MELANOMA RESEARCH AND THERAPY IN NEW ZEALAND: RAISING THE BAR THROUGH COLLABORATIVE ACTION

QMB Melanoma Abstracts

M1: Melanoma epidemiology and risk assessment

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Cutaneous melanomas are very common cancers for which the incidence is rising in most fair-skinned populations. The population of New Zealand now has the highest national incidence rates of melanoma in the world, and rates are projected to continue rising in future decades(1). Most of the burden will be experienced in older people. In absolute terms, it is estimated that the numbers of New Zealanders newly diagnosed with melanoma each year will rise from around 2200 currently to nearly 3500 by 2031. This burden will impose considerable economic costs, particularly if new therapies for melanoma are approved to treat patients with advanced disease.

In an effort to reduce the morbidity, mortality and economic burden of melanoma, there is great interest in improving strategies for earlier detection. One approach is to stratify the population according to predicted future risk, with the aim of screening only the subset of the population which contributes the majority of cases. We have developed a risk assessment tool within the QSKIN Study – a prospective cohort of 43,000 Queensland residents being followed for skin cancer events. Briefly, we split the cohort into derivation (67%) and validation subsets (33%), and then used stepwise Cox regression techniques to derive the best performing model for predicting 3-year risk of melanoma. The final model included terms for 8 factors, and performed with high discrimination and good calibration. By optimising the thresholds for screening, a streamlined intervention could be implemented in which approximately 40% of the population could be screened, yielding almost 80% of all melanoma cases. The impact of these tools will be discussed.

1. Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. J Invest Dermatol. 2016;136(6):1161-71.

M2: Blood based biomarkers for diagnosis, prognosis and monitoring of patients with melanoma

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Current methods of melanoma diagnosis and prognosis are at times problematic and limited to observation of tumour tissue by histology or imaging. The analysis of blood based, tumour specific products, including autoantibodies, circulating tumour DNA (ctDNA) and circulating tumour cells (CTCs), now provide early rapid, accurate and quantitative measurements of tumour presence and /or burden.

In our studies, we utilised protein arrays, mutation-specific droplet digital PCR and microfluidic devices to measure autoantibodies, mutant tumour DNA (ctDNA) and circulating tumour cells (CTCs), respectively, in patients with very early (in situ) to advanced stage metastatic melanoma. Autoantibodies were detected in early stage patients (n=150) at significantly higher concentrations than those in healthy controls (n=150). A diagnostic combination of 10 autoantibodies has been identified that can be utilised as an accompaniment to current clinical measures.

For metastatic melanoma we utilised ctDNA and CTCs to detect and monitor tumour burden during treatment of patients with targeted therapies (n=47) and/or immunotherapies (n=48). CTCs and/or ctDNA were detected in 70% to 80% of samples prior to treatment. Levels of ctDNA and CTCs decreased in response to therapies, prior to, or concurrently with radiographic response. Moreover, patients with no, or low, levels of ctDNA and CTCs at baseline had significantly longer PFS. In addition, CTC subtypes, including those positive for PDL1, predicted response.

In conclusion, our studies demonstrate the utility of blood based liquid biopsies to assist with diagnosis, prognosis and monitoring of melanoma patients.

1. J. Freeman, E. Gray, M. Millward, R. Pearce, **M. Ziman** (2012). *Evaluation of a multi-marker immunomagnetic enrichment assay for the quantification of circulating melanoma cells.* J Transl Med, 10:192**.** 2.
2. A. Reid, M. Millward, R. Pearce, M. Lee, M. H. Frank, P. Heenan, A. Ireland, L. Monshizadeh, T. Rai, S. Medic, **M. Ziman**. (2013) *Markers of circulating tumour cells in the peripheral blood of melanoma patients correlates with disease recurrence and progression.* BJD, 168: 85-92.
3. E. Gray, A. Reid, S. Bowyer, L. Calapre, K. Siew, R. Pearce, L. Cowell, M. H. Frank, M. Millward, **M. Ziman** (2015) *Circulating melanoma cell subpopulations: Their heterogeneity and differential responses to treatment.* J Invest Dermatol, 135(8): 2040-2048.
4. E. Gray, H. Rizos, A. Reid, S. Boyd, M. Pereira, J Lo, V Tembe, J Freeman, J. Lee, R. Scolyer, K. Siew, C. Lomma, A. Cooper, M. Khattak, T. Meniawy, G. Long, M. Carlino, M. Millward, **M. Ziman.** (2015) *Circulating tumor DNA to monitor treatment response and detect acquired resistance in patients with metastatic melanoma,* Oncotarget, 6(39):42008-18.

