Predictive and prognostic molecular markers in solid tumors

Ph.D. thesis

Brauswetter Diána M.D.

Semmelweis University
Doctoral School of Molecular Medicine

Supervisor: Dr. Peták István, Ph.D., senior research fellow

Official reviewers: Dr. Törőcsik Beáta, Ph.D., senior lecturer
Dr. Tátrai Péter, Ph.D., senior scientist

Theoretical exam committee:
Chairman: Dr. Tretter László, D.Sc., professor
Members: Dr. Csermely Péter, D.Sc., professor
Dr. Reményi Attila, Ph.D., senior research fellow

Budapest
2017

1. Introduction

In the past decades, a change of attitude can be observed in cancer therapy. The Human Genom Project has given an insight into the human DNA and many researches begun to focus on the genetic background of diseases, such as malignances. This process made it possible to define specific targets in tumor subgroups and with their inhibition we can achieve greater efficacy in tumor treatment. These one or multiple target specific therapeutic agents are also called “target therapies”. However, tumor subgroups rarely follow anatomy, histology, but rather are based on other biomarkers (mutation, expression, genealogy change), on the molecular background of the tumor. Recently similar shifts in the design of clinical trials can be observed. In addition to the histological diagnosis and location-based studies, molecular biomarker-based so-called “genotyped matched” clinical trials are slowly gaining ground in which the survival of the selected patients was significantly better than the whose therapy was chosen based on the conventional, anatomical or histological criteria. In addition, besides retrospective, prospective biomarker analyzing studies are also appeared where, in addition to the outcome of the disease, the detailed analysis of tumors is also the goal of the clinical trials.

In our research, we studied head-neck and pancreatic cancers based on their frequency observed in cancer statistics and their extremely unfavorable incidence and mortality rates in Hungary.

For both examined tumor types, there are only a few approved and in the clinical practice available tyrosine-kinase inhibitors. The erlotinib EGFR-inhibitor could not reach relevant improvement in survival when using in the treatment of pancreatic adenocarcinoma, which can maybe explained with the very common KRAS mutations (70-90%) that can lead to the resistance to EGFR-inhibítors in these tumors. KRAS could be an ideal target, but its inhibition is currently possible only indirectly, for example with MEK-inhibitors. However, these therapies also failed in clinical trials, remission was only observed in a small fraction of patients. This can be due to a feedback mechanism through EGFR/PI+K/Akt pathway. We also need adequate molecular markers to select the sensitive tumors. For head and neck cancers, the only target therapy, that is used at the clinics is cetuximab, an anti-EGFR monoclonal antibody. Currently we do not have an appropriate biomarker to predict its efficacy. In addition, localization-specific or HPV-status (which occurs often in this tumor types) associated molecular markers could also

contribute to the use of more potent therapeutic agents with less toxicity in certain molecular subgroups. Besides EGFR, PIK3CA, MET and Src protein could be possible targets, whose mutation or overexpression can often be observed in these tumors.

The more accurate analysis of tumors and patients, revealing their genetic background, makes it possible to choose the most effective, personalized anti-cancer treatment.

2. Aims

We investigated archived (formalin-fixed, paraffin-embedded, FFPE) tumor tissue samples that provided a better insight into the differences in DNA, mRNA and protein-level in tumors. We used them to identify the genetic background of tumors. (in various localization.) So we could identify possible targets that can be the same even in the tumors of the head and neck region or in pancreatic cancers regardless of localization. In vitro experiments with tumor cell lines were used to investigate the found biomarkers as possible targets of anti-cancer therapy.

Our aims were the following:

1. **Investigation of archived FFPE tissue samples at DNA and protein levels to find molecular markers that are potential targets or can be used prognostic or predictive markers in tumor therapy.** We aimed to examine the change in the copy number of PIK3CA and MET genes with the use of fluorescent in situ hybridization. In addition, our goal was to compare the prognostic value of $p16^{INK4}$ immunohistochemistry and HPV DNA detection.

