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Standing Committee on Health
House of Representatives
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Dear Mr Nelson

Thank you for the opportunity to provide a presentation to the House of Representatives Standing Committee on Health's inquiry into skin cancer in Australia: awareness, early diagnosis and management.

For melanoma, there appears to be gap in the utilisation of blood based melanoma markers in the clinical setting, for improved monitoring of patients. Given the significant benefits that could be obtained with the use of blood based 'liquid biopsies' there is a need for increased research in this area.

The primary aim of our research is to optimise methodologies for detection of blood based melanoma markers both for diagnosis and prognosis.

BLOOD BASED AUTOANTIBODIES AS DIAGNOSTIC BIOMARKERS

Despite significant effort in diagnosis and treatment of melanoma during the past decade, diagnosis remains extremely challenging, particularly for some early stage melanomas. It is a well-established fact that early diagnosis significantly influences prognosis of melanoma. If a melanoma lesion is diagnosed and removed while it is *in situ*, the cure rate can approach 98%. However, in those patients diagnosed in late stages the five-year survival rates are very low (<50% for stages III and IV melanoma) (1). Currently, biomarkers in melanoma lack reliability for screening. There are no validated serum biomarkers for melanoma; although S100 and LDH are used for prognosis in advanced disease, these have no clinical utility in early detection (1, 2). The incidence of melanoma is increasing rapidly; 133,000 new cases are being reported worldwide each year (3). Therefore, a reliable, highly sensitive and specific screening blood based diagnostic test is truly an unmet medical need for melanoma. Such a test could be used for routine patient screening of those at risk and as an accompaniment to normal diagnostic procedures in cases of uncertain diagnosis, and for those in remote areas. Such a test could dramatically reduce unnecessary surgical procedures and associated costs.

Cancer patients frequently develop autoantibodies upon exposure to tumour-associated antigens. These autoantibodies are emerging as promising biomarkers for the ***early*** detection of cancers since they are secreted, despite the presence of a small amount of the corresponding antigen, in relatively large quantities in the serum, prior to detection of the antigens and prior to the first clinical signs of cancer (4).

Autoantibodies also have persistent concentrations and long half-lives (between 7 and 30 d), making them ideal biomarkers for melanoma diagnosis.

There is increasing evidence that protein arrays are a powerful tool for testing biological samples to find biomarkers. The high-throughput simultaneous, rapid analysis of thousands of proteins on an array allows biomarker discovery that is relatively inexpensive (5). New antigen arrays allow identification of multiple biomarkers for reliable clinical testing with improved sensitivity and specificity. Once identified and validated, melanoma biomarkers can be applied for diagnostic screening of high-risk individuals worldwide.

Using a functional protein array comprised of 1636 antigens, in a proof of principle experiment we identified 8 biomarkers with high specificity and sensitivity (95%) for melanoma. The results indicate that further investigations are warranted in a larger cohort of patients. We are progressing preliminary research findings with the aim of developing an accurate and sensitive autoantibody blood test for melanoma diagnostics.

Such a test could be used in a coordinated national approach to diagnostic screening of at risk patients, those with difficult to diagnose tumours and those in remote areas.

Recommendation to improve early stage diagnosis:

A more intense and concentrated search for multiple novel blood-based diagnostic markers is required.

REFERENCES

1. Balch, C.M., et al., *Final version of 2009 AJCC melanoma staging and classification.* J Clin Oncol, 2009. **27**(36): p. 6199-206.
2. Sullivan, R. *The challenge of developing useful blood-based biomarkers in melanoma.* Br J Dermatol, 2013. **168**: p. 3
3. Vetvicka, V., et al., *Autoantibody profiling as a diagnostic marker for cancers.* World J Clin Oncol, 2013. **4**: p. 1-3
4. Lu, H., et al., *Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer.* J Proteome Res, 2008. **7**: p. 1388-94
5. Ramachandran, N., Srivastava, S., Labaer, J. *Applications of protein microarrays for biomarker discovery.* Proteomics Clin Appl, 2008. **2**: p. 1444-59

CIRCULATING MELANOMA CELLS TO INFORM PERSONALISED TREATMENT

Circulating tumour cells (CTCs) are tumour cells shed from primary or metastatic tumours into the blood stream. These circulating cells invade distant tissues and form secondary metastatic tumours. (1). There is increasing evidence that circulating tumour cells (CTCs) in blood are an important indicator of the potential for metastatic disease and poor prognosis (2-5). In fact, the presence and quantity of CTCs have been included as factors in the international tumour staging systems of several carcinomas (6) and the Cell Search System has been approved by the US FDA for monitoring progression in breast, colorectal and prostatic cancer (2,7,8).

With an ever increasing number of patients diagnosed with melanoma, particularly at early stages, routine surveillance of this population presents a significant logistical and financial challenge. Melanoma patients require routine skin examinations, and those at highest risk for recurrence require costly and potentially harmful radiologic imaging (PET/CT scans and brain MRI). These methods are only effective in finding metastases once they are visible on imaging or by dermatological examination. Monitoring of CTCs may provide important prognostic and diagnostic information at an **earlier** time.

