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Background: Melanoma is one of the most common cancer types in the United States and accounts for 90% of the mortality due to skin cancers. Approximately 60% of melanoma patients develop resistance to immunotherapy, and roughly 82% to targeted therapies. The ErbB receptor family comprises the melanoma dedifferentiation marker EGFR, HER2, HER3, and HER4. These receptors play a crucial role in the onset and propagation of a variety of cancer entities. In a recent study we identified EGFR in therapy-naïve metastatic tissue of stage III/IV melanoma patients as a negative predictor for response to adjuvant ICI[1]. Here we investigate the ErbB receptor family as a whole in the context of melanoma.

Methods: In a cohort of melanoma patients receiving adjuvant anti-PD-1 therapy (n = 37) we assessed the ErbB receptor expression and dimerization patterns in FFPE tumors by IHC, IF and digital image analysis. We performed the same assessment in a second cohort of melanoma patients diagnosed with various stages of disease for which the primary and multiple metastatic tissues were available (n = 18). In four metastatic melanoma cell lines (WM793, UACC257, MEWO, WM266-4) we assessed ErbB expression by IF, dimerization patterns by PLA and co-IP, and tested the effect of 8 FDA-approved TKIs. We assessed the effect of afatinib, neratinib, and osimertinib in a mouse melanoma model. In the A375, MEWO, and WM266-4 melanoma cell lines we assessed the production of ErbB ligands NRG-1, HB-EGF, and TGF-alpha.

Results: In the melanoma cohorts we identify EGFR and HER3 as the major players of the ErbB receptor family, in both primary and metastatic tumors. We confirm the same to receptors as the only ones being expressed in metastatic melanoma cell lines. In both patient samples and in vitro we identify specific ErbB dimerization patterns. In melanoma cell lines we show that afatinib, neratinib, and osimertinib have the most significant effect on cell proliferation and apoptosis. In NSG mice injected with the MEWO cell line we show that treatment with afatinib leads to reduced tumor growth and a significant increase in survival compared to controls. We show that in melanoma EGFR-HER3 dependant pathways can be activated in an autocrine manner.

Conclusions: Here we demonstrate the relevance of investigating the ErbB receptor family in melanoma as a whole, we provide insight into the mechanisms involving the ErbB receptors, and we provide a therapeutic option by targeting these receptors and their associated signalling pathways.

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Harnessing machine learning to the immuohistochemical expression of AMBRA1 and Loricin to identify non-ulcerated AJCC Stage I/II melanomas at high-risk of metastasis

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Background: Precision-based personalised biomarkers able to identify both low-risk and high-risk patient subpopulations with localised cutaneous melanoma are urgently needed to guide clinical follow up and treatment stratification.

We recently validated the combined immunohistochemical expression of AMBRA1 and Loricrin (AMBLor) in the epidermis overlying non-ulcerated AJCC stage I/II melanomas as prognostic biomarker able to accurately identify genuinely low-risk patient subpopulations (NPV > 96%, clinical sensitivity > 95%, Ewen et al Brit J Dermatol. 2024). To further identify distinct subsets of patients with non-ulcerated AJCC stage I/II melanomas ar high risk of metastasis, the present study aimed to develop a machine learning (ML) risk-prediction model combining AMBLor 'at -risk' status with specific patient clinical and tumour pathological features.

Methods: Using commonly and widely used ML models, ML algorithms were trained and tested using three internationally distinct retrospective-prospective cohorts of AMBLor at-risk non-ulcerated AJCC stage I/II melanomas (n = 552).

Results: Based on a training: test data split of 50:50, 20% of patients were defined as high-risk, with a 5-year recurrence-free survival (RFS) probability of 56% (Log-rank [Mantel-Cox) $P < 0.0001$, HR 6.88, 95% CI 3[PL1].03-15.63, clinical specificity 87.2%, PPV 44.4%).

Further validation of the ML algorithms in a 4th independent retrospective-prospective cohort of 120 AMBLor at-risk non-ulcerated localised melanomas derived from the UK identified 24% patients as high-risk, with a 5-year RFS of 56.3% (Log-rank [Mantel-Cox) $P < 0.0001$, HR 7.59, 95% CI 2.94-19.6, clinical specificity 82.1%, PPV 50%).

Conclusions: Through the proven negative predictive power of AMBLor with the cumulative power of prognostic clinical and pathological features these novel translationally relevant data provide an improved risk- prediction model to stratify patients with non-ulcerated localised melanomas at low or high risk of tumour recurrence thereby aiding optimal personalised patient management and treatment stratification.

Keywords: AMBRA1, Loricrin, clinicopathological features, machine learning, Prognostic Biomarker, High Risk Early stage melanoma

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