

## Review

# Predictive pathology in routine diagnostics of solid tumors

Silke Lassmann and Martin Werner

Institute of Pathology, University Medical Center, Albert-Ludwigs University, Freiburg, Germany

**Summary.** For decades, macroscopic and microscopic analysis of human tissue specimens by pathologists has been the basis for disease classification. In recent years, there has been an increasingly better understanding of molecular alterations underlying the pathogenesis of cancers as well as the establishment and integration of novel molecular analyses into a histomorphological-based workflow. This has dramatically extended the possibilities of diagnostic pathology - from its descriptive role to a clinical advisory role on cancer classification including prognostic and predictive molecular pathological information.

This review will focus on the recent developments of molecular pathological techniques and the current tools and applications of predictive pathology in view of targeted therapies in solid cancers.

**Key words:** Pathology, Molecular, Predictive markers, Solid tumors

### Integrated workflows of histomorphological and molecular pathological analyses

The basis for predictive pathology is the macroscopic and microscopic analysis of mainly formalin-fixed and paraffin-embedded (FFPE) tissue derived from surgical resection specimens or from biopsies. From these, the use of serial sections for protein, mRNA and/or DNA analyses ensures the validity of the results in the specific histological context.

Two approaches allow this close correlation of morphology and molecular analysis (Fig. 1): 1) “in situ” approaches directly assessing mRNA and/or DNA within the tissue section and 2) “extract-based” approaches with prior morphology-guided micro-dissection of (tumor)

cells from the tissue section. For both approaches, a trained pathologist must control the selection of appropriate tissue block and/or mark the relevant tissue area for subsequent molecular analyses as well as “signing out” a final diagnostic synopsis encompassing the histomorphological and molecular findings.

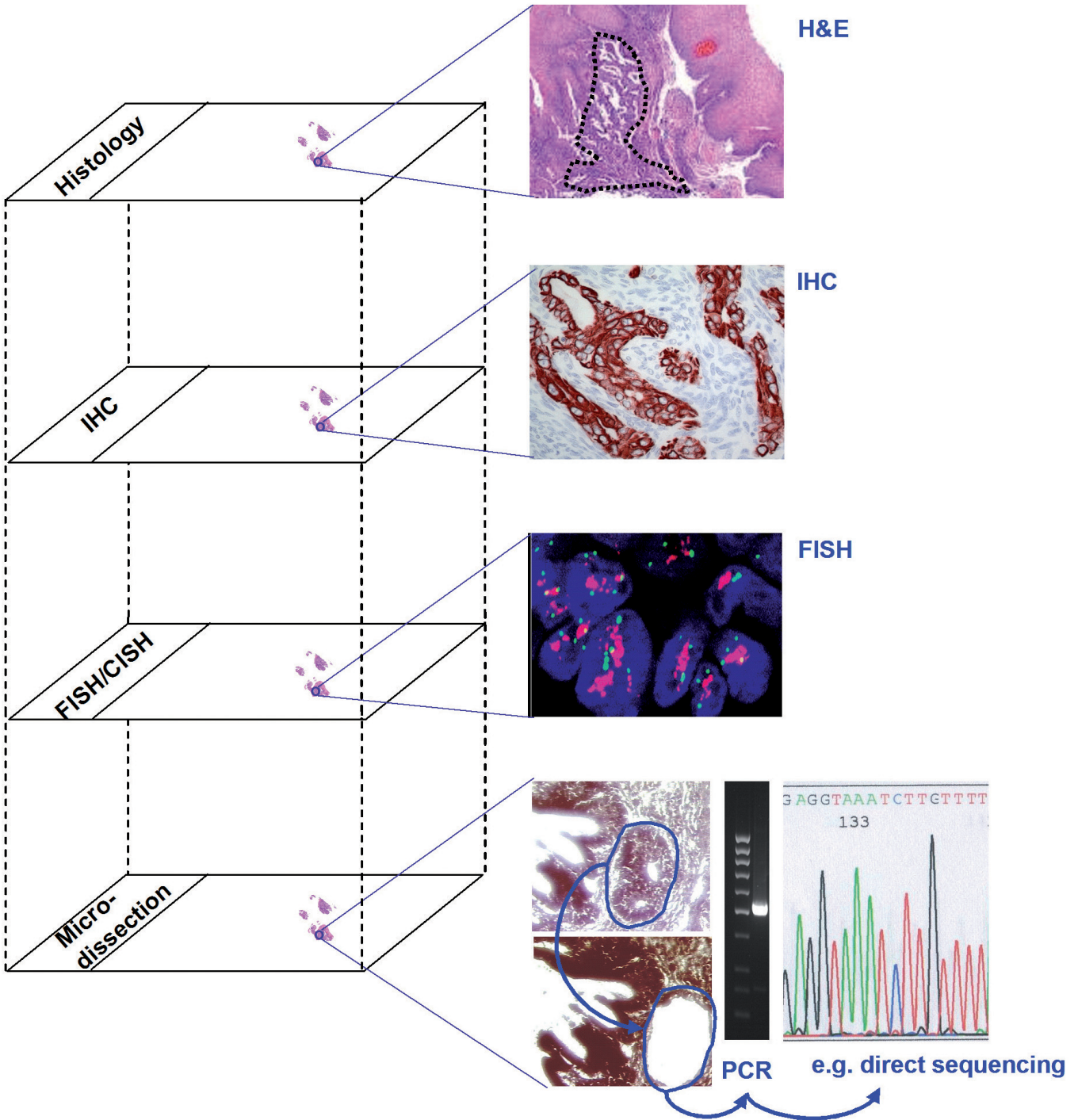
With an increasing need for stratification of patients according to histomorphological and molecular analyses on the basis of small FFPE processed biopsies with limited tissue, a major goal of the above integrated workflow is to reduce the amount of cells required for each step. Since such FFPE processed tissues are and will remain the gold standard for diagnostic pathology, it is therefore necessary to optimise routine molecular analyses to work in small quantities of FFPE tissue specimens, respective microdissected (tumor) cells, instead of adapting tissue sampling and processing to research-derived molecular biology protocols.

