BIOMARKERS IN SOLID TUMORS

PhD thesis

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BIOMARKERS – IN GENERAL

Cancers are one of the most feared enemies for the individuals and also for the society in the „western world". This fear is melting rather slowly, because the successes in therapy are questioned by the painful failures. Nevertheless, we can witness improvements, step-by-step both in the survival and in the quality of life. As a result, more and more cancer patients are with us, which is explained also by the longer age and the earlier diagnosis.

Currently emphasis is given to the „personalized care", especially on the field of chemotherapy. This is based on the continuously expanding molecular knowledge on the identification of those gene errors and products which play important role in the appearance, growth and progression of tumors. These molecular errors could serve as targets for the pharmaceutical industry to produce newer and newer drugs for the clinical area. Further steps require many tasks today and tomorrow for the scientists and clinical oncologists. Fortunately, the gap between the bench and bedside – in case of correct cooperation - is narrowing due to the increasing quality of molecular techniques, preclinical testsystems, and refined clinical trials. All of these help to identify more and more details in the regulatory networks approaching the wanted patients' selection for individualized therapy. Among these informations a family of molecules, called biomarkers (or here tumormarkers), can predict the designed clinical response or the prognosis of the given malignancies.

Biomarkers are already available and can be classified in several ways: they can appear int he circulation (usually measured by laboratory tests) or in the tissues (mainly in the tumorous tissue). Our studies aimed to extend the knowledge on the role of such biomarkers.

AIMS

Studies were focused on three different topics related to biomarkers in cancer. The first two deal with the regulatory disturbances, and the third discusses the markers in circulation. The main problems are the followings

(1) How the predictive role of mutant KRAS is reflected in the anti-EGFR therapy of our colorectal cancer patients?
(2) What is the relationship between the regulatory changes in cell functions and the patients' survival, i.e. what marker or marker-panels have prognostic capacity?

(3) Whether the factors produced by the disturbed hemostasis associated with cancer can signal – at least as effectively as the recently used tumor markers – the prognosis of the disease?

KRAS MUTATIONS IN COLORECTAL CANCERS

Today the clinical management of colorectal cancers is based mainly on certain clinicopathoological characteristics: the stage (according to TNM), histology, the edge of the surgical sample and the general condition of the patients. Further informations can be obtained from the lymphoid invasion, involvement of the peritoneum, degree of differentiation (judged by histology), and from clinical events as obstruction or perforation at the time of diagnosis (ASCO risk factors). In the past years some proteins and genetic markers were introduced to make the patients' selection much better, to select those who may have the most advantage from the systemic therapy. KRAS (member of the RAS family) is one of these markers and used as a critical information on the effectiveness of the anti-EGFR monoclonal antibodies against the colorectal cancers.

The overproduction of EGFR in certain tumors was considered as a bad prognostic sign, and suggested that genetic errors of EGFR (amplification, mutation, changes in copy number) can indicate the sensitivity of colorectal cancers to EGFR-inhibitors. The effectiveness of the monoclonal antibodies (cetuximab, panitumumab) has been proved in advanced, metastatic cases, especially in combination, while the small molecular weight tyrosine-kinase inhibitors failed. The mechanism of action is still unknown, since the overproduction of EGFR shown by immunohistochemistry is not an acceptable marker for the clinical response. On the contrary, the activating mutation of KRAS has negative predictive marker, because in the presence of such mutations the inhibition of EGFR is ineffective. In that case the tumor cells with mutated KRAS actively stimulate proliferation independently from the EGFR-signal.

Today, the anti-EGFR monoclonal antibodies can be used in the treatment of colorectal cancer, if the tumor cells carry wild-type and not mutated KRAS.

Our study – Data and samples of 47 patients were available. Markers were determined either from the primary tumor or from the metastases (in 3 cases samples from both sources from the same patients could be included). Activity of EGFR was measured by

immunohistochemistry (Pathology, St. Imre Hospital), KRAS mutation by sequencing (KPS Kft). In two cases the samples contained insufficient amount of tumor cells. (The first sample for sequencing was sent at March 4, 2010.)