2. **In vitro studies with cancer cell lines, analyzing their genetic background and using them to model the effect of possible tyrosine-kinase inhibitor treatments.** We aimed to determine the persisting mutations in the examined cell lines by next-generation sequencing. We planned to investigate the protein expression and signaling activity as well.

3. **Determination of subgroups based on molecular biomarkers in which a certain monotherapy or combination therapy has better effect**. We aimed to determine the sensitivity of the cell lines to specific inhibitors by viability assays. In case of resistance, we aimed to reveal the mechanism and find potentially effective combinational treatments.

3. Methods

Histological methods

Data collection, patients

Two cohort of head and neck cancer patients were included into our study. The first cohort consisted 152 patients being treated between 2000 and 2008 at the Department of Oto-Rhino-Laryngology of Jahn Ferenc Hospital. The other cohort included 124 patients having head and neck squamous cell carcinoma. Each patient underwent treatment between 2012 and 2014 at the Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Semmelweis University.

Sanger sequencing and next generation sequencing data of 138 pancreas tumors were collected anonymously from the database by the molecular diagnostic laboratory of Oncompass Medicine Hungary Kft. All the sequencing reactions were carried out between 2012 and 2016.

Histological processing, staining and evaluation

In case of both head and neck cancer cohort 7-7 TMA-blocks were created from the FFPE tumor samples. Tissue sections were cut and mounted on adhesion slides and used for histological methods.

Immunohistochemical (IHC) staining (p53, $p16^{INK4}$, EGFR, Ki-67, connexin 43) and fluorescent in situ hybridization (FISH) (*MET* + centromere 7, *PIK3CA* + centromere 3) were carried out.

Slides were digitized and analyzed with Panoramic Viewer software (3DHISTECH). In case of Ki-67, p53 and connexin 43 scoring was performed using a 4-grade system (Score 0: <5% weak staining; score 1: 6-20% positive tumor cells, score 2: 21–60% positive tumor cells; and score 3: >60% positive tumor cells). Cut-off for p16 positivity was set at 75% of cytoplasmic or nuclear staining. An alternative 3-grade scoring approach was used for the evaluation of EGFR protein expression: the percentage of stained cells was multiplied by the grade intensity of staining (in four grades). Cores with scores 0 to 200, 201 to 300 and 301 to 400 were referred to as negative, intermediate or high protein expression, respectively. In case of FISH, the evaluation system of Cappuzzo et al. was used. This scoring method is based on the number of chromosome and locus specific signals in the tumor cells. Patients were classified into the following groups: disomy, low trisomy, high trisomy, low polysomy, high polysomy and gene amplification.

HPV (human papillomavirus) Genotyping

During the second cohort HPV genotyping was carried out. DNA was extracted from FFPE tissue sections in case of p16 positivity. High-risk HPV DNA detection was performed using CONFIDENCE™ HPV test in NEUMANN Diagnostics, Hungary.

In vitro methods

Cell culturing

Pancreas adenocarcinoma cell lines MiaPaCa2, BxPC3 and Panc1 and head-neck cancer cell lines FaDu and Detroit562 were obtained from ATCC and cultured in appropriate medium and conditions.

DNA extraction and next generation sequencing of cancer cell lines:

The same method was used for the next generation sequencing of cell lines as in case of FFPE tumor samples. Following the DNA extraction DNA libraries were prepared using Ion AmpliSeq™ Library Kit 2.0 This target enrichment method allows the analysis of 207 gene fragments in 50 genes. After barcode adapter ligation libraries were purified and sequenced on Ion 318 Chip by Ion PGM equipment in the laboratory of Seqomics Kft. The average depth coverage of the amplicons was about 200 000 reads per sample. Variants were detected in the libraries and listed.

Tyrosine-kinase inhibitors

In our experiments FDA approved or drugs in Phase III clinical trials were used. Cells were treated with EGFR (HER-family)-inhibitors: afatinib, erlotinib, MEK-inhibitors: trametinib, refametinib, PD0325901, selumetinib, Akt-inhibitor: triciribine and Src- inhibitor: dasatinib.