This can be ensured by “in situ” analyses, such as Fluorescence/Chromogen-In-Situ Hybridization (FISH, CISH), where a single 5  $\mu$ m thick serial section suffices for single gene (Hicks and Tubbs, 2005; Gruver et al., 2010) or multiple gene (Hicks et al., 2005) analysis. Nevertheless, these “in situ” analyses mostly address chromosomal rearrangements/translocations and/or gene amplifications/deletions. Although some reports exist as to the *in situ* detection of mutations in cell lines (Larsson et al., 2010), the routine application of “in situ mutation detection” to FFPE tissues awaits realization. Since several specific gene mutations have shown to be therapeutically relevant (see below), “extract”-based approaches currently dominate the field.

The methodology for studying limited amounts of RNA/DNA isolated from FFPE tissues by “extract”-based technologies is already available in most molecular pathology laboratories. Above all this includes (quantitative, q) Polymerase Chain Reaction (PCR) based detection of bacterial or viral pathogens as well as the detection of tumor cell related gene fusions (or even chromosomal translocations), altered

microsatellite-loci and gene mutations. For the latter, sequencing analysis is an important final step in the diagnostic workflow. Several novel approaches of (q)PCR and sequencing methodologies have recently

been reported for the detection of single gene (Franklin et al., 2010) or multiple-gene (Sarasqueta et al., 2011) alterations, also for the application to microdissected FFPE-tissue derived samples (Weichert et al., 2010).



**Fig. 1.** Workflow of predictive pathology. Basis for all analyses are formalin-fixed and paraffin-embedded tissue specimens. From these, serial sections are used for the different steps of diagnosis and predictive marker evaluation at the levels of morphology (HE), immunohistochemistry (IHC) and/or molecular analyses (FISH; microdissection/PCR/sequencing).

However, this excludes recent interest in genome-wide next generation sequencing technologies (Voelkerding et al., 2010), for which the “proof of principle” application and implementation within a daily routine diagnostic molecular pathology laboratory setting is still missing. In contrast, recent studies, including our own (Lassmann et al., 2009), have shown that genome-wide mRNA expression profiling by microarray technology is possible from microdissected (colorectal) tumor cells of FFPE-tissues and from as little as 10-100ng of isolated (FFPE-fragmented) RNA.

It is still a matter of debate whether or not sampling and analysis of low numbers of tumor cells (from small biopsies) reduces the specificity and sensitivity of molecular analysis in terms of the predictive value of the molecular analysis for the entire tumor-, respective patients therapeutic response.

Finally, a major consideration for the future of integrated workflows of histomorphological and molecular analyses is the standardization in diagnostic pathology institutes, including measurements of quality assurance, e.g. approval/certification of workflows and/or equipment, and issues of reimbursement.

In the following, only current state-of-the-art routine diagnostic applications of predictive pathology are outlined with an emphasis on solid tumors treated by (receptor) tyrosine kinase and associated signalling pathway inhibitors (Table 1).

### Current diagnostic applications of predictive pathology

#### Breast cancer

The histological analysis of biopsies and/or resection specimens of breast carcinomas already yields important information about prognostic patient sub-groups. This is further supported by parallel analysis of proteins of hormone and growth factor receptors, which are of predictive and partly prognostic value. Specifically,

these are estrogen (ER) and progesterone (PR) receptors, whose expression correlates with reduced tumor growth as well as a better response to adjuvant hormone therapy with tamoxifen. In addition, strong and membranous protein expression of the growth factor receptor HER2/neu is a negative prognostic factor for nodal positive patients and a positive predictive factor for the response to HER2/neu targeted therapy by monoclonal antibody trastuzumab (Herceptin). In the case of unclear immunohistochemical staining patterns for HER2/neu, supportive molecular analysis of HER2/neu gene copy numbers by fluorescence-in-situ hybridization (FISH) (Hicks and Tubbs, 2005), respective light-microscopic in-situ-hybridization, such as Chromogen-ISH (CISH) (Gruver et al., 2010) can be performed. These analyses yield information on whether or not the HER2/neu gene is amplified, a result considered as having a positive predictive value for adjuvant therapy by trastuzumab.

