Modern Pathology (2005) 18, 1146–1147

www.modernpathology.org

Response

Modern Pathology (2005) **18,** 1146–1147. doi:10.1038/modpathol.3800443

In reply: To the Letter to the Editor by Brigitte Bruun Rasmussen regarding our article 'Influence of slide aging on results of translational research studies using immunohistochemistry' in *Modern Pathology* (2004) 17, 1414–1420, we would like to answer as follows.

We are embarrassed to see that Dr Rasmussen identified an unpleasant large number of typographical errors in our manuscript. It is indeed true that the *P*-values described in the text differ from those shown in the curves and that the numbers in the figure showing the impact of *HER2* on survival have been mixed up. The true *P*-values for associations with survival are for ER, $P=0.11$ instead of 0.009; for PR, $P=0.14$ instead of 0.11; and for *HER2*, $P=0.12$ instead of 0.019. In Figure 3, the *P*-value for '*HER2* old' should be 0.1166 instead of 0.2920. In addition, the curve labels in the *HER2* analysis are mixed up: The upper curve represents the '*HER2*-negative' and the lower curve the '*HER2*-positive' cases. We sincerely apologize to the readers of *Modern Pathology* for these mistakes.

It is important, however, to understand that the conclusions to be drawn from our study do not at all change because of these typos. In fact, the data presented in the Mirlacher paper show that the immunoreactivity of slides decreased significantly after 6 months of storage at 4°C for all five examined antibodies. This is consistent with previous studies[1–4] and currently probably undisputed. Based on this observation, we clearly share Dr Rasmussens opinion that fresh sections are preferable for translational studies. However, fresh sections are not always available and our data do also clearly show that old sections are still very useful for research. Highly significant clinicopathological associations could be found on old sections, despite a decreased level of immunostaining. This especially applied to almost all associations between molecular parameters and grade in old and new sections (*HER2*: $P<0.0001$; ER: $P<0.0001$; *CCND1*: $P<0.03$). Most of Dr Rasmussens concerns on our conclusions were based on the fact that no significant associations with survival were observed in our old section analyses. For judging the significance of this 'disappointing' observation, it is important to understand the inherent reproducibility problem of IHC in general. Many factors, unfortunately, have a strong impact on IHC results including tissue processing, epitope retrieval, staining protocol and slide interpretation. These variables regularly lead to interlaboratory variations that are much higher than the 7–26% decrease of positive results in old sections as compared to new sections. For example, published frequencies of *HER2* postivity range from 0 to 100% in prostate cancer, from 0 to 93% in head and neck cancer and from 4 to 100% in non-small-cell lung cancer (reviewed in Sauter *et al*[5]). It is important to know that IHC results never provide absolute values on the fraction of 'positive' tumors but only numbers that reflect the selected experimental conditions. It is beyond question that modified experimental conditions in our old section study could lead to a higher rate of positivity than seen in our initial 'fresh section' experiment. It is also clear that the use of another staining protocol or antibody could have led to even less significant results than seen in our old slide analysis. From our experience, survival curves showing clearcut differences should always raise suspicion on true survival differences, even if the *P*-value is 'not significant'. Sometimes, significant survival differences can only be identified after optimization of IHC protocols. This applies to traditional large section studies, but especially also to TMA studies. In other words, in case of not very strong associations between protein expression changes and patient survival, mild variations of experimental conditions (including slide age) can easily lead to a change of *P*-values from over 0.05 to under 0.05, or *vice versa*. We therefore strongly reject interpreting our data as strong argument against the research use of old tissue sections. However, researchers using old tissue sections for translational research studies should be aware of the aging problem and carefully consider this issue during their protocol optimization process.

Martina Mirlacher, Ronald Simon and Guido Sauter
Department of Pathology, Medical University Center, University Clinics Eppendorf, Building N30, Martinistrasse 52, Hamburg, Germany

References

1 Jacobs TW, Prioleau JE, Stillman IE, *et al.* Loss of tumor marker-immunostaining intensity on stored paraffin slides of breast cancer. J Natl Cancer Inst 1996;88: 1054–1059.
2 Bertheau P, Cazals-Hatem D, Meignin V, de Roquancourt A, *et al.* Variability of immunohistochemical reactivity

on stored paraffin slides. J Clin Pathol 1998;51: 370–374.
3 Manne U, Myers RB, Srivastava S, *et al.* Re: loss of tumor marker-immunostaining intensity on stored paraffin slides of breast cancer. J Natl Cancer Inst 1997;89:585–586.
4 Henson DE. Loss of p53-immunostaining intensity in breast cancer. J Natl Cancer Inst 1996;88:1015–1016.
5 Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Disc 2003;2:962–972.