Cell viability assay and drug synergism

Cell viability was measured with the CellTiter-Glo Luminescent Cell Viability assay. Cell lines were left overnight to attach, then treated with decreasing concentrations of the kinase-inhibitor alone or in combination. 72 hours after treatment, appropriate amount of CellTiter-Glo Reagent was added to the cell culture medium in each well and luminescent signal was recorded.

Potential drug synergism was confirmed and combination index at different effective doses (ED) was calculated with Compusyn software, which is based on the Median-Effect Principle and the Combination Index-Isobologram Theorem.

Combination indexes generated by Compusyn indicate drug synergism under 1 and additive effect between 0.75 and 1.25. In this research combination indexes were calculated in a constant concentration ratio of the used drugs.

Western blot analysis

The protein expression and phosphorylation of the certain cell lines were investigated by Western blot analysis. Cells were allowed to attach and treated or not with inhibitors. Then, cells were lysed in lysis buffer and ysates were centrifuged with 13 000 *g* at 4°C for 15 minutes. 5-30 μg protein was subjected to SDS-PAGE and electrotransferred to polyvinylidene-difluoride (PVDF) membranes. Membranes were incubated with the diluted primary antibodies [EGFR, Phospho-EGFR (Tyr1068), Akt (pan), Phospho-Akt (Ser473), p44/42 MAPK (Erk ½), Phospho-p44/42 MAPK (Thr202/Tyr204), connexin 43 and α-tubulin antibodies. Bands were visualized by Enhanced Chemiluminescence (ECL) detection system and quantified by ImageJ v1.48 software.

Fluorescent immunocytochemistry

Cells were grown on coverslips and fixed with 4% buffered formalin. Following the permeabilization with 0.1% Triton-X containing buffer connexin 43 and α-tubulin primary antibodies were applied. Fluorescent secondary antibodies (Alexa-Fluor 488 and Alexa-Fluor 546) and DAPI were used and slides were examined by fluorescent microscopy.

4. Results

Predictive and prognostic molecular markers in head and neck cancers

PIK3CA and MET copy number gains have prognostic role

Six different groups were distinguished based on the Cappuzzo criteria. In order to assess differences in disease-specific survival, the groups were compared using Kaplan-Meier estimations with log-rank tests and Cox proportional hazards model. We found that patients having a tumor with *PIK3CA* amplification or high polysomy of chromosome 3 and *PIK3CA* had a worse survival rate compared to the other groups In case of *MET* gene, no tissue sample was found harboring gene amplification. However, in our cohort patients suffering from head and neck cancer with high polysomy of chromosome 7 and *MET* had the worst survival rates, but low polysomy of this gene was also associated with unfavorable outcome.

We therefore distinguished two groups based on the prognostic value of the gene status. For *PIK3CA*, high polysomy and amplification were called "copy number gain (CNG)", whereas for *MET*, CNG included patients having tumors not only with high, but also with low polysomy.

We found a significant correlation between *MET* and *PIK3CA* gene status and disease-specific survival. Patients with a head and neck cancer with normal gene status (without CNG) had significantly longer survival (MET: 31 vs 19 months; p=0.013 and PIK3CA: 23 vs. 16 months; p=0.037)

Absence of p16^{INK4} expression is associated with copy number gain of PIK3CA

Low or absent p16 expression was associated with CNG of *PIK3CA*, but not with CNG of *MET* (p=0.036 and p=0.279, respectively, Table 4.). This finding implies that HPV negative tumors multiply this gene more often than those associated with HPV infection.

EGFR expression and MET copy number gain are negatively correlated

A three-tier scoring system was applied to distinguish groups differing in IHC staining intensity for EGFR. Low or intermediate protein expression in tumor samples was not associated with the presence of CNG. In contrast, high EGFR protein expression was associated with a significantly lower rate of *MET* CNG (p=0.019). No correlation between EGFR expression and *PIK3CA* CNG was observed.