With increasing knowledge on receptor-associated signalling pathway and more precise mechanisms of action of inhibitors, a wave of novel predictive markers as well as novel targets for breast cancers refractory to HER2/neu inhibition has recently evolved (Jones and Buzdar, 2009; Pohlmann et al., 2009). The latter not only include monoclonal antibodies directed against HER2/neu, but also inhibitors that interfere with the dimerization of HER2/neu, essential for its function, or inhibitors that target intracellular “down-stream” signalling partners of Her2/neu, such as RAS/MEK, or inhibitors targeting other signalling pathways, such as PI3K/AKT or VEGF (Rosen et al., 2010). Particularly, a therapeutic monoclonal antibody directed against the vascular endothelial growth factor (VEGF) - “bevacizumab” is already undergoing testing and approval for HER2-negative breast cancers patients. However, very recent clinical trial results have caused removal of breast cancer as FDA indication for “bevacizumab”.

For these novel therapeutic approaches, specific information derived from molecular pathological

**Table 1.** Current diagnostic markers in molecular pathology of solid tumors – Focus on receptor-tyrosine kinase associated signalling pathways. For a complex list of diagnostic translocations in soft tissue sarcomas the reader is referred to recent reviews (Ordoñez et al. 2010; Wardelmann et al. 2010).

(Sporadic) Cancer	Gene	Alteration	Method	Reporting
Breast	HER2/neu	gene amplification, protein “over”expression	FISH/CISH, IHC	prognostic, predictive
Gastric Cancer	HER2/neu	gene amplification, protein “over”expression	FISH/CISH, IHC	predictive
Colorectal	KRAS	mutation	direct sequencing, pyrosequencing	predictive
	BRAF	mutation	direct Sequencing, pyrosequencing	predictive
Lung	EGFR	mutation	direct sequencing, pyrosequencing	predictive
	KRAS	mutation	direct sequencing, pyrosequencing	predictive
	ALK	translocation	FISH, IHC, RT-PCR	predictive
Melanoma	BRAF	mutation	direct sequencing, pyrosequencing	predictive
	NRAS	mutation	direct sequencing, pyrosequencing	predictive
GIST	KIT	mutation	direct sequencing, pyrosequencing	predictive
	PDGFRA	mutation	direct sequencing, pyrosequencing	predictive

analyses will almost certainly play a central role in patient management.

#### *Gastric cancer*

In view of the recent approval of the HER2 inhibitor “trastuzumab” also for targeted therapy of gastric cancer, analysis of HER2 gene amplification (by CISH, FISH) and/or HER2 protein “over” expression (by immunohistochemistry) is now part of predictive pathology. Due to different molecular-pathological conditions in gastric as compared to breast cancer, the interpretation of HER2 immunohistochemistry follows other guidelines (Rüschoff et al., 2010).

Whether analysis of the receptor-tyrosine kinase MET by immunohistochemistry and/or analysis of molecular alterations of the MET gene have a predictive role for the currently discussed MET-targeted therapies has still to be validated.

#### *Colorectal cancer*

Metastasized colorectal cancer can be targeted by monoclonal antibodies, cetuximab or panitumumab, which interfere with the growth factor receptor EGFR thereby inducing tumor cell death. However, in contrast to HER2/neu in breast cancer, analysis of EGFR protein expression by immunohistochemistry or determination of EGFR gene copy numbers by ISH approaches in the clinical trials of EGFR-inhibitors showed no predictive value (Cunningham et al., 2004; Saltz et al., 2004; Chung et al., 2005; Italiano et al., 2008).

Instead, in colorectal cancer (or lung cancer, see below) mechanisms of resistance and hence therapeutic response have already been identified as residing in mutations of “down-stream” signalling partners of EGFR (Banck and Grothey, 2009). These are specific mutations of KRAS, BRAF or PI3K, which lead to their constitutive activation and hence independent signalling from EGFR. Interestingly, these genes are involved in the carcinogenesis of colorectal cancers and are therefore frequently found to be mutated, for example KRAS mutations in up to 40% and BRAF mutations in up to 10% of cases.

Thus, current recommendations for predictive pathology to EGFR targeted therapy in metastasized colorectal cancer foresee the analysis of mutational status of KRAS in a specific region of the gene (exon 2, codon 12/13) (Allegra et al., 2009). This generally involves microdissection of tumor cells followed by DNA extraction, PCR and sequencing analysis. However, protocols for PCR and sequencing analysis steps and associated laboratory equipment, e.g. direct sequencing versus pyrosequencing, differ between Institutions (Van Krieken et al., 2008), so that KRAS mutation analysis awaits national/international standardization. This also includes an ongoing discussion about how many tumor cells need to be analyzed, which can be seen from a technical point of

view (e.g. as few as 100 cells may suffice for PCR and sequencing) and more importantly from a predictive pathology view point (e.g. content of tumor versus other cells). More importantly, one critical issue is still whether or not low numbers of tumor cells are really representative of the (heterogeneous) tumors’ response to EGFR therapy.

With the approval of the VEGF-targeted therapy “bevacizumab” for metastasized colorectal cancer (see above breast cancer), it is conceivable that also for this therapy novel molecular markers will become part of predictive pathology for colorectal cancers.