Patients. Forty five patients were evaluated: 29 males (median age 66 yr, range: 37-73 yr), 16 females (59 yr; 47-76). Patients after the removal of the primary received adjuvant chemotherapy, those with metastases received palliative treatment. Most frequently used protocols: de Gramont, FOLFOX, FOLFIRI, capecitabin, bevacizumab + FOLFIRI.

Anti-EGFR treatment. Anti-EGFR treatment was performed almost always after multiple chemotherapeutic protocols in patients with ECOG 0-2, and following EGFR and KRAS determinations. As mentioned, only those patients with advanced, metastatic disease received anti-EGFR treatment, whose tumor expressed wild-type KRAS. Cetuximab was used in combination with FOLFIRI, panitumumab as monotherapy. The dose of cetuximab was 400 mg/m2 body surface for induction, followed by 250 mg/m2 body surface for maintenance. Treatment was continued until progression or the onset of unwanted side-effects. In the latter case the non-treatment period was lengthened. The most frequent side effects (grade 3/4) were the acne-like skin rash (4 cases) and neutropenia (6 cases).

Anti-EGFR treatment was introduced in 5 out 11 patients with wild-type KRAS primary tumor (4 cetuximab and 1 panitumumab). In 2 cases the tumor showed regression after 6 months cetuximab treatment, progression happened in 1 case, and the tretment is still two short (less than 2 months) in other 2 cases. In this group the median survival (overall survival: time passed between the diagnosis and death or the last visit) was 21 months (12-72 months). In the metastatic group 8 patients out of 9 received anti-EGFR treatment. In 1 patient the liver metastases bacame operable after cetuximab. In another case after 6 months cetuximab treatment the tumor progressed, but in the next 6 months panitumumab was given and the progression was stopped, the tumor is now under regression. (This patients showed the longest survival, 108 months, but when anti-EGFR therapy was introduced metastases in the lung and adrenals were already present.) In this group the median overall survival was 45 months (21-108 months). As a whole, 11 patients out of 13 treated with anti-EGFR drugs were evaluated, and after 6 months treatment period 4 of them showed clinical advantage (36.3%). This result can be considered really positive, with two comments: (a) the number of patients were rather low to make firm conclusions, (b) in almost all cases the patients received multiple chemotherapy with temporary regressions followed by progressions before anti-EGFR-treatment (as 2nd line line and above).

Cases carrying mutant KRAS. Mutant KRAS occurred in 19 patients with primary tumors out of 30 patients (63.3%). The frequency was even higher in samples of colonic cancers (13/18, 72.1%). It can be mentioned that among the 19 mutant primary cases 15 were males and 4 females, while in metastatic cases this ratio was much lower (6/15, 40%), especially in patients with liver metastases (2/7, 28.6%). Various types of activating mutations were detected. In spite of the low number of cases the G13D mutation showed the highest frequency, and now reports emphasized the relative sensitivity of this type of mutation to anti-EGFR therapy in contrast to the other types.

Discussion **–** Although the number of cases in this KRAS study is not high, certain conclusions can be made:

(a) The ratio of survivers (at the time of the study) was 60% in patients carrying tumor with wild-type KRAS (12/20), and 52% (13/25) in the mutant counterparts. The close values could indicate that KRAS-status does not influence survival (i.e. it has no prognostic power), but it is very important as predictive marker to select patients for anti-EGFR therapy.

(b) The percentage of mutant KRAS in the 45 patients is relatively high (55.5 %, 25/45), compared to others data (35-45%). The ratio could be increased by lung metastases, because in these patients the mutated KRAS is more frequent than in other locations (liver metastases have much more wild-type KRAS, 71.5%) This results indicate that increased attention should be paid to primary tumors with mutant KRAS in order to discover lung metastases as early as we can. In the 3 cases when samples from both primeries and metastases were available, the KRAS-status agreed.