Correlation between copy number variations of MET and PIK3CA and clinicopathological parameters

Chi-square test and Fisher test were used to evaluate correlations between clinicopathological parameters (such as tumor site, stage, grade, T-, N- and M-stages) and copy number variations.

High copy number rate of *PIK3CA* was observed in tumors of the hypopharynx but this did not reach statistical significance using chi-square test. Additionally, supraglottic cancers showed CNG of both studied genes significantly more often than tumors of the glottic region (by Fisher's exact test for PIK3CA p=0.036; for MET p=0.033).

p16^{INK4}-expression in different localization and its prognostic role

In our former study we found significant correlation between disease-specific survival (DSS) and p16^{INK4}-status. p16^{INK4}-positive patients had significantly better prognosis then the p16^{INK4}-negative group. (55 vs. 20 months, p=0.0049)

In case of the 2. cohort 17.3% of the cases proved to be p16^{INK4}-positive. The highest proportion of p16^{INK4} positivity was observed in oropharyngeal tumors (38.1%), whereas other locations showed much lower (larynx: 4.8%, hypopharynx: 4.2%) or no (oral cavity: 0%) p16^{INK4} positivity rate (chi-square test: p<0.001).

Involving all tumor locations, p16^{INK4} status was not significantly associated with DSS (median survival: 17.5 vs. 30.3 months, p=0.107).

In contrast, p16^{INK4}-positive oropharyngeal tumors showed an improved DSS compared to the p16-negative oropharyngeal cancers (median survival: 30.3 vs. 8.8 months, p<0.001) We found no correlation between p16^{INK4} expression and T, N or M status, stage, tumor grade or patients' age. This latter observation was true comparing both tumors of all regions and tumors of the oropharynx.

Prognostic role of HPV-status and the specificity of p16^{INK4}-staining for its detection

The p16^{INK4}-positive cases were tested for 7 high-risk HPV subtypes using DNA PCR method. Out of 19 cases, 9 patients proved to be HPV DNA positive (HPV-positive). HPV 16 was present in 8 cases, HPV 33 in one single case. All the HPV positive samples originated from the oropharynx. This means an overall 21.4% HPV positivity among the 42 patients with oropharyngeal cancer. Thus, the

specificity of p16 to detect oropharyngeal HPV-positivity was 56,3% (out of the 16 $p16^{INK4}$-positive oropharyngeal tumors, 9 cases tested positive for HPV as well). HPV-positive patients' DSS was significantly better when compared with HPV-negative cases (median survival: 25.9 vs. 9.5 months, p=0.024)

$P16^{INK4}$/HPV DNA Positivity and Smoking

We investigated smoking habits among $p16^{INK4}$-positive cases dividing them into HPV-positive and HPV-negative subgroups. Interestingly, none of HPV-positive patients were current smokers, and 55.4% of them had no history of smoking. On the other hand, 67.7% of HPV-negative patients belonged to current smokers and only 7% of them had no history of tobacco use.

There was a significant difference between the groups (Fisher's exact test: p=0.002).

Association between the molecular background and their sensitivity to tyrosine-kinase inhibitors of head neck cancer cell lines

Western blot analysis was used to evaluate protein expression and phosphorylation of FaDu and Detroit 562 cell lines. The most significant difference was found in MET expression and even more in MET activity, which was more pronounced in Detroit562.

The treatment with dasatinib, a low molecular weight tyrosine-kinase inhibitor, was only effective on the Dertoit562 cell line while we observed resistance in case of FaDu. We found only a minor difference in IC50 values when using afatinib treatment. In both cases, the combination of the two drugs had a synergistic effect, but this did not indicate significant dose reduction for Detroit 562 compared to dasatinib monotherapy. We examined the phosphorylation of the EGFR protein and observed that the relative EGFR phosphorylation was almost doubled when using dasatinib treatment on the FaDu cell line, while in case of Detroit 562 this change was not relevant. It is assumed that in the case of FaDu, which cell line expresses all members of the HER-family in a significant way, the found synergistic effect of the two agents is due to this feedback activation of EGFR-receptor. For the MET-dependent Detroit 562, this feedback does not have a significant role, dasatinib can be effective in monotherapy. The possible success of dasatinib monotherapy can be found in the capability of the Src protein to activate MET, while in FaDu cell line, the blockade of the MET receptor leads to the rearrangement of signal pathways to the direction of EGFR.