#### *Lung cancer*

As with colorectal cancers, in metastasized lung cancers the molecular pathological analysis of EGFR-associated signalling has evolved as a supportive tool for therapy prediction. For non-small-cell lung cancer therapeutic inhibition of EGFR has been approved. Instead of monoclonal antibodies directed against EGFR (e.g. cetuximab, see above), small molecule inhibitors (Gefitinib, Erlotinib) that target the intracellular tyrosine kinase domain of EGFR are used.

In NSCLC patients, alterations of EGFR protein expression and/or DNA copy numbers are of no predictive value for response to EGFR-targeted therapy. Instead, primary (or secondary acquired) mutations in EGFR itself are indicative for therapeutic response to EGFR-targeted therapy (Hammermann et al., 2009; Pao and Chmielecki, 2010): activating point mutations in Exon 18 (G719S) or Exon 21 (L858R) as well as deletions in Exon 19 (747-752) are associated with a positive therapeutic response, whereas insertions in Exon 20 (761-774) or a point mutation in Exon 20 (T790M) predict poor therapeutic response. For molecular pathology this means that mutation analysis of selected EGFR exons is performed by microdissection of tumor cells and the use of PCR and (Pyro-) sequencing. Although not yet standardized in all laboratory aspects (see above), a recent European workshop laid out some consensus recommendations regarding EGFR mutation testing for NSCLC patients in pathology institutes (Pirker et al., 2010).

In view of the close association of EGFR with “down-stream” signalling partners KRAS, BRAF and PI3K as well as the known predictive value of KRAS mutational status for EGFR-targeted therapy in colorectal cancer (see above), there are ongoing discussions as to whether mutational testing for e.g. KRAS should also be included in the molecular pathological assessment of NSCLCs (Roberts et al., 2010).

Finally, still another receptor-tyrosine kinase – Anaplastic Lymphoma Kinase (ALK) - has recently evolved as a distinct altered molecular feature of NSCLCs (Soda et al., 2007; Shaw et al., 2009), appears to be predictive for treatment of NSCLC patients with the folic acid analogue Pemetrexed (Camidge et al.,

2011) and may even be a potential novel therapeutic target itself (Kwak et al., 2010; Wellstein and Toretzky, 2011). The unique mechanisms of ALK alterations in NSCLC are chromosomal translocations in up to 5% of NSCLC cases. ALK is located on chromosome 2p23 and translocation partners can be genes on chromosome 3q11 (TFG), chromosome 10p (KIF5B). However, the most frequent alteration is an inversion of the p-arm of chromosome 2, causing different variants of EML4-ALK fusions according to the chromosomal break points. Detection of EML4-ALK translocations can be performed by FISH or RT-PCR of fusion transcripts, as previously described for ALK translocations in Anaplastic Large Cell lymphoma (Cataldo et al., 1999). Since FISH and RT-PCR analyses are laborious and time consuming, efforts to establish IHC-based detection of ALK fusions have recently been successfully designed (Takeuchi et al., 2009; Mino-Kenudson et al., 2010) and may be used as a supportive screening tool prior to specific genomic characterization. Since a recent study detected EML4-ALK fusion transcripts in about 15% normal, non-cancer associated lung cancer tissues using these analyses (Martelli et al., 2009), the role of ALK as a therapeutic target is still controversial.

### Melanoma

As with breast, colorectal and lung carcinomas, melanomas also have distinct molecular alterations in receptor tyrosine kinase associated signalling pathways. Specifically, mutations in BRAF and NRAS occur in about 50% and 25% of sporadic melanomas, respectively. Mutations of BRAF or NRAS are almost mutually exclusive and act in the same RAS/MAPK/ERK signalling pathway, which normally prevents apoptosis and stimulates cell proliferation. The characteristic mutations of BRAF (cT1799A/pV600E) and NRAS (cA182T/pQ61L or cC181A, pQ61K) result in constitutive activation of this pathway. A recent meta-analysis showed that the occurrence of BRAF and NRAS mutations are associated with the histological types of melanoma and sun-exposure, whereby superficial melanomas are associated with BRAF mutations and nodular melanomas with NRAS mutations (Curtin et al., 2005; Lee et al., 2010). Importantly, BRAF or NRAS mutations are predictive markers for therapeutic response and resistance to RAF-inhibitors (Bollag et al., 2010; Ellerhorst et al., 2011; Nazarian et al., 2010). Currently, BRAF mutation detection, most commonly by PCR-based direct sequencing or pyrosequencing of microdissected cell populations, is incorporated in molecular pathological workflows. In the near future, this may also be the case for NRAS mutation analyses.

In addition, alterations of other (receptor-) tyrosine kinase associated signalling pathway molecules - PI3K/AKT or KIT - are of increasing interest in malignant melanoma. Although PI3K (mutation), AKT (gene amplification) or KIT (mutations) alterations can

also be found in benign nevi, the significant occurrence of an activating KIT mutation (pL576P) in malignant melanomas (Curtin et al., 2006) and the associated positive response to KIT-targeted inhibition (e.g. imatinib mesylate or dasatinib) provides new therapeutic opportunities for patients with malignant melanoma (Hodi et al., 2008; Woodman et al., 2009). A molecular pathological analysis of KIT alterations, as seen for Gastrointestinal Stromal Tumours (see below), will most likely be included as a supportive predictive tool to histopathological classification of melanomas in the near future.