To determine CTC sensitivity for monitoring patient status, tumour CTC-based clinical decision-making is currently being evaluated in ongoing trials in other cancers and should be assessed in melanoma particularly since a significant percentage of early stage patients develop metastatic spread; approximately 8% of patients with lesions <1 mm thick, 20% of patients with 1-2 mm lesions, 25–40% of patients with 2–4 mm lesions, and 50–75% of patients with lesions >4 mm thick develop metastatic disease and die within 10 years (9,10). Evidently then, diagnosing patients as clinically disease free after surgical removal of early-stage melanomas may be inaccurate, as tumour cells dispersed into the bloodstream may at any time, form distant metastases.

Currently there are no prospectively validated biomarkers for monitoring melanoma progression after surgical removal of the primary lesion. Primary tumour thickness and histological tumour features from a baseline biopsy are still used for prognosis in the absence of metastases (1,10). The biopsy does not however reflect tumour heterogeneity, nor the current status of the patient if taken previously, nor the composition of new tumours should there be progression.

Circulating tumour cells (CTCs) provide a prognostic tool for patient monitoring that can be performed in real time - when treatment decisions need to be made. Notably, a proportion of early stage melanoma patients show persistent, detectable CTC levels (9,11,12). It has been demonstrated that the persistence of CTC levels is indicative of early recurrence in chemo-naive non-metastatic breast cancer patients (13,14). For melanoma, follow up studies are needed to determine whether CTCs in early stage patients predict metastatic spread. It is likely that CTCs will provide a less invasive and potentially more sensitive method than those currently available, such as surgery and radiographic scans.

Overall, analysis of circulating melanoma cells from patient blood has the potential to identify patients at risk of disease progression who may benefit from additional treatment following surgical removal of their tumour. Thus CTCs may be suitable for routine monitoring of melanoma patients after surgical tumour removal.

Using circulating tumour cells we can also monitor treatment response allowing patients who fail on one therapy to be switched early to another treatment,

avoiding unnecessary side effects. We have shown that CTC quantification provides an early indication of treatment efficacy and disease free survival.

We can also test the captured melanoma cells for various DNA mutations that are targeted by the latest therapies including BRAF inhibitors– thus we can perform a 'liquid biopsy' in real time. Identification of such mutations, necessary for making personalised treatment decisions, are currently made using archival tissue or an invasive biopsy of current metastatic tumours, which is not possible in all cases. We are also developing methodologies that will allow us to determine disease recurrence using markers of treatment resistance in CTCs.

Finally we can grow the isolated cells in culture for further experimentation and drug testing. Thus we envision that characterisation of CTCs might uncover new biomarkers of metastatic propensity and guide the development of new cancer treatments.

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Recommendation to improve monitoring of melanoma patients:

1. The prognostic value of CTC quantification for disease free survival in early stage melanoma after primary tumour resection now needs to be evaluated for implementation of this methodology into clinical practice.
2. CTC characterisation should be evaluated for real time treatment decisions, monitoring of treatment efficacy and treatment resistance

REFERENCES

1. Ireland, A., et al., *Genetic factors in metastatic progression of cutaneous melanoma: the future role of circulating melanoma cells in prognosis and management.* Clin Exp Metastasis, 2011. **28**(4): p. 327-36.
2. de Bono, J.S., et al., *Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer.* Clin Cancer Res, 2008. **14**(19): p. 6302-9.
3. Krebs, M.G., et al., *Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer.* J Clin Oncol, 2011. **29**(12): p. 1556-63.
4. Hou, J.M., et al., *Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer.* J Clin Oncol, 2012. **30**(5): p. 525-32.
5. Lucci, A., et al., *Circulating tumour cells in non-metastatic breast cancer: a prospective study.* Lancet Oncol, 2012. **13**(7): p. 688-95.
6. Harris, L., et al., *American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer.* J Clin Oncol, 2007. **25**(33): p. 5287-312.
7. Cohen, S.J., et al., *Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer.* J Clin Oncol, 2008. **26**(19): p. 3213-21.
8. Cristofanilli, M., et al., *Circulating tumor cells, disease progression, and survival in metastatic breast cancer.* N Engl J Med, 2004. **351**(8): p. 781-91.

9. Reid, A.L., et al., *Markers of circulating tumour cells in the peripheral blood of patients with melanoma correlate with disease recurrence and progression.* Br J Dermatol, 2013. **168**(1): p. 85-92.
10. Balch, C.M., et al., *Final version of 2009 AJCC melanoma staging and classification.* J Clin Oncol, 2009. **27**(36): p. 6199-206.
11. Freeman, J.B., et al., *Evaluation of a multi-marker immunomagnetic enrichment assay for the quantification of circulating melanoma cells.* J Transl Med, 2012. **10**: p. 192.
12. Clawson, G.A., et al., *Circulating tumor cells in melanoma patients.* PLoS One, 2012. **7**(7): p. e41052.
13. Lucci, A., et al., *Circulating tumour cells in non-metastatic breast cancer: a prospective study.* Lancet Oncol, 2012. **13**(7): p. 688-95.
14. Molloy, T.J., et al., *The prognostic significance of tumour cell detection in the peripheral blood versus the bone marrow in 733 early-stage breast cancer patients.* Breast Cancer Res, 2011. **13**(3): p. R61.