

## Prognostic Biomarkers in Cutaneous Melanoma

RITA GRUDE LADSTEIN

Department of Dermatology, Haukeland University Hospital, Bergen, Norway. E-mail: rita.grude.ladstein@helse-bergen.no

Rita Grude Ladstein defended her thesis “Prognostic markers in cutaneous melanoma with emphasis on proliferation and tumour necrosis” at the University of Bergen, Norway, on February 1<sup>st</sup> 2013. Her main supervisor was Professor Lars A. Akslen and her co-supervisor was Associate Professor Ingeborg M. Bachmann. The evaluation committee members and opponents were Professor Ole Petter Fraas Clausen, University of Oslo, Associate Professor Sonja Eriksson Steigen, University of Tromsø, and Professor Leiv Hove, University of Bergen, Norway.

Cutaneous malignant melanoma is the most frequent cause of death from skin cancer, and therapeutic opportunities in advanced cases are limited. The incidence has increased, and melanoma is among the most common malignancies in younger adults. There is a need for increased knowledge of the biology of this disease and improved prognostication for more precise management of patients.

In the present study, the aims were to explore the prognostic impact of different tumour cell proliferation markers and tumour necrosis in cutaneous melanoma, and to explore the expression and impact of the stem cell associated marker nestin in these tumours.

The thesis is based on four separate articles (**Paper I–IV**). A patient series of consecutive cases of cutaneous nodular melanoma was examined. In **Paper I**, 202 cases (1981–1997) were included, and prior to **Paper II–IV**, the series was expanded to also include cases from 1998–2008 (255 additional cases). In **Paper I**, mitotic count (mitoses per mm<sup>2</sup>) was assessed on H&E sections, and Ki-67 expression was estimated by immunohistochemistry on standard sections. Phosphohistone H3 (PHH3), MCM4 and mitotin were examined by immunohistochemical staining on tissue microarrays (TMA) sections. In **Paper II**, both mitotic count and PHH3 count were recorded on standard sections, in the most mitotically active areas, in areas of at least 1 mm<sup>2</sup>. Original H&E sections were reexamined for morphologic tumour necrosis, and recorded as significant when an area of at least ¼ high power field (HPF) (field diameter 0.61 mm; area 0.07 mm<sup>2</sup>) was occupied by necrotic cells and sparse when clusters of at least 5 necrotic cells were observed (**Paper III**). The nestin expression was examined by immunostaining of TMA sections (**Paper IV**).

In **Paper I**, increased mitotic count and elevated Ki-67 expression were associated with unfavourable features like increased tumour thickness and tumour ulceration. High expression of PHH3 and MCM4 was correlated with high mitotic count, elevated Ki-67 expression and ulceration. Uni-

variate survival analyses showed that increased mitotic count and Ki-67 expression were associated with reduced survival, whereas PHH3, MCM4 and mitotin did not predict patient outcome. Ki-67 expression was the only proliferation marker to be significant by multivariate analysis. In **Paper II**, high PHH3 count assessed on regular slides and increased mitotic count were both associ-

ated with thicker tumours and presence of tumour ulceration. Both markers showed highly significant prognostic impact by univariate analysis, whereas multivariate analysis indicated PHH3 to be a stronger prognostic indicator than mitotic count. In multivariate analysis on a subset of cases with data on Ki-67 expression as well as PHH3 counts on standard sections, both Ki-67 and PHH3 remained in the final model. Assessment of mitotic activity by PHH3 had important practical advantages compared with standard mitotic counting. In **Paper III**, tumour necrosis (present in 26% of the cases) was associated with increased tumour thickness, high mitotic count, presence of tumour ulceration, and reduced survival. Stratified analyses (univariate and multivariate) indicated the strongest prognostic impact in tumours thicker than 4 mm. Notably, in the stratum of pT4 tumours, presence of necrosis was a stronger prognostic predictor than ulceration. Finally, in **Paper IV**, nestin expression was found in the majority of primary nodular melanomas, and strong expression was associated with aggressive melanoma features, with independent prognostic impact in comparison with clinico-pathologic factors by multivariate analysis.



PHOTOGRAPHER: ANNE SIDSEL HERDLEVER,  
UNIVERSITY OF BERGEN, NORWAY.

In conclusion, Ki-67 expression was superior to mitotic count but not PHH3 as a prognostic factor. Increased mitotic count and PHH3 frequency showed prognostic impact, but multivariate analysis indicated PHH3 to be a stronger prognostic predictor than mitotic count. Assessment of mitotic activity is facilitated by the use of the mitotic marker PHH3. Tumour necrosis is associated with histopathologic markers of aggressive melanomas and has prognostic impact. In pT4 melanomas, presence of tumour necrosis appears to be a stronger prognostic factor than mitotic count and ulceration. High nestin expression in tumour cells is associated with unfavourable melanoma features and reduced survival by multivariate analysis.

### List of publications

1. Ladstein RG, Bachmann IM, Straume O, Akslen LA. Ki-67 expression is superior to mitotic count and novel proliferation markers PHH3, MCM4 and mitotin as a prognostic factor in thick cutaneous melanoma. *BMC Cancer* 2010; 10: 140.
2. Ladstein RG, Bachmann IM, Straume O, Akslen LA. Prognostic importance of the mitotic marker phosphohistone H3 in cutaneous nodular melanoma. *J Invest Dermatol* 2012; 132: 1247–1252.
3. Ladstein RG, Bachmann IM, Straume O, Akslen LA. Tumor necrosis is a prognostic factor in thick cutaneous melanoma. *Am J Surg Pathol* 2012; 36: 1477–1482.
4. Ladstein RG, Bachmann IM, Straume O, Akslen LA. Nestin expression is associated with aggressive cutaneous melanoma. Submitted.