(c) Anti-EGFR monoclonal antibodies as 2nd line or higher treatments produced clinical advantage (regression, operability) in 36.3% of colorectal tumors with wild-type KRAS. It is conceivable, suggested by some clinical trials that these or similar drugs would be more effective in selected patients at an earlier stage of treatment. (Licensing these drugs in first line treatment of advanced colorectal cancers is now reality or under consideration.)

(d) G13D was the most frequent mutation, and according to some current reoports, tumors with this type of mutation can show certain response to anti-EGFR treatment. (International recommendations are not available yet.)

STUDY ON SIGNALING PATHWAYS

The identification of tumormarkers is rather difficult due to the accumulation of genetic errors during the tumor growth (more than 100 cancer genes are suggested), by the appearance of subclones leading to heterogeneity and the variability of the regulatory pathways with many interactions. These explain the intensive search for more useful biomarkers or marker panels, partly using the already known relationships between the markers (involving usually few genes or gene products), partly using high technology to „fish out" the most „suspicious" molecules from hundreds or thousands of candidates. The techniques could be manyfold. The changes at gene level often require the validation at protein level trying to approach the functional significance. Such method is the immunohistochemistry with some advantages (the used antibodies usually specicific and rather sensitive) and drawbacks (the difference in the „preparation" of samples – formaline fixation, opening up the epitopes, subjective evaluation). The problems could be overcome – at least to a certain extent – using tissue microarray (TMA), where several samples are placed in a common block for the reaction (e.g. for immunohistochemistry).

Our studies – Using immunohistochemistry on TMA blocks an attept was made to find molecules or family of molecules which can be related to the progression of the colorectal cancers and patients' survival. Consequently, the aim was to identify prognostic factors.

TMA – Formalin-fixed and paraffin embedded samples of colorectal cancers (1996-2004) was used to construct 3 TMA blocks with the help of Panoramic TMA Master (3D Histech, Budapest). All TMA-blocks contained 70 tissue cylinders of 2 mm of diameter.

Patients – Samples were collected from 95 patients (52.6% males and 47.4% females), median age 62 yr (34-78 yr). Chemotherapy and monitoring were performed in the Oncological Profile of St. Imre Hospital. The mean follow-up was 78 months (8-181 months). The clinical characteristics were: stage of the disease, overall survival, progression-free survival, and progressive disease. If the cause of death was independent from cancer the case was disregarded.

Immunohistochemistry – For immunihistochemistry (IHC) 5 μ thin sections were made from the TMA blocks. Following antibodies were used:

- EGFR/RAS/PI3K pathway - *EGFR* (DX, Ventana, Zymed, NC384, pY1068, pY1173), *HER2* (SP3), *RAS* (AB1), *RAF* (pRAF-9421), *MAPK* (4376), *ERK1/2* (pMEK1/2-2338), *AKT* (pAKT, panAKT), *mTOR, PTEN* (Ab-6)
- Other ligands/receptors - *MET* (NCL-cMET-S), *PDGFRa, PDGFRb, STAT2* (RB-10458-P0), *STAT3* (RB-9237-P0), *IGFR*

• Cell cycle regulators - *cyclin D1* (RM-9104-S0), *cyclin D3* (MS-215-P0), *Ki67* (N1633)

• Apoptosis regulators - *p53* (DO7, FP2-3, SP5), *Bax* (Ab-1), *Bad* (*75*, RB10376-P19; *99*, RB10377-P1), *Bcl-2* (Ab-3), *cytochrome C* (Ab-2), *caspase 8* (Ab-4), *FLIP* (Ab4042), *survivin*, *NFκB* (*p50*, Ab-2; *p65*, RB-1648-PO)

• Adhesion/invasion regulators - *E-cadherin* (MS-1479-S0). *β-catenin* (MS-1763-S0), *CD44v6* (Ab-1), *CD138* (syndecan-1), *MMP2* (RB-9233-PO)

• Others - *CD10* (CALLA, Ab-2, MS.728-S0), *Cox2*, *HLA-DR, timidilát-szintáz* (MAB4130)

Reactions were evaluated by 3 pathologists (from 1st Department of Pathology and Experimental Cancer Research, Semmelweis University; dr. Tamás Micsik, dr. László Fónyad, dr. Tibor Krenács), independently from each other. Common opinion was made if it was necessary. All reactions were characterized by a number (0-9) based on the intensity of the reaction and the percentage of the positive cells.