### Gastrointestinal stroma tumours (GIST)

Gastrointestinal stroma tumors are the most frequent mesenchymal tumors of the gastrointestinal tract and have distinct features of molecular alterations, whose analyses not only support classification and prognostication, but also provide the basis for therapy selection and prediction.

The characteristic molecular alterations involved in GIST affect (receptor) tyrosine kinases, specifically mutations of KIT or PDGFRA in about 80% and 8% of cases, respectively. Different types of mutations of KIT or PDGFRA can result in their ligand independent autophosphorylation and activation of down-stream signalling pathways with associated increased cell proliferation. The type of KIT or PDGFRA mutations observed in GISTs correlate with clinico-pathological features, such as KIT exon 9 or 11 mutations being associated with more aggressive behaviour or PDGFRA mutations frequently being associated with an epithelioid morphology and clinically more indolent behaviour (Antonescu, 2011; Liegl-Atzwanger et al., 2010).

Importantly, KIT and PDGFRA mutations are predictive for targeted therapy of GIST patients: A mutation in the juxtamembrane domain of KIT (exon 11 mutation) results in constitutive KIT activation and improved therapeutic response to imatinib mesylate. In contrast, mutation of the kinase II domain of PDGFR (exon 18) confers resistance to imatinib mesylate. Thus, molecular pathological assessment of KIT (exon 9,11,13,17) and PDGFR (exon 18) mutations has become an integral part of the pathological assessment of GISTs. Once again, microdissection and PCR-based direct sequencing analyses are the methods of choice for these molecular pathological analyses.

### Soft tissue sarcomas

Although soft tissue sarcomas have a common mesenchymal origin, they are a very heterogeneous group of tumours, in which molecular pathology is supportive for sub-type classification.

This is possible by FISH or PCR-based analyses for a group of sarcomas with characteristic chromosomal alterations, among them Ewing Sarcoma (t(11,22) or t(21/22)), Rhabdomyosarcoma (t(2,13) or t(1;13)),

Desmoplastic Small Round Cell Tumour (t(11;22)) and Synovial Sarcoma (t(X;18)). In addition, characteristic translocations also assist in the classification of inflammatory myofibroblastic tumours, where the receptor tyrosine kinase ALK (see lung cancer above) is involved. In contrast to lung cancer, the fusion partners of ALK in inflammatory myofibroblastic tumours can differ, but most frequently involve TPM3-ALK (t(1;2), also occurring in carcinomas or hematologic neoplasms) or CLTC2-ALK (t(2;17)) or RANBP2-ALK (t(2;2)) (Griffin et al., 1999; Lawrence et al., 2000). However, for several other sarcomas (e.g. osteosarcoma, fibrosarcoma, leiomyosarcoma) specific molecular alterations and associated molecular pathological analyses have not yet been identified and/or validated.

As described for epithelial (melanoma) and mesenchymal (GIST) tumours above, there is increasing interest in predictive molecular testing for alterations associated with therapeutic responses, specifically with recent studies of the BCR-ABL-KIT-, PDGFR-inhibitor Imatinib Mesylate in patients with dermatofibrosarcoma protuberans (McArthur et al., 2005) and the positive predictive value of the t(17;22) translocation, present in up to 80% of cases and encoding the COL1A1-PDGFB fusion gene (Segura et al., 2011). Future predictive analysis of t(17;22) translocations may be of routine diagnostic value.

For further detailed discussion of molecular alterations and their clinical relevance for soft tissue sarcomas, the reader is referred to recent reviews exclusively addressing this topic (Ordoñez et al., 2010; Wardelmann et al., 2010).

## Conclusion

From the above examples it is clear that the pathologist is increasingly more involved in the molecular analysis of complex molecular signalling networks to provide the clinician with appropriate therapy recommendations. This clearly requires the synopsis of histological and molecular interpretation.

Whether specific molecular alterations of predictive value actually result in histomorphologically recognizable phenotypes is yet to be proven. However, it is known that specific molecular sub-classes of cancers exist, whose (epi)genetic alterations are detectable at the morphologic level: e.g. microsatellite-unstable colorectal cancers (frequently mucinous, lymphocytic infiltration) or others (e.g. breast cancer: lobular with defect in E-cadherin). Still, it is the histomorphology of a tissue, which will reflect the sum of all (epi-) genetic alterations.

Clearly, supportive immunohistochemical analyses for detection of oncogenes, such as HER2/neu, or for specific detection of altered proteins resulting from gene-fusion (e.g. ELM-ALK) are now possible. One may even envisage that in situ evaluation of molecular pathways and therapeutic response associated with point mutations (KRAS, EGFR, BRAF, KIT, PDGFR) may

become possible by immunohistochemical analyses of mutation-specific antibodies.

In summary, the expertise in morphologically- and molecular-based analyses of human tissue specimens is the pathologists' treasure and will be of utmost clinical-relevance in terms of personalized medicine.

~~Acknowledgements.~~ We thank the Mushett-Family-Foundation (Chester, New Jersey, US) for substantial long-term support of our efforts in further developing predictive molecular pathology for translational research and routine diagnostics. The authors are supported by research grants of the German Research Foundation (DFG SFB850, project C5, Z1) and the Landes-Stiftung Baden-Württemberg. S.L. is associated member of BIOS Centre for Biological Signalling Studies, Albert-Ludwigs University, Freiburg, Germany.