M3: Melanoma heterogeneity – the elephant in the room

Prof. Mark Shackleton[1]
[1]Alfred Health and Monash University, Melbourne, Australia

Progress has been breathtaking in the treatment of advanced melanoma with molecularly targeted therapies and immunotherapy. This has fundamentally transformed an almost-universally and rapidly fatal disease into one in which expectations are now high not only of gaining control over progressing disease, but more importantly of substantially increasing the length and quality of life of those affected. However, despite these gains, most patients with advanced melanoma still die from their disease. Although in some patients this is due to primary resistance to modern therapies, particularly immunotherapy, the vast majority of patients who respond well initially to treatment unfortunately develop secondary resistance. A key driver of this is the highly adaptive nature of this disease, which is driven by dynamic intratumoral epigenetic and genetic variation that generates resistance mechanisms amongst melanoma cells. Elucidating the causes and consequences of this variation, which drives the cellular heterogeneity evident in most melanomas, will be key to availing the therapy advances of the modern era.

Towards this, our laboratory has identified multiple mechanisms through which melanomas become heterogeneous. While epigenetic variation drives plastic changes in some phenotypes that would be predicted to undermine cell marker-specific therapeutic targeting, we also find evidence of irreversible phenotypic changes associated with loss of tumorigenic potential. Mechanisms that drive this indicate therapeutic opportunities to drive senescence in melanoma cells. Underpinning these epigenetic changes, however, we also find striking degrees of mutational instability during melanoma progression in patients. Unravelling the perturbed mechanisms that fail to maintain genomic integrity in proliferating melanoma cells has the potential to turn intratumoral heterogeneity, currently the unassailable armour of melanoma, into its Achilles heel.

M4: Understanding and facilitating clinical research in melanoma

Dr. Michelle Wilson[1], **Mr Paul O'Neil**
[1]Auckland City Hospital, Auckland, New Zealand

Cancer remains the leading cause of mortality in New Zealand and is likely to be the defining health issue for the next decade. Clinical trials are designed to improve patient outcomes with the ultimate global priority to reduce cancer related morbidity and mortality. Importantly, they offer access to new therapies and have been shown to impact patient outcomes. ***Despite the recognition of their importance, less than 5% of adult patients with cancer are involved in clinical trials***.

In contrast, over 60% of children with cancer in the United States are enrolled in clinical trials.[1] Survival rates in paediatric tumours has quadrupled over the past four decades driven by the introduction of new therapies as a consequence of the high rate of clinician and patient involvement in research.[1] This model allows for rapid evaluation of new therapies, and delineation of the sub-populations that do benefit.[1]

Multiple barriers to enrolment in clinical trials have been recognised including patient, clinician and institutional factors. Key factors include paucity of trials, cost (financial and time), belief in the study, age and comorbidities.[2-4] While it is important for us to draw on the experience from other countries, we need to understand the barriers relevant to our patient population and health care system. ***This will help us implement strategies relevant to our patients and systems.***

This session will explore the barriers to clinical trials in New Zealand focussing on melanoma. Patients' experiences and perspectives on clinical trials will be discussed. We will explore overseas models and consider the relevance of these to our patients in New Zealand. Ultimately, this session aims to develop a model for the conduct of clinical research in melanoma in New Zealand to improve equity and opportunities at a national level.

1. Gelijns AC, Gabriel SE. *Looking beyond translation--integrating clinical research with medical practice.* The New England journal of medicine 2012;366:1659-61.
2. Ross S, Grant A, Counsell C, Gillespie W, Russell I, Prescott R. *Barriers to participation in randomised controlled trials: a systematic review. Journal of clinical epidemiology* 1999;52:1143-56.
3. Townsley CA, Selby R, Siu LL. *Systematic review of barriers to the recruitment of older patients with cancer onto clinical trials.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:3112-24.
4. Tournoux C, Katsahian S, Chevret S, Levy V. *Factors influencing inclusion of patients with malignancies in clinical trials.* Cancer 2006;106:258-70.