Statistical analysis - Statistica 9.1 (StatSoft Inc., Tulsa, USA) and MedCalc (1.2.1.0. (MediCals Software, Mariakerke, Belgium) softwares were used to analyse clinical and immunohistochemical data.

Results – Connections were searched between the activity of the above listed regulators and the clinical characteristics.

• *Connection between the activity of antibodies against a common target* – Significant correlation (R>0.7) was found (a) between the activity of the two phosphorylated-EGFR antibodies (89 cases, 0.74), (b) between the 3 anti-p53 antibodies (30 cases, 0.85-0.87), (c) between the two groups above (a, b) (30 cases, 0.74-0.82), (d) between 2 anti-EGFR antibodies (Pharm-DX and Ventana) (23 cases, 0.76).

• *Connection between the activity of the different regulators* – Significant correlation (R>0.7) was found: (a) between p53 and pEGFR (35 cases), (b) between NFkB p65 and survivin (32 cases, 0.76), (c) between panAKT and NFκB or PDGFb (0.88), however these were limited to very few cases (8 and 7).

• *Connection between the clinical data and IHC activities* – For this comparison the IHC activities were divided into 2 groups: a group with strong values (7-9), and another with weak values (0-3), while the intermediers (4-6) were disregarded. Further criterias were: at least 8 cases should belong to the evaluated group and at least 70% of the cases should show either high or weak activity. It turned out that very few statistically acceptable correlations – related to progression - were found between clinical data and IHC values.

However, EGFR was highly expressed independently from the survival, suggesting that even in colorectal cancers the normal regulatory EGFR-pathway is very important. This conclusion is supported by the fact that other members of EGFR-pathway (RAS, RAF, ERK) are also strongly expressed. The results indicate that EGFR-expression itself has no prognostic value (similarly to EGFR mutations in lung cancer). It is very conceivable, that in colonic cancer either the inhibitory molecules of the normal regulation for intestinal epithelium lost their functions or the protooncogenes are mutated. A further task is to clarify the role of STAT3 – which is also connected to the EGFR-pathway.

Connection between IHC activities and progression

	Strong activity	*Weak activity*
Overall survival		
>10 yr	EGFR(V), BAX ---	
6-10 yr	EGFR(V), RAS, STAT3, NFkB	p53, CD44v6
3-5 yr	EGFR(V), RAS, BAX, p53 caspase 8, E-cadherin, survivin	CD44v6
0-2 yr	EGFR(V), ERK, RAF	----
PFR (progression free survival)		
> 6 yr	EGFR(V), RAS, STAT3	CD44v6
3-5 yr	---	
1-2 yr	EGFR(V), ERK, RAS	CD44v6
0-1 yr	EGFR(V)	---

Concerning other markers, E-cadherin activity showed some correlation with better survival, and CD44v6 with worse prognosis, in harmony with others' results. Markers for apoptosis (e.g. BAX, p53, caspase-8, survivin, NFkB) expressed very little or no correlation with prognosis.

Discussion – The first part of the study compared the activity of the potential markers using IHC. According to the results the 6 diagnostic antibodies against EGFR showed very similar activities. The closest connections were between PharmDX and Ventana and between the phosphorylated antibodies. Since phosphorylation indicates not only the presence but also the functional activity, it is advised to use them whenever possible. Although the data are limited

for general statements, it seems that the IHC reactions are acceptable to identify certain markers.

