EDITORIAL

Melanoma treatment guided by a panel of miRNA biomarkers

"...the melanoma-related seven miRNA was found to characterize overall survival of melanoma patients better than both serum lactate dehydrogenase and S100B..."

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Melanoma incidence is continuing to rise in Australia [1], US [2] and in the UK and Europe [3]. Metastatic patients have a poor prognosis and are expensive to treat whereas if melanoma is caught in its *in situ* stage (or in precursor lesions), the 5-year survival is >99%. In melanoma, primary prevention strategies and early detection of primary lesions offer the best outcome but rely heavily on self-monitoring and the expertise of medical professionals. After surgical excision of the lesion, patients are asked to return to their treating clinician at regular intervals which spans from months to years, dependent upon the American Joint Committee on Cancer tumor stage (I–IV) [4]. If the lesion has invaded beyond the epidermis, a sentinel lymph node is commonly biopsied, which is important for accurate staging of the patient to devise clinical follow-up and treatment regimens. In most cases the sentinel lymph node has no detectable cells, therefore, the patient is staged as I or II depending on the level of invasion. In patients with stage II tumors, their relative 5-year survival rates range from approximately 80% (stage IIA) to 50% (stage IIC) [4]. These differences in survival strongly indicate that in a large proportion of patients, metastatic spread has occurred and was not detected by conventional methods or the patient was lost to follow-up. It is evident that these practices are not absolute and disease can be missed, reoccur or manifest as metastatic disease without the observation of a primary or palpable lesion. In clinical practice, there is currently a lack of reliable, sensitive and specific predictive biomarkers for detecting early melanoma progression. Considering the complexities of offering a diagnosis of the degree of spread beyond the primary site, having the ability to rapidly identify predictors of melanoma progression in patients would therefore be a significant clinical tool.

Potential markers to detect melanoma progression have been investigated for many years with varying levels of success. Serum lactate dehydrogenase (LDH) levels have been reported to be elevated in melanoma patients but the performance of this marker reduces as disease progresses [5–8]. Due to these limitations, it is not routinely used in

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all centers. To address this clinical unmet need, we recently identified a panel of highly specific and sensitive miRNA biomarkers that have the potential to monitor melanoma progression in both tissue and serum in a clinical setting [9]. In this study, we first showed that a panel of 'melanoma-related' miRNAs (MELmiR-17) were able to predict the stage, recurrence and survival of patients when its expression was measured in melanoma tissues derived from metastases (stages III and IV) [9]. Currently, we are assessing if this biomarker panel could be used as a companion diagnostic tool to assist in histopathological diagnosis of ambiguous early melanocytic lesions. Additionally, we found that in a minimally invasive blood test, a seven-miRNA panel (MELmiR-7) derived from the 'MELmiR-17' panel, detected the presence of melanoma (relative to no melanoma controls) with high sensitivity (93%) and specificity (≥82%) [9]. Importantly, in stage IV patients, members of the MELmiR-7 panel were able to detect an increase in tumor burden in 100% of cases [9]. One particular miRNA from the panel, miR-211, has long been associated as a melanocyte/melanoma related miRNA [10]. In a conditional inference tree analysis, we showed that stage at blood draw together with miR-211 expression could be used to triage patients based on overall survival (OS) status [9]. Specifically, upon diagnosis with stage IV disease and prior to therapy, miR-211 expression was able to discern survival based on high (median survival = 4.8 months, CI: 4.5–5.9) and low expression (median survival = 2.7 years, CI: 1.7–NA) [9]. Taken together, the MELmiR-7 panel was found to characterize OS of melanoma patients better than both serum LDH and S100B (delta-log likelihood = 11; $p < 0.001$) [9].

The accurate staging of individuals is of the utmost importance to devise appropriate follow-up and treatment regimens. We found that the circulating miRNA expression of our panel was able to discern between disease-free controls and stage IV disease with a near perfect area under the curve (AUC; AUC = 0.99) [9]. Moreover, members of the panel could discriminate between stages with an AUC for stages I/II versus IV and stages III versus IV of 0.99 respectively [9]. With further development, this panel has the potential to be able to upstage patients without observable disease. This may mean they could qualify for the current targeted therapies. It has been well documented in the literature that patients with a lower disease burden, treated

with first-line therapy, exhibit an improvement in OS [11–13]. However, generally speaking, patients present to their oncologist with varying degrees of metastatic spread. It is therefore evident that a more precise way to monitor disease burden and to predict treatment response is required to complement and improve current practices.

Until relatively recently, metastatic melanoma patients had no viable treatment options for disseminated melanoma. With the advent of targeted- (BRAF and MEK inhibition) and immuno-therapy (anti-PD1/PDL1 and anti-CTLA4 antibodies), we are witnessing improvements in progression-free survival. However, in a fair proportion of patients, there has not been a dramatic improvement in patient's OS, with many experiencing a relapse of disease. This relapse is often caused by an acquired or inherent resistance to the therapy, but in some cases it can also be due to the late detection of recurrence in patients undergoing treatment. Measured at baseline (prior to treatment), serum LDH levels have recently been observed to be a prognostic marker for the OS of patients treated with dabrafenib and trametinib [14]. This study showed that trial patients with lower disease burden (n = 237) had a lower serum LDH and an improved OS (75% CI: 70–81) measured at 2 years compared with patients with at least two-times the upper limit of normal (7% OS [3–19%]) [14]. Since our miRNA panel was found to be superior at determining OS in treatment-naive patients [9], further investigation into our panel's clinical utility is currently being assessed.

Diagnostic imaging (e.g., CT scan, PET, and MRI) is the mainstay to record baseline images to observe the whereabouts of metastatic deposits. Appropriate therapy is then commenced with 'target' and 'non-target' lesions assessed at intervals of 2–3 months based upon the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) or the immune-related response criteria (irRC) guidelines [15,16]. These scans are assessed by the radiologist and the patients are categorized as having a complete response (CR), a partial response (PR), progressive disease (PD) or stable disease (SD). This type of monitoring is limited as it can only be used intermittently during the course of treatment due to the risks of increased radiation exposure (e.g., a CT scans radiation is ~60× greater than a chest x-ray) [17]. The various imaging techniques also have limits of detection. For example, lesions can only be accurately measured if they are >10 mm [15]

and FDG-PET scans can only detect metabolic activity [18]. In sum, this means that if the lesions are <10 mm or they have low metabolic activity, then tumours may not be detected. Lastly, no scans are sensitive enough to detect micrometastases [19]. To address these limitations in monitoring of patients under therapy, we are currently assessing the utility of the MELmiR-7 panel, in comparison with diagnostic imaging, using serially collected bloods. Additionally, in a head-to-head comparison, circulating tumor DNA will also be compared.

Armed with these improved surveillance methodologies, we hope to see better clinical management of disease, which in turn could increase objective clinical responses and survival times. Future melanoma follow-up and treatment regimens should consider the utility of miRNAs as a companion diagnostic and prognostic aid in a clinical setting.

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