## References

- Allegra C.J., Jessup J.M., Somerfield M.R., Hamilton S.R., Hammond E.H., Hayes D.F., McAllister P.K., Morton R.F. and Schilsky R.L. (2009). American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J. Clin. Oncol.* 27, 2091-2096.
- Antonescu C.R. (2009). The GIST paradigm: lessons for other kinase-driven cancers. *J. Pathol.* 223, 251-261.
- Banck M.S. and Grothey A. (2009). Biomarkers of resistance to epidermal growth factor receptor monoclonal antibodies in patients with metastatic colorectal cancer. *Clin. Cancer. Res.* 15, 7492-7501.
- Bollag G., Hirth P., Tsai J., Zhang J., Ibrahim P.N., Cho H., Spevak W., Zhang C., Zhang Y., Habets G., Burton E.A., Wong B., Tsang G., West B.L., Powell B., Shellooe R., Marimuthu A., Nguyen H., Zhang K.Y., Artis D.R., Schlessinger J., Su F., Higgins B., Iyer R., D'Andrea K., Koehler A., Stumm M., Lin P.S., Lee R.J., Grippo J., Puzanov I., Kim K.B., Ribas A., McArthur G.A., Sosman J.A., Chapman P.B., Flaherty K.T., Xu X., Nathanson K.L. and Nolop K. (2010). Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 467, 596-599.
- Camidge D.R., Kono S.A., Lu X., Okuyama S., Barón A.E., Oton A.B., Davies A.M., Varela-Garcia M., Franklin W. and Doebele R.C. (2011). Anaplastic Lymphoma Kinase Gene Rearrangements in Non-small Cell Lung Cancer are Associated with Prolonged Progression-Free Survival on Pemetrexed. *J. Thorac. Oncol.* 6, 774-780.
- Cataldo K.A., Jalal S.M., Law M.E., Ansell S.M., Inwards D.J., Fine M., Arber D.A., Pulford K.A. and Strickler J.G. (1999). Detection of t(2;5) in anaplastic large cell lymphoma: comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue. *Am. J. Surg. Pathol.* 23, 1386-1392.
- Chung K.Y., Shia J., Kemeny N.E., Shah M., Schwartz G.K., Tse A., Hamilton A., Pan D., Schrag D., Schwartz L., Klimstra D.S., Fridman D., Kelsen D.P. and Saltz L.B. (2005). Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J. Clin. Oncol.* 23, 1803-1810.
- Cunningham D., Humblet Y., Siena S., Khayat D., Bleiberg H., Santoro A., Bets D., Mueser M., Harstrick A., Verslype C., Chau I. and Van