Patients participated in the study received conventional chemotherapy, and it is possible, that the response of tumor cells was independent from the EGFR-pathway. In general, this pathway was responsible „only" for the continuous production of the tumor cells but not for the survival. The clinical results that anti-EGFR monoclonal antibodies (cetuximab, panitumumab) are effective in 10-20% of cases (carrying wild-type KRAS), indicate that some other factors should play important role in the response. One can suggest, that the substantial heterogeneity between the cases can explain that why other factors besides the members of EGFR-pathway were not able to reach the statistically acceptable level. It could be part of that hypothesis that tumors at the early stages of progression, when the heterogeneity of the molecular errors is not so divergent, would be more sensitive to an anti-EGFR-pathway therapy (which means that not only EGFR can serve as target).

HEMOSTASIS AND TUMOR PROGRESSION

Since the first descriptions it has been reinforced many times that bidirectional connections exist between venous thromboembolism (VTE) and malignancies. From one side the risk for VTE is highly increased in tumorous patients (7-28-times), especially within few months after the diagnosis or in the presence of distant metastases, and on the other side, the risk for a malignant tumor is higher within 2 years after an idiopathic VTE. In hospitalized patients VTE is the second cause of death, pulmonary embolism develops in every seventh patients. Surgery can increase the risk as well.

In tumorous patients several factors can increase the coagulation activity (e.g. TF, uPA, CP), which could be resistant against the normal anticoagulation. With tumor progression the procoagulation will be accompanied by the increased activity of the fibrinolytic system. The latter is indicated by several markers, including enhanced level of D-dimer (DD), calling the attention on the elevated risk for thrombosis and/or embolism.

The widely used drugs for the prevention of the development and also the therapy of thrombosis in tumorous (and non-tumorous) patients are the unfractionated heparin (UFH) and the low molecular weight heparins (LMWH). Recently some reports claimed that heparin and LMWH besides their prophylactic capacity could improve the survival of cancer patients. According to the preclinical data this effect is the result of the inhibition of metastatization, but the exact mechanism is unknown. It is possible, that the anticancer phenomenon is

independent from the anticoagulant action, although heparin has connections to those factors which are important in the metastasis formation. Such factor could be a specific enzyme (e.g. heparanase), adhesion molecules (e.g. P- and L-selectin), growth factors, cytokines. Using these relationships the heparin/LMWH could inhibit or decrease the protection of tumor cells e.g. in the circulation. Such protection can be achieved by a fibrin- or thrombocyte coat around the traveling tumor cells or can increase the success of adhesion of tumor cells to the endothelial cells (i.e. extravasation). The effect of heparin/LMWH on the local growth of a tumorous foci is even less known, but some data support the inhibition of angiogenesis as an important mechanism.

Our studies – In this section the results of two experiments are described.

• ***1st study***

Patients, therapy. In this randomized, open, restrospective study LMWH-treated and non-treated patients with solid tumors – 62 breast cancer, 29 colorectal cancer, 5 ovarian cancer – were evaluated. Nadroparin (Fraxiparin, GlaxoSmith Kline) was used as LMWH during the chemotherapy, and between the cycles, at least for 6 months. LMWH was given s.c., daily, in a dose estimated upon the body mass.

The aim of the study was to evaluate progression-free and overall survival (overall survival was considered as a period lasted from the diagnosis till the death or the last visit). TNM-classification was used for staging.

Results – Among the LMWH-treated patients those showed statistically advantageous effect, i.e. longer survival who had a tumor stage T3 or T4. The involvement of lymphnodes (N) did not make any differences between the two groups. Stages M0 and M1 occurred in both groups. As a surprise, the LMWH treatment caused longer survival, if metastases were already present (with an exception of central nervous system).

The median follow-up was 4.9 yr. The changes after diagnosis - regression, progression, steady state – were evaluated by imaging techniques and markers. At the end of the studied period (death or last visit) the tumor progressed in 32.3% of LMWH-treated patients, while in 50.7% of control patients. The progression-free survival was 35 months in the treated and 26 months in the control groups, while regression (more than 25% decrease in tumor volume) was registered in 12.5% and 3.1% respectively. There was no change clinically in the 55.2% of the LMWH-treated and 46.2% of control patients.

