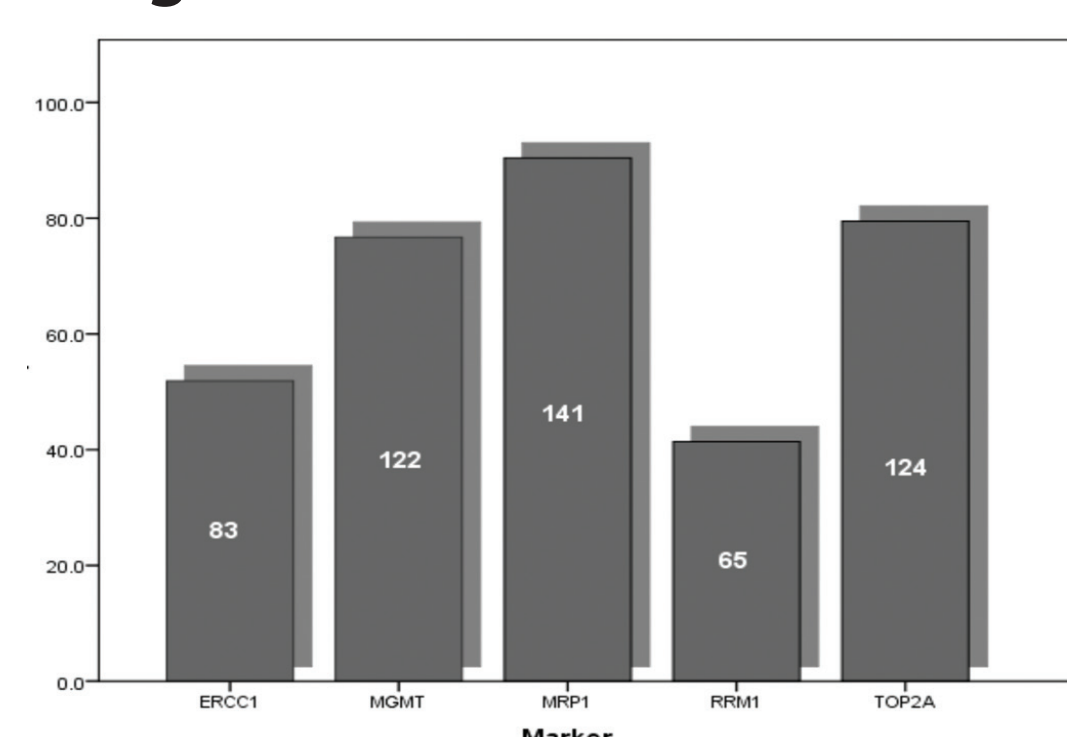


Figure 1c

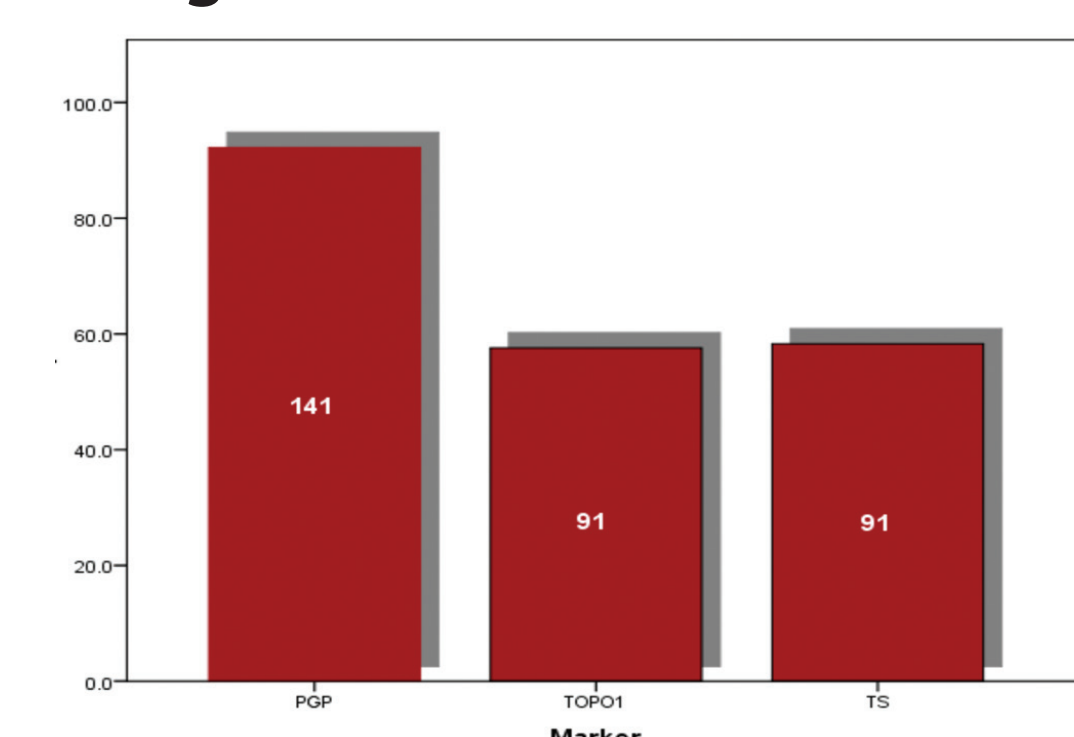


Figure 1a. Molecular profile of the total sample (N=166). Histogram displays the distribution of above threshold (grey), below threshold (pattern fill), or negative (red) IHCs, including cases that yielded no result (black). Markers are listed in order of decreasing number of IHC stains.

1b-1c. Graphical representations of biomarkers with the highest relative overexpression (b) and underexpression (c). Y axis denotes percent, numbers in stacks denote case number (n).

Expression of the classical drug pumps, PGP and MRP1 displayed a marked inverse relationship (Figures 2a.-2b.). The results of the correspondence analysis (bi-dimensional representation) are shown in Figure 2c. Overexpression of MRP1 likely contributes to resistance to doxorubicin, etoposide and methotrexate, whereas PGP suggests a potential benefit from taxanes. Collectively, our data suggest 5-fluorouracil, pemetrexed, docetaxel and paclitaxel are potential treatment choices for HNSCC based on the preponderance of negative expression values of TS and PGP, respectively.