*Predictive pathology of solid tumors*

- Cutsem E. (2004). Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351, 337–345.
- Curtin J.A., Fridlyand J., Kageshita T., Patel H.N., Busam K.J., Kutzner H., Cho K.H., Aiba S., Bröcker E.B., LeBoit P.E., Pinkel D. and Bastian B.C. (2005). Distinct sets of genetic alterations in melanoma. *N. Engl. J. Med.* 353, 2135-2147.
- Curtin J.A., Busam K., Pinkel D. and Bastian B.C. (2006). Somatic activation of KIT in distinct subtypes of melanoma. *J. Clin. Oncol.* 24, 4340-4346.
- Ellerhorst J.A., Greene V.R., Ekmekcioglu S., Warneke C.L., Johnson M.M., Cooke C.P., Wang L.E., Prieto V.G., Gershenwald J.E., Wei Q. and Grimm E.A. (2011). Clinical correlates of NRAS and BRAF mutations in primary human melanoma. *Clin. Cancer. Res.* 17, 229-235.
- Franklin W.A., Haney J., Sugita M., Bemis L., Jimeno A. and Messersmith W.A. (2010). KRAS mutation: comparison of testing methods and tissue sampling techniques in colon cancer. *J. Mol. Diagn.* 12, 43-50.
- Griffin C.A., Hawkins A.L., Dvorak C., Henkle C., Ellingham T. and Perlman E.J. (1999). Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. *Cancer Res.* 59, 2776-2780.
- Gruver A.M., Peerwani Z. and Tubbs R.R. (2010). Out of the darkness and into the light: bright field *in situ* hybridisation for delineation of ERBB2 (HER2) status in breast carcinoma. *J. Clin. Pathol.* 63, 210-219.
- Hammerman P.S., Jänne P.A. and Johnson B.E. (2009). Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer. *Clin. Cancer Res.* 15, 7502-7509.
- Hicks D.G. and Tubbs R.R. (2005). Assessment of the HER2 status in breast cancer by fluorescence *in situ* hybridization: a technical review with interpretive guidelines. *Hum. Pathol.* 36, 250-261.
- Hicks D.G., Yoder B.J., Pettay J., Swain E., Tarr S., Hartke M., Skacel M., Crowe J.P., Budd G.T. and Tubbs R.R. (2005). The incidence of topoisomerase II-alpha genomic alterations in adenocarcinoma of the breast and their relationship to human epidermal growth factor receptor-2 gene amplification: a fluorescence *in situ* hybridization study. *Hum. Pathol.* 36, 348-356.
- Hodi F.S., Friedlander P., Corless C.L., Heinrich M.C., Mac Rae S., Kruse A., Jagannathan J., Van den Abbeele A.D., Velazquez E.F., Demetri G.D. and Fisher D.E. (2008). Major response to imatinib mesylate in KIT-mutated melanoma. *J. Clin. Oncol.* 26, 2046-2051.
- Italiano A., Follana P., Caroli F.X., Badetti J.L., Benchimol D., Garnier G., Gugenheim J., Haudebourg J., Keslair F., Lesbats G., Lledo G., Roussel J.F., Pedeutour F. and François E. (2008) Cetuximab shows activity in colorectal cancer patients with tumors for which FISH analysis does not detect an increase in EGFR gene copy number. *Ann. Surg. Oncol.* 15, 649-654.
- Jones K.L. and Buzdar A.U. (2009). Evolving novel anti-HER2 strategies. *Lancet Oncol.* 10, 1179-1187.
- Kwak E.L., Bang Y.J., Camidge D.R., Shaw A.T., Solomon B., Maki R.G., Ou S.H., Dezube B.J., Jänne P.A., Costa D.B., Varela-Garcia M., Kim W.H., Lynch T.J., Fidias P., Stubbs H., Engelman J.A., Sequist L.V., Tan W., Gandhi L., Mino-Kenudson M., Wei G.C., Shreeve S.M., Ratain M.J., Settleman J., Christensen J.G., Haber D.A., Wilner K., Salgia R., Shapiro G.I., Clark J.W. and Iafrate A.J. (2010). Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* 363, 1693-1703.
- Lassmann S., Kreutz C., Schoepflin A., Hopt U., Timmer J. and Werner M. (2009). A novel approach for reliable microarray analysis of microdissected tumor cells from formalin-fixed and paraffin-embedded colorectal cancer resection specimens. *J. Mol. Med.* 87, 211-224.
- Larsson C., Grundberg I., Söderberg O. and Nilsson M. (2010). *In situ* detection and genotyping of individual mRNA molecules. *Nat. Methods* 7, 395-397.
- Lawrence B., Perez-Atayde A., Hibbard M.K., Rubin B.P., Dal Cin P., Pinkus J.L., Pinkus G.S., Xiao S., Yi E.S., Fletcher C.D. and Fletcher J.A. (2000). TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. *Am. J. Pathol.* 157, 377-384.
- Lee J.H., Choi J.W. and Kim Y.S. (2010). Frequencies of BRAF and NRAS mutations are different in histological types and sites of origin of cutaneous melanoma: a meta-analysis. *Br. J. Dermatol.* 164, 776-784.
- Liegl-Atzwanger B., Fletcher J.A. and Fletcher C.D. (2010). Gastrointestinal stromal tumors. *Virchows Arch.* 456, 111-127.
- Martelli M.P., Sozzi G., Hernandez L., Pettirossi V., Navarro A., Conte D., Gasparini P., Perrone F., Modena P., Pastorino U., Carbone A., Fabbri A., Sidoni A., Nakamura S., Gambacorta M., Fernández P.L., Ramirez J., Chan J.K., Grigioni W.F., Campo E., Pileri S.A. and Falini B. (2009). EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. *Am. J. Pathol.* 174, 661-670.
- McArthur G.A., Demetri G.D., van Oosterom A., Heinrich M.C., Debic-Rychter M., Corless C.L., Nikolova Z., Dimitrijevic S. and Fletcher J.A. (2005). Molecular and clinical analysis of locally advanced dermatofibrosarcoma protuberans treated with imatinib: Imatinib Target Exploration Consortium Study B2225. *J. Clin. Oncol.* 23, 866-873.
- Mino-Kenudson M., Chirieac L.R., Law K., Hornick J.L., Lindeman N., Mark E.J., Cohen D.W., Johnson B.E., Jänne P.A., Iafrate A.J. and Rodig S.J. (2010). A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin. Cancer Res.* 16, 1561-1571.
- Nazarian R., Shi H., Wang Q., Kong X., Koya R.C., Lee H., Chen Z., Lee M.K., Attar N., Sazegar H., Chodon T., Nelson S.F., McArthur G., Sosman J.A., Ribas A. and Lo R.S. (2010). Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 468, 973-977.
- Ordóñez J.L., Osuna D., García-Domínguez D.J., Amaral A.T., Otero-Motta A.P., Mackintosh C., Sevillano M.V., Barbado M.V., Hernández T. and de Alava E. (2010). The clinical relevance of molecular genetics in soft tissue sarcomas. *Adv. Anat. Pathol.* 17, 162-181.
- Pao W. and Chmielecki J. (2010). Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat. Rev. Cancer* 10, 760-774.
- Pirker R., Herth F.J., Kerr K.M., Filipits M., Taron M., Gandara D., Hirsch F.R., Grunenwald D., Popper H., Smit E., Diel M., Marchetti A., Manegold C., Schirmacher P., Thomas M., Rosell R., Cappuzzo F. and Stahel R; European EGFR Workshop Group (2010) Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. *J. Thorac. Oncol.* 5, 1706-1713.
- Pohlmann P.R., Mayer I.A. and Mernaugh R. (2009). Resistance to Trastuzumab in Breast Cancer. *Clin. Cancer Res.* 15, 7479-7491.
- Roberts P.J., Stinchcombe T.E., Der C.J. and Socinski M.A. (2010). Personalized medicine in non-small-cell lung cancer: is KRAS a useful marker in selecting patients for epidermal growth factor receptor-targeted therapy? *J. Clin. Oncol.* 28, 4769-4777.