2nd study

Patients. Twohundred ninty nine patients – breast cancer, colorectal cancer, pancreatic cancer, ovarian cancer – were selected randomly in this retrospective study. The main aim was the evaluation of the LMWH treatment, and also the potential prognostic role of D-dimer.

Markers. Tumormarkers – CEA, CA125, CA15-3, CA19-9 - and markers of hemostasis were measured by routine laboratory methods performed by the Central Laboratory and the Pathology of St. Imre Hospital).

Treatment. As thromboprofilaxis the patients were given nadroparin, enoxaparin, (Clexane, Sanofi-Aventis), or dalteparin (Fragmin, Pfizer). Patients were treated with LMWH during the adjuvant therapy (usually with 6 months cycles and 3 months longer thereafter), or parallel with palliative therapy. Severe side effects (grade 3/4) occurred only in 4 cases. Chemotherapy followed the usual standard protocols.

Results – The range of age of the tumorous patients showed the usual values. It is not surprising, unfortunately, that more than 80% of pancreatic cancers were discovered at a later stage, which is reflected in the short survival. Even in breast cancer more than 25% was identified in stages III or IV, which calls the attention on the problems of screening.

• *Connectios between the LMWH-treatment and disturbed hemostasis* – Disturbed hemostasis (vein thrombosis, embolism – VTE) was observed in 11.5% of LMWH treated patients (24/208), but in 15.8%, if arterial thrombosis and embolism are also considered. Differences in the frequencies are remarkable between tumor types (e.g. breast cancer 6.6% - lowest, pancreatic cancer 22.7% - highest).

It is a question whether connection exists between data mentioned above and the level of D-dimer (further: DD), since DD reflects a shift towards procoagulation. If all cases are evaluated as one group the DD-level indicates much worset prognosis than the normal DD-level (alive / died patients 30.1% versus 88.4%) during the 12 yr observation period.

• *Connections between LMWH-treatment, DD-level and survival* – Only those patients were included at the time of the evaluation of survival (Nov 2011), who had the diagnosis between Jan 2000. and Dec 2009. Therefore the patients were divided into two groups: those who died within 2 yr after diagnosis (<2yr), and those who did not (>2yr). There was no major difference between the two groups if all LMWH-treated patients were considered (47.4% versus 52.6%), however the ratios differed between tumor types (long survivors in breast cancer patients 76.5%, while in pancreatic cancer 26%). These values are in harmony with distribution of the patients in different stages at the time of diagnosis. The survival data showed similar values in increased and normal DD-levels collecting all cases or considering tumor types separately.

Stages reflect the progression of the disease. In the LMWH-treated group the increase of DD-level was more frrequent in the advanced stages versus earlier stages suggesting a link between increased DD-level and progression. The differences were significant. When the DD-level proved to be normal, the values connected more to the better prognosis. All of these underline the potential prognostic role of the DD-level in malignancies.

• *Connections between „classical" tumormarkers and DD-levels* - In those tumor types which were included in this study, the use of tumormarkers is an everyday part of tumor management, mainly to estimate progression, to monitor the efficiency of the treatment – essentially they have prognostic power. During evaluation those values were used which were measured at diagnosis or in some cases during follow-up the patients.

Following conclusions were made from the results: (a) In pancreatic and ovarian cancers the frequency of increased DD-level was similar to the „classical" ones (pancreatic cancer: CA19-9, ovarian cancer: CA125/CEA), but it was much higher in colonic cancer and breast cancer (colonic cancer: CEA, breast cancer: CA15-3/CEA). (b) The same tendency was observed when the progression was characterized by stages. (c) It may happen that the levels of classical markers and that of DD move together, but this was very rare, far from all cases. (d) It seems that the determination of CEA in breast cancers and ovarian cancers is questionable.