M5: An online melanoma risk prediction tool specifically for New Zealanders

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[3]Department of General Practice, University of Otago, Dunedin, NZ,
[4]Best Practice Advocacy Centre, Dunedin, NZ.

New Zealand and Australia have the highest rates of cutaneous melanoma in the world. One method of improving melanoma control is to target primary and secondary prevention activities according to an individual's risk of disease.

Mary Jane will briefly cover trends in incidence, mortality and thickness of melanoma in New Zealand, including differences by age, gender and ethnicity. She will also present an overview of the development and implementation of a New Zealand-specific risk prediction tool for melanoma. This tool allows physicians to estimate an individual's probability of developing melanoma in the next 5 years.

Sneyd MJ, Cameron S, Cox B (2014). *Individual Risk of Cutaneous Melanoma in New Zealand: Developing a Clinical Prediction Aid*. BMC Cancer, 14:359

M6: Can research involving NZ melanoma patient participation and extensive networking by researchers help reduce the melanoma burden in NZ?

Eccles, M.R.[1, 4], Chatterjee, A.[1, 4], Rodger, E.J.[1, 4], Ahn, A.[1], Leichter, A.[1], Motwani, J.[1], Stockwell, P.[2], Parry, M. [3], Jones, A.[4], Ferguson P.[5], Gardner, J.[6], Sutton, T.[7], Sarwar, M.[8], Emanuel, P.[9], Shepherd, P.[10], multiple other members of The Maurice Wilkins Centre. [11]

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With the highest per capita rate of melanoma in the world in NZ, facilitating high quality melanoma research in NZ is of critical importance. Frequently, involvement of patients, tissue samples, and networking amongst researchers helps facilitate an inter-disciplinary approach to research that enhances research outcomes. I will discuss two projects where we have combined an extensive networking approach between NZ researchers, as well as the use of NZ melanoma patient material, and how this approach is leading to significantly enhanced outcomes. In the first example, we have collaborated in a network of NZ researchers to identify mutations in >500 melanomas in NZ patients, and we found a surprisingly high rate of *NRAS* mutations, as well as previously unrecognised, although relatively commonly occurring mutations in the *EPHB6* gene, encoding the ephrin type B receptor 6 protein, in NZ melanomas. These data suggest that melanomas in NZ patients do not necessarily conform to similar patterns of mutations as those in Europe or North America for instance. In the second example, we are collaborating with a network of NZ researchers to generate a comprehensive "omic" database associated with a well characterised large panel of between 50-100 NZM melanoma cell lines generated by Prof Bruce Baguley, derived from NZ melanoma patients. Included in this "omic" data are exomes, transcriptomes and methylomes, as well as characterisation of the cell lines by analysis of immune response, invasiveness, and also growth response and drug resistance profiles. These data will significantly enhance melanoma research efforts both nationally and internationally. In summary, a short-term goal might be to encourage NZ melanoma patient participation (including tissue samples) as well as networking by melanoma researchers, including researchers associated with translation of research into clinical practice in NZ. These approaches may enhance overall knowledge of the melanoma burden in NZ, which can be applied to reduce melanoma mortality rates.

M7: Making immune therapy work for more melanoma patients

Prof. Rod Dunbar[1]
[1]Maurice Wilkins Centre & School of Biological Sciences, University of Auckland, Auckland, New Zealand

Although anti-PD1 is revolutionising medical care for patients with advanced melanoma, many patients fail to respond. In some cases patient tumour samples show signs that other immune inhibitory mechanisms are active within their tumours, and it's logical to assume that many of these patients are likely to respond to systemic agents targeting these molecular pathways. However the success of all these therapies is predicated on the patients generating a spontaneous T cell response against their melanoma – and this does not happen in all patients, even at late disease stages. Strategies to induce effective T cell responses against patients' melanomas include non-specific immune stimulators such as anti-CTLA4, as well as agents targeted at specific antigens within melanomas, from antigen-specific vaccines to adoptive T cell therapy. Recent clinical data suggest that targeting unique antigens ("neo-antigens") within each melanoma for specific T cell attack may generate durable clinical responses in many patients, and render others newly responsive to anti-PD1. However challenges remain in rolling out these highly personalised therapies to large numbers of patients. We are pursuing both vaccination and adoptive cell therapy approaches to such highly personalised therapy, and will describe why we remain optimistic that these therapies could eventually be made available to melanoma patients as part of routine care.