- Rosen L.S., Ashurst H.L. and Chap L. (2010). Targeting signal transduction pathways in metastatic breast cancer: a comprehensive review. *Oncologist* 15, 216-235.
- Rüschhoff J., Dietel M., Baretton G., Arbogast S., Walch A., Monges G., Chenard M.P., Penault-Llorca F., Nagelmeier I., Schlake W., Höfler H. and Kreipe H.H. (2010). HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch.* 457, 299-307.
- Saltz L.B., Meropol N.J., Loehrer P.J., Needle M.N., Kopit J. and Mayer R.J. (2004). Phase II trial of Cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J. Clin. Oncol.* 22, 1201-1208.
- Sarasqueta A., Moerland E., de Bruyne H., de Graaf H., Vrancken T., van Lijnschoten G. and van den Brule A.J. (2011). SNaPshot and StripAssay as valuable alternatives to direct sequencing for KRAS mutation detection in colon cancer routine diagnostics. *J. Mol. Diagn.* 13, 199-205.
- Segura S., Salgado R., Toll A., Martín-Ezquerria G., Yébenes M., Sáez A., Solé F., Barranco C., Umberto P., Espinet B. and Pujol R.M. (2011). Identification of t(17;22)(q22;q13) (COL1A1/PDGFB) in dermatofibrosarcoma protuberans by fluorescence *in situ* hybridization in paraffin-embedded tissue microarrays. *Hum. Pathol.* 42, 176-184.
- Shaw A.T., Yeap B.Y., Mino-Kenudson M., Digumarthy S.R., Costa D.B., Heist R.S., Solomon B., Stubbs H., Admane S., McDermott U., Settleman J., Kobayashi S., Mark E.J., Rodig S.J., Chirieac L.R., Kwak E.L., Lynch T.J. and Iafrate A.J. (2009). Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J. Clin. Oncol.* 27, 4247-4253.
- Soda M., Choi Y.L., Enomoto M., Takada S., Yamashita Y., Ishikawa S., Fujiwara S., Watanabe H., Kurashina K., Hatanaka H., Bando M., Ohno S., Ishikawa Y., Aburatani H., Niki T., Sohara Y., Sugiyama Y. and Mano H. (2007). Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 448, 561-566.
- Takeuchi K., Choi Y.L., Togashi Y., Soda M., Hatano S., Inamura K., Takada S., Ueno T., Yamashita Y., Satoh Y., Okumura S., Nakagawa K., Ishikawa Y. and Mano H. (2009). KIF5B-ALK, a novel fusion oncokinas identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin. Cancer Res.* 15, 3143-3149.
- van Krieken J.H., Jung A., Kirchner T., Carneiro F., Seruca R., Bosman F.T., Quirke P., Fléjou J.F., Plato Hansen T., de Hertogh G., Jares P., Langner C., Hoefler G., Ligtenberg M., Tiniakos D., Tejpar S., Bevilacqua G. and Ensari A. (2008). KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. *Virchows Arch.* 453, 417-431.
- Voelkerding K.V., Dames S. and Durtschi J.D. (2010). Next generation sequencing for clinical diagnostics-principles and application to targeted resequencing for hypertrophic cardiomyopathy: a paper from the 2009 William Beaumont Hospital Symposium on Molecular Pathology. *J. Mol. Diagn.* 12, 539-551.
- Wardelmann E., Schildhaus H.U., Merkelbach-Bruse S., Hartmann W., Reichardt P., Hohenberger P. and Büttner R. (2010). Soft tissue sarcoma: from molecular diagnosis to selection of treatment. Pathological diagnosis of soft tissue sarcoma amid molecular biology and targeted therapies. *Ann. Oncol.* 21 (Suppl 7), vii265-vii269.
- Weichert W., Schewe C., Lehmann A., Sers C., Denkert C., Budczies J., Stenzinger A., Joos H., Landt O., Heiser V., Röcken C. and Dietel M. (2010). KRAS genotyping of paraffin-embedded colorectal cancer tissue in routine diagnostics: comparison of methods and impact of histology. *J. Mol. Diagn.* 12, 35-42.
- Wellstein A. and Toretsky J.A. (2011). Hunting ALK to feed targeted cancer therapy. *Nat. Med.* 17, 290-291.
- Woodman S.E., Trent J.C., Stemke-Hale K., Lazar A.J., Pricl S., Pavan G.M., Fermeglia M., Gopal Y.N., Yang D., Podoloff D.A., Ivan D., Kim K.B., Papadopoulos N., Hwu P., Mills G.B. and Davies M.A. (2009). Activity of dasatinib against L576P KIT mutant melanoma: molecular, cellular, and clinical correlates. *Mol. Cancer Ther.* 8, 2079-2085.

Accepted October 21, 2011