Discussion **–** According to some studies LMWHs (and heparin) besides their anticoagulant effect can act against cancer. The inhibitory effect could be exercized locally (as suggested in the 1st experiment where advanced local growth and metastases were the better targets) or during the progression. The later is mainly about the inhibition of certain steps of the metastatic cascade, e.g. by preventing the formation of a protective fibrincoat around the tiny tumor cell aggregates. Inhibition could be the consequence the downregulation of the procoagulant activity of the thrombocytes and endothelial cells. The influence on the local growth could be the result of an antiangiogenic effect of LMWH, but this explanation is still contradictory. One reason of this uncertanity that angiogenesis has several mechanisms requiring different approach.

The quality (sensitivity and specificity) and functional usefulness (diagnostic, predictive, prognostic) of the markers are very important to design the best therapeutic strategy. We have markers but far to be perfect, therefore to extend the markerlist is mandatory. One can suggest, that if the procoagulation is good for the tumor, then a marker (e.g. D-dimer) alarming the disturbances of hemostasis could be a signal for the bad prognosis. Our study supports this idea: when all tumors were counted the elevated level of

DD was associated with worse prognosis. Elevation of DD was less frequent in breast cancer patients, and most frequent in pancreatic cancer. This made reasonable to compare the changes of widely used classical tumormarkers in different tumor types with DD. The comparison is rightful because the effectiveness of recent tumormarkers is not very high, and using them we have to be familiar with their limitations. As an example: at early stages of ovarian cancer CA125 is elevated in about the half of the cases (in our study 34.0%), which gave the reason to search for additional markers, as HE4/WFDC2-t. Combination these markers can increase the accuracy of the estimation of the prognosis. In our 2nd study the level of DD increased at least similarly or even better at early stages (I/II) of tumor growth than the classical markers. With an exception of pancreatic cancer (which were almost always discovered at a later stage – III or IV) in the other tumors the elevation of DD proved to be a prognostic marker. The study also found the use of CEA in breast and ovarian cancer unnecessary.

The search for new and more useful markers is going on, and the goal is not only to identify single markers but also marker-panels (e.g. Oncotype DX, or Mammaprint in breast cancer – but more and more panels were published recently in other tumor types). The D-dimer as a prognostic marker is new, and could be useful in the clinical practice (a good aspect, which is not neglibile, is the cost-effectiveness).

SUMMARY

In the past decade the revolutionary development of molecular technology contributed a lot to the increase of our knowledge on cancer. These informations led to the discovery and understanding of those key regulatory changes in the genesis and progression of malignancies which can serve as targets in tumor diagnostics and therapy. One of the main challenges in the research field is to identify the most important molecular networks, the molecuar targets, the markers (biomarkers) which can predict therapeutic responsiveness in order to select the appropriate patients, as well as markers to judge the prognosis of the disease. The aims of our study approached some details of the biomarker area and reached certain conclusions:

(1) The anti-EGFR therapy – used in the second-line or even further – proved to be effective, providing clinical advantage (operability, regressioní) in 36% af patients carrying wild-type KRAS. G13D mutations were the most frequent among the KRAS-mutants, which - according to current data – could react to anti-EGFR therapy. (2) Extended

immunohistochemical (IHC) analysis on colorectal cancer samples (using tissue microarray) found rather few correlations between the IHC estimation and the clinical characteristics related mainly to survival. According to the results with anti-EGFR antibodies in the diagnostic histological samples suggested that the regulatory pathway which rules the proliferation of normal colonic mucosa is also present in colonic cancer cells. This finding is supported the increased ativity of the downstream members (as RAS, RAF, ERK) of the EGFR signalling. (3) The level of D-dimer increased at least as much as the level of classical tumor marker in the early stages of tumor growth. D-dimer can be considered as a prognostic factors in tumor types studies (breast-, colorectal-, ovarian cancers) and its measurement is advised besides the classical markers. Additional observation, that CEA has no help e.g. in breast- and ovarian cancers.

It is our hope that these results may contribute to the design of a more individual-based and more effective antitumor strategy.

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