M8: The promise and challenges of melanoma genomics

Print, C[1,2], Blenkiron C[1,2], Fitzgerald S[1], Knowlton N[1,2], Lasham A[1], Shields P[1], Trevarton A[1], Chatterjee A[2,3], Eccles M[2,3], Rodger EJ[2,3], Stephens R[4], Mathy JE[5], Mathy JA[6], Dunbar R[5], Braithwaite A[2,3] and Black M[2,7]

[1]Department of Molecular Medicine and Pathology, University of Auckland; [2]Maurice Wilkins Centre, NZ; [3]Department of Pathology, University of Otago; [4]Auckland District Health Board; [5]School of Biological Sciences, University of Auckland; [6]Counties Manukau District Health Board; [7]Department of Biochemistry, University of Otago.

Genomic research has taught us a great deal about melanoma biology and immunology, which in turn has brought dramatically changed outcomes for melanoma patients[1]. Genomics is also making in-roads into pathology and oncology to improve patient care[2]. However, the genomic variability between melanomas from different patients[3] makes precision oncology for melanoma challenging. This talk will describe our research group's attempts to use genomics to understand variability between patients in terms of melanoma drivers and immune response. We will also discuss NZ's first attempts to use blood genomics to identify melanoma relapse.

1. Rajkumar S, Watson I. *Molecular characterisation of cutaneous melanoma: creating a framework for targeted and immune therapies*. Br J Cancer. 115(2), 145-5570(1), 2016.
2. Harris G, O'Toole S, George P, Browett P and Print C. *Massively Parallel Sequencing of Solid Tumours – Challenges and Opportunities for Pathologists*. Histopathology. 70(1), 123-133, 2017.
3. Trevarton A, Mann MB, Knapp C, Araki H, Wren JD, Stones-Havas S, Black M and Print CG. *MelanomaDB: integrative melanoma genomic analysis*. Frontiers in Cancer Genetics. , art 184, 2013.

M9: Growing melanoma cells from surgical samples: what have we learnt?

Bruce C Baguley[1]
[1]Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand

In 1989, a small group of clinicians and scientists started a project where surgical samples of metastatic melanoma were grown in the laboratory. It was thought that such samples might reflect the sensitivity of the original tumour to anticancer drugs and radiotherapy, and that the system could be used to match therapy to individual patients, as well as to test new potential therapies before they progressed to clinical trial. Melanoma patients were a critical part of the team and the project was explained before requesting permission to provide material. The work also entailed continuing discussions with ethical committees. A talented cell biologist, Elaine Marshall liaised with surgeons, pathologists and patients, as well as leading the laboratory work. Funding was successively from the Health Research Council and the Cancer Society of New Zealand, from its Auckland Division and from a Manning Fellowship. Even the most aggressive of melanomas is completely unable to grow without growth factors, which are normally provided by host cells in the tumour microenvironment. As the cultures proceed, host cells are lost and growth factors must be added to compensate. Since chopping up the surgical sample into tiny pieces prior to culture induces a massive wounding response, leading to fibroblast proliferation, we had to devise strategies to stop the cultures being overrun by fibroblasts[1]. Our initial goal was to get a result in 7 days, providing a time frame relevant to clinical management. However, we found that most melanoma samples could be coaxed to grow indefinitely, allowing us to develop and store a collection of melanoma lines. The results suggest that the cell lines mimic many but not all of the properties of the original tumours in melanoma patients. Today, the melanoma line collection forms an important resource that is being used in both national and international studies.