Changes in the phosphorylation and membrane localization of connexin 43

We investigated the changes in expression and phosphorylation of connexin 43 protein in the FaDu head and neck cancer cell line when using afatinib and dasatinib treatment. With Western blot analysis we found two other bands over the total-connexin 43 protein-specific signal (P1 and P2) in case of control and dasatinib-treated cells, while when using afatinib these signals disappear and only the unphosphorylated protein was present in the sample . The examined phosphorylation sites are the targets of the Akt protein. Western blot analysis revealed that afatinib treatment decreased the phosphorylation of Akt protein, while after dasatinib treatment, the Akt activity remained significant.

Other researchers have observed that the decreased phosphorylation of connexin 43 was associated with less protein stabilization in the membrane. Fluorescence immunocytochemistry was performed to investigate the changes –caused by afatinib and dasatinib– in membrane localization of connexin 43. We acknowledged with fluorescent microscopy, that dasatinib-treated cells showed more connexin 43 plaques on their surface, than for the afatinib-treated cells.

Predictive molecular markers in pancreatic cancers

The investigation of the NGS results of pancreatic cancer cell lines and tumors

We found the mutation of KRAS in 76.8% of the cases. In 68.9% of the examined tumors, the mutation affected the hotspot at codon 12. Other alterations were found in codon 19 (0.7%), codon 13 (0.7%) and codon 61 (6.5%) the remaining about 23% of the patients had tumors with wild type KRAS. KRAS G12D represented the highest percentage (31.2%) and G12C was relatively rare (1.5%). The rest of codon 12 mutation divided between G12V (21.7%) and G12R (14.5%).

Based on the next generation sequencing results of the 3 cell lines: MiaPaCa2 (homozygote mutations in KRAS G12C, TP53 R248W, NOTCH1 L2457V), Panc1 (homozygote mutation in TP53 R273H and heterozygote mutation in KRAS G12D) and BxPC3 (homozygote mutation in TP53 Y220C and KDR Q472H) their in vitro tumor model can cover the mutational status of 44.4% of the pancreas tumor biopsies.

Connection between the KRAS mutation subtype and the sensitivity to MEK-inhibitors of pancreatic cancer cell lines

We analyzed the inhibitory effect of four frequently used MEK inhibitors. In our in vitro model MiaPaCa2 (KRAS-G12C) showed a very high sensitivity to these inhibitors. The KRAS wild type BxPC3 cell line showed moderate sensitivity while in case of Panc1 (KRAS-G12D), all the MEK-inhibitors proved to be

absolutely ineffective. The lowest IC_{50} concentration was experienced when we treated MiaPaCa2 and BxPC3 cells with the drug trametinib. In order to increase efficiency, we first combined trametinib with an EGFR inhibitor. Afatinib was much more effective in vitro than erlotinib, which is the FDA approved drug in pancreatic cancer therapy [Fig 2A], therefore we used afatinib in combination with trametinib. We could reach extreme low clinically relevant IC50 values and strong synergic effect (CI:0.11) in KRAS wild type BXPC3 cell line. In case of Panc1 there was only additive effect and the IC50 concentrations were very high even in combinations. We also measured the effect of trametinib in combination with the Akt inhibitor triciribine. This combined application of the two drugs reduced the total applied dose in case of both cell lines (BXPC3 and Panc1), and were synergistic both cell lines particularly in Panc1. But the absolute IC_{50} concentration of trametinib, was not under the clinically applicable limit in this cell line.

Molecular subtype specific efficacy of MEK-inhibitors in pancreatic cancers

Western blot analysis was used to examine the expression and phosphorylation of EGFR and downstream ERK and Akt proteins in the three cell lines.