Figure 2a

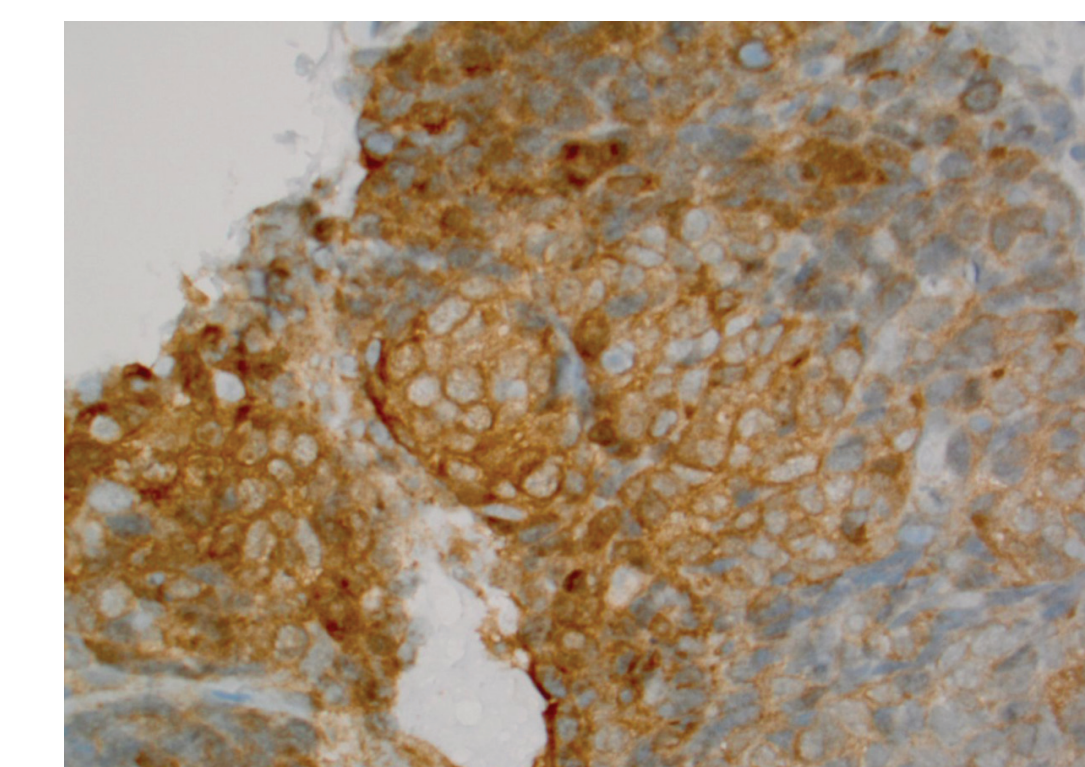


Figure 2b

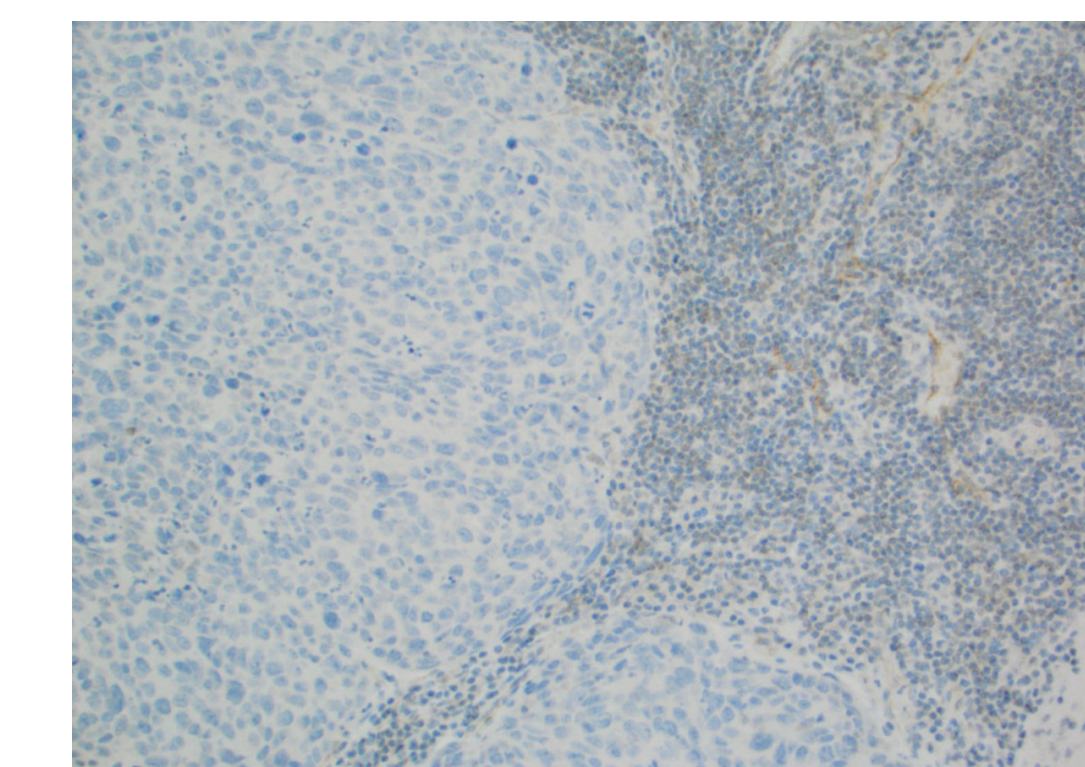


Figure 2a. and 2b. – representative IHC stains for MRP1 over-expression (40x) and PGP underexpression (20x).