[1]Marshall ES, Finlay GJ, Matthews JHL, Shaw JHF, Nixon J, Baguley BC. *Microculture-based chemosensitivity testing: a feasibility study comparing freshly explanted human melanoma cells with human melanoma cell lines*. J Natl Cancer Inst 1992; 84: 340-345

M10: Lessons for melanoma therapy gained from studies in the NZM melanoma cell line panel

Baguley B[1], Kolekar S[1], Tran K[1], Hunter F[1], Shepherd P[1]
[1]Auckland Cancer Society Research Centre, University of Auckland

We have performed extensive phenotypic analysis and exome sequencing studies in a panel of 102 early passage patient derived melanoma cell lines obtained at the Auckland Cancer Society Research Centre. These studies have included extensive analysis of how the cells respond to a range of small molecule drugs, both in vitro and in in vivo xenograft models. The major findings to date from these studies are that most of the lines are sensitive to lipophilic statins. Toidentify the mechanisms involved we have used genome wide screening studies using CRISPR/Cas9 Gecko libraries and results of these studies will be described. We have also discovered a drug combination that greatly improves the efficacy of the BRAF inhibitor vemurafenib. What is more, this drug combination is surprisingly highly efficacious against melanomas that have wild type-BRAF, a tumour type that is normally resistant to vemurafenib. These findings suggest new avenues for clinical trials for patients who are refractory to other treatments.

M11: Working towards a molecular classification of melanoma

Dr Jacqui Gardner[1], Dr David Gibbs[2], Dr Matthew Strother[2], Tracey King[3]

[1]Department of Pathology, CDHB, Christchurch, NZ, [2]Department of Medical Oncology, CDHB, Christchurch, NZ, [3]Research Nurse, Department of Medical Oncology, CDHB, NZ.

At Christchurch hospital patients with high risk primary and stage 3 and 4 metastatic melanoma are discussed at the regular major skin malignancy multidisciplinary meetings. As part of a baseline data set all primary and metastatic melanoma have immunohistochemical staining for the BRAF V600E activating mutation. In addition since 2014 all cases of metastatic melanoma have extended BRAF testing. This consists of DNA extraction and next generation sequencing of melanoma specific mutations using mass array at IGENZ in Auckland. We have collected data on 80 cases. This testing is performed to identify mutations, which may have implications for patient treatment and/or inclusion in clinical trials. Mutation frequency against wild type is also reported. These results are entered into an oncology database by one of our oncology research nurses Tracey king. The classification of melanoma for many years has been a clinical one but it is quite likely that future systems will include important genomic information to stratify patients into groups, which may reflect prognosis and predictive responses to specific inhibitor and immunotherapy.

1. Cancer Genome Atlas Research Network (2015). *Genomic Classification of Cutaneous Melanoma.* Cell. 161(7): 1681-1696. DOI: http://dx.doi.org/10.1016/j.cell.2015.05.044
2. *Agena Bioscience* website agenabio.com (last accessed 27/07/17).

M12: What role does the surgeon have in contemporary melanoma management and research?

Richard CW Martin
Melanoma Unit and Waitemata District Health Board, Auckland, New Zealand

Melanoma was once a purely surgical disease, radical resections were the norm but local, regional and distant recurrences were common. Incredible advances in the medical management of melanoma in the past 10 years with targeted therapy and immunotherapy has revolutionised melanoma management.

This presentation will look at the role of the surgical oncologist in the contemporary melanoma patient and research environment.

M13: Strategies to improve access to melanoma clinical research in New Zealand

Dr. Rosalie Stephens[1]
[1]*Auckland City Hospital, Auckland, New Zealand*

There are limited systemic agents available to New Zealanders with advanced melanoma, due to the high cost of treatments such as BRAF and immune checkpoint inhibitors. In addition, there remain many unresolved issues with respect to the optimal use of the newly introduced drugs. Despite these compelling reasons to undertake clinical trial research in melanoma, the proportion of patients participating in drug development or translational studies is extremely low. Barriers to participation have been described in a previous talk; this session will utilise that information to explore strategies in New Zealand for improving trial access. I will describe the Auckland experience of employing a dedicated melanoma clinical trial coordinator for industry-supported melanoma research and propose an extension of this role to a national co-ordinator. We will discuss a broader mechanism to facilitate more research, which is to develop a national framework for melanoma research including the formation of a New Zealand special interest group and fellowship opportunities.