The MiaPaCa2 cell line showed extremely low level of EGFR expression and activation. Furthermore the G12C mutant KRAS activated primarily the MEK/ERK pathway so the feedback activation of the EGFR-AKT pathway was expected to be less pronounced.

BXPC3 cells exhibited the highest EGFR expression and activity. While –probably due to the wild type KRAS- ERK and Akt phosphorylation were in balance but the significant EGFR activity could lead to the activation of the EGFR-AKT feedback loop upon MEK inhibition –as assumed by the previously observed synergism of EGFR or AKT and MEK inhibitors in this cell line.

Whereas Panc1 cells expressed high amount of EGFR, its activity wasn't prominent. However, both expression and phosphorylation of Akt were remarkable in the KRAS G12D mutant Panc1, in turn the activity of ERK was the lowest of all cell lines. This indicates that G12D mutation in KRAS protein activates mainly the PI3K/Akt pathway rather than MEK/ERK signaling, which is in line with the effective growth inhibition of the MEK-Akt inhibitor combination in this cell line.

Next we analyzed the effect of trametinib on EGFR and Akt activity to corroborate the presence of a potential EGFR-feedback activation loop. In line with our hypothesis, trametinib treated MiaPaCa2 cell line showed no increase in Akt activity when compared to the control sample. However, the EGFR overexpressing

BXPC3 cell line responded with an increased Akt activity to trametinib treatment. While Panc1 showed remarkable baseline Akt activity both in control cells and trametinib treated samples, further increase due to the treatment wasn't observed. These observations are in concert with only MiaPaCa2 being highly sensitive to MEK inhibitor monotherapy.

5. Conclusions

The summarized conclusions of my PhD study are the following:

1. The presence of *PIK3CA* and *MET* gene copy number gain (CNG) is a potential target for tyrosine kinase inhibitors. It also has a role in primary and secondary resistance to certain kinase-inhibitors. Based on our results, the increase in the copy number of the two examined genes is frequent in the head and neck tumors, so their use as a biomarker or target in therapy may be considered.
2. The increase in copy number *PIK3CA* and *MET* has prognostic significance in head and neck tumors. The presence of both *PIK3CA* and *MET* CNG is associated with significantly worse disease-specific survival for this tumor type.
3. There is a significant correlation between *PIK3CA* CNG and p16^{INK4} -expression. In case of tumors with low p16^{INK4}-expression, increased *PIK3CA* CNG was more common. P16^{INK4}-expression is used as a surrogate HPV marker, we concluded that *PIK3CA* CNG is more relevant in HVP-negative tumors. Since several studies aimed to look for molecular markers associated with HPV-status and define molecular subtypes, our results can contribute to a more precise characterization of subgroups.
4. There is a reverse relationship between *MET* CNG and EGFR-expression (IHC) in head and neck cancers. This result may indicate the dominance and dependence of tumors to specific signaling pathways.
5. The CNG of *MET* and *PIK3CA* genes differs in the subgroups of the laryngeal region. In the case of glottic tumors, this phenomenon was less frequent than observed in the supraglottic region. Among others, this could explaine the better survival parameters of glottic tumors.
6. Expression of p16^{INK4} is of prognostic value in oropharyngeal tumors irrespective of HPV-positivity.
7. Investigating HPV-positivity in p16^{INK4}-immunopositive cases, the specificity of p16INK4 to predict the presence of HPV was 56% in oropharyngeal region. In p16^{INK4}-positive, but HPV-negative patients the etiology of smoking proved to have significant role.
8. In the in vitro model of head and neck tumors, dasatinib and afatinib sensitivity can be predicted with their genetic background (mutations and protein expression), and the combination of the two drugs has a synergistic effect.
9. The expression of connexin 43 protein was previously shown to have prognostic significance in head and neck tumors. The decrease in connexin 43 expression was associated with a worse disease-specific survival. In our studies we

observed, that the change in the phosphorylation of the protein –due to tyrosine-kinase inhibitors can affect the presence of connexin protein 43 into the membrane. This result can be important from the aspect of connexin 43 modulation.