Figure 2c

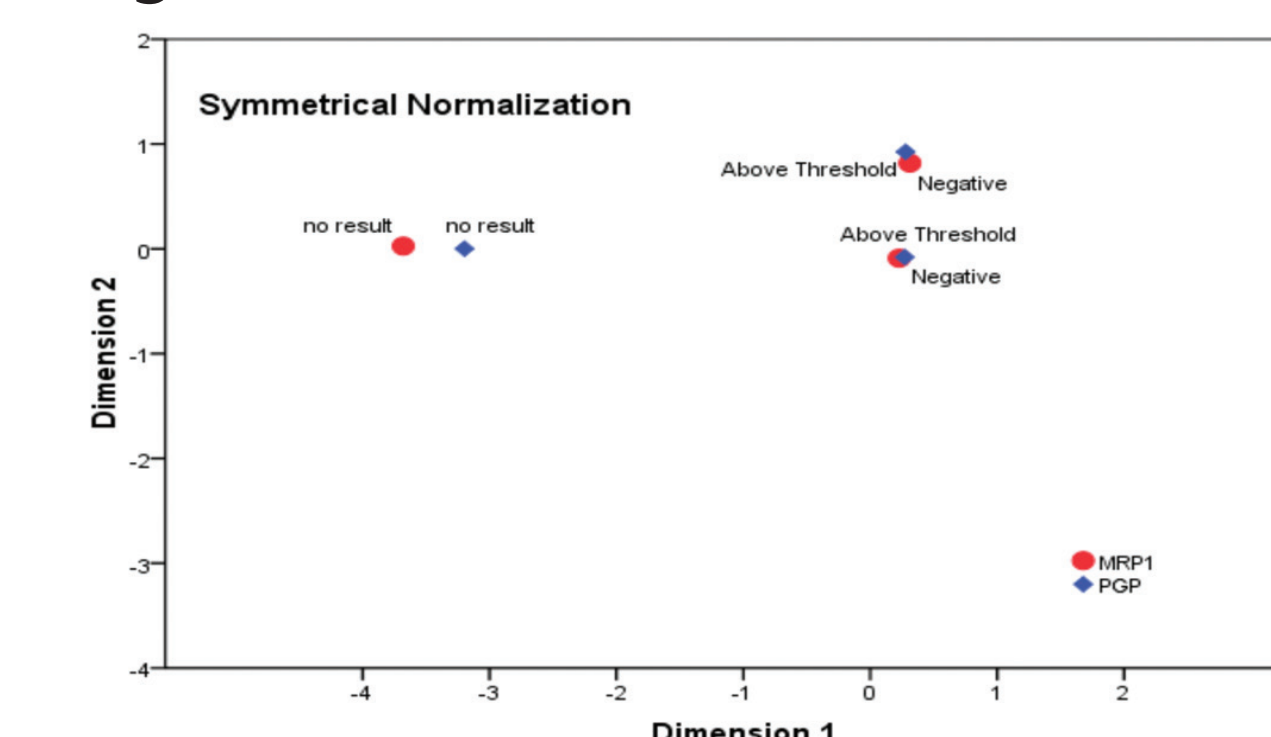


Figure 2c. – correspondence analysis regarding the relationship between PGP and MRP1 (bi-dimensional representation).

Study Highlights

- HNSCC is an aggressive disease where first-line treatment failure is common.
- 166 HNSCC tumor samples were profiled for insight on treatment options based on expression of biomarkers predictive of various chemotherapies.
- Differences in relative distribution were significant for ERCC1, MGMT, MRP1, PGP, RRM1, TOPO1, TOPO2A and TS.
- Expression data suggest this HNSCC cohort shows a trend towards resistance to platinum agents, anthracyclines, temozolomide, gemcitabine, irinotecan or topotecan and sensitivity to fluoropyrimidines and taxanes.
- Expression of the classical drug pumps displayed a marked inverse relationship with most of the HNSCC cohort having overexpression of MRP1 and underexpression of PGP.

Conclusions

- The most frequently over-expressed biomarkers were MRP1 (90.4%), TOPO2A (79.5%), MGMT (76.7%), ERCC1 (51.9%) and RRM1 (44.1%). Overexpression data may suggest a lack of response from temozolomide, gemcitabine, and platinum agents and therapeutic benefit from anthracyclines.
- The most frequently under-expressed biomarkers in HNSCC are PGP (92.2%), TS (58.3%) and TOPO1 (57.6%). Under expression of TS in more than half of the tumor samples may indicate therapeutic benefit from pemetrexed and fluorouracil in HNSCC patients.
- Although PGP is under-expressed in 92.2% of HNSCC tumor samples, multiple drug resistance is active in HNSCC as MRP1 is over-expressed in over 90% tumors. A superior treatment option may be identified by understanding the substrate specificity differences between PGP and MRP1.
- Our study provides data that might be used to aid in selecting primary or adjuvant therapy, evaluating new therapies, determining prognosis and assessing outcome of HNSCC patients.

References

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