M14: Establishment of an early phase clinical trials unit

Dr. Sanjeev Deva[1]
[1]Cancer and Blood, Auckland Hospital, Auckland, New Zealand

In order to improve health outcomes for patients with melanoma, we require medical research to prove the utility of newly discovered therapies. A key components of the drug development pathway are early phase clinical studies. These studies bridge the gap between the laboratory and the clinic. The aim is to test safety and find the optimal dosage, although increasingly they also assess efficacy in specific patient groups. Once its dose has been established as optimal, the medicine is further tested in larger later-phase trials to determine whether it will become the new standard of care.

Early phase studies are onerous on both patients and institutions alike, and ideally require a dedicated clinical research facility and specific research team. An Auckland Academic Health Alliance (Auckland District Health Board and the University of Auckland) initiative has resulted in the establishment of such a clinic in order to run such studies.

This talk will discuss key aspects of the establishment of the clinic, factors required for its future success and examples of melanoma studies in the portfolio.

Poster Abstracts

M15: Genomic variations of the mevalonate pathway and roles of bisphosphonate in melanoma

Tran, K.B.[1,2,3], Kolekar, S.[1,3], Jabed, A.J.[1,2], Hutchens, J.[2], Buchanan, C.M.[1,2,3], Baguley, B.C.[1,2,3], and Shepherd, P.R. [1,2,3]

[1]Auckland Cancer Society Research Centre, University of Auckland.

[2]Department of Molecular Medicine and Pathology, University of Auckland.

[3]Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, New Zealand

Bisphosphonates, inhibitors of the mevalonate pathway, have been found to inhibit osteoclasts and prevent bone loss; they therefore are common treatment to prevent the risk of skeletal relevant complications in bone-metastatic cancers. Recently, inhibition of the mevalonate pathway has been linked to improvement of the treatment outcome for melanoma patients. In addition, new evidence suggested that bisphosphonates not only acted on osteoclasts but might also have direct inhibitory effects on cancer cells. However, it remains largely unclear what roles the mevalonate pathway takes in melanoma biology and what effects bisphosphonates have on the growth of melanoma cells. Therefore, we aimed to analyse the status of key genes of the mevalonate pathway, assess the effects of bisphosphonate in melanoma and whether the addition of bisphosphonates to melanoma therapies could improve the treatment outcome.

We used an in-house panel of early passage cell lines derived from metastatic melanomas. DNA from melanoma cells was isolated and subjected to whole-exome sequencing. Sequence data were analysed by the Torrent Suite and Ion Reporter software package. The potency of zoledronate and alendronate was assessed in assays of proliferation, apoptosis, cell cycle and migration. BRAF-mutant cell lines were induced to become resistant to vemurafenib by chronic treatment. Our data demonstrated that genomic variations were frequently found in the mevalonate pathway. In addition, we found that the bisphosphonate attenuated the growth of melanoma cells at low-micromolar concentrations in most cell lines tested. Interestingly, the effect was observed in different genotypes of cell lines. In addition, combination of zoledronates and vemurafenib was effective in vemurafenib-acquire resistant NZM12 cell line. Our ongoing experiments will assess whether zoledronate and alendronate could induce apoptosis, arrest cell cycle, and inhibit the migration of melanoma cells. These data will provide a rationale for further mechanistic studies of the effects of bisphosphonates in melanoma treatment.

M16: Development of single-cell methodologies to interrogate DNA methylation patterns at a whole genome scale

Leichter, A.L.[1], Day, R.C.[2], Stockwell, P.A.[2], Wilson, M.[3], Guilford, P.[2], Eccles, M.R.[1] & Chatterjee, A.[1*]

[1]Department of Pathology, University of Otago, Dunedin School of Medicine, NZ, [2]Department of Biochemistry, University of Otago, School of Biomedical Sciences, [3]Department of Microbiology and Immunology, University of Otago, School of Biomedical Sciences. *for correspondence

To date DNA methylation studies have been carried out on bulk cell populations providing an averaged snapshot of methylation but failing to detect individual cell-specific or rare cell subpopulation changes. There is enormous potential for biomedical applications of single-cell epigenomic technologies. Using a single cell methylation (SCM) technique would enable profiling of circulating tumour cells, exfoliated cells and cancer stem cells and would provide critical detection and dynamic measurement of epigenetic abnormalities during disease initiation