10. In the in vitro model of pancreatic tumors, KRAS mutational subtypes and tumor protein-expression can determine the activity of RAS/Raf/MEK/ERK and PI3K/Akt signaling pathways and thus influence their sensitivity to MEK-inhibitors.

11. In case of pancreatic cancers, the molecular subtype of the tumor can predict the most effective, subtype-specific drug-combination in vitro.

6. Author's own publications

Publications related to the dissertation

1. **Brauswetter D**, Birtalan E, Danos K, Kocsis A, Krenacs T, Timar J, Mihalyi R, Horcsik D, Polony G, Tamas L, Petak I. (2017) p16INK4 expression is of prognostic and predictive value in oropharyngeal cancers independent of human papillomavirus status: a Hungarian study. Eur Arch Otorhinolaryngol, 274: 1959-1965. **(IF: 1.660)**
2. **Brauswetter D**, Danos K, Gurbi B, Felegyhazi EF, Birtalan E, Meggyeshazi N, Krenacs T, Tamas L, Petak I. (2016) Copy number gain of PIK3CA and MET is associated with poor prognosis in head and neck squamous cell carcinoma. Virchows Arch, 468: 579-587. **(IF: 2.848)**
3. Danos K, **Brauswetter D**, Birtalan E, Pato A, Bencsik G, Krenacs T, Petak I, Tamas L. (2016) The Potential Prognostic Value of Connexin 43 Expression in Head and Neck Squamous Cell Carcinomas. Appl Immunohistochem Mol Morphol, 24: 476-481. **(IF: 1.634)**
4. Szentkuti G, Danos K, **Brauswetter D**, Kiszner G, Krenacs T, Csako L, Repassy G, Tamas L. (2015) Correlations Between Prognosis and Regional Biomarker Profiles in Head and Neck Squamous Cell Carcinomas. Pathol Oncol Res, 3: 643-650. **(IF: 1.94)**

Publications not related to the dissertation

1. Varga A, Gyulavari P, Greff Z, Futosi K, Nemeth T, Simon-Szabo L, Kerekes K, Szantai-Kis C, **Brauswetter D**, Kokas M, Borbely G, Erdei A, Mocsai A, Keri G, Vantus T. (2015) Targeting vascular endothelial growth factor receptor 2 and protein kinase D1 related pathways by a multiple kinase inhibitor in angiogenesis and inflammation related processes in vitro. PLoS One, 10: e0124234. **(IF: 3.057)**
2. Dános K, Birtalan E, **Brauswetter D**, Tamás L. (2015) A humán (emberi) papillomavírus (HPV) szerepe a szájgarat daganatainak kialakulásában, kórisméjében és kezelésében – áttekintő közlemény. Nőgyógyászati Onkológia, 20: 8-11. **(IF: 0)**
3. Szentkúti G, **Brauswetter D**, Dános K, Birtalan E, Tamás L. (2014) A fej-nyaki daganatok magas incidenciájának és mortalitásának okai hazánkban. OTORHINOLARYNGOLOGIA HUNGARICA, 60: 77-80. **(IF: 0)**

4. Schwab R, Petak I, Kollar M, Pinter F, Varkondi E, Kohanka A, Barti-Juhasz H, Schonleber J, **Brauswetter D**, Kopper L, Urban L. (2014) Major partial response to crizotinib, a dual MET/ALK inhibitor, in a squamous cell lung (SCC) carcinoma patient with de novo c-MET amplification in the absence of ALK rearrangement. Lung Cancer, 83: 109-111. **(IF: 3.958)**

5. Tamas L, Szentkuti G, Eros M, Danos K, **Brauswetter D**, Szende B, Zsakovics I, Krenacs T. (2011) Differential biomarker expression in head and neck cancer correlates with anatomical localization. Pathol Oncol Res, 17: 721-727. **(IF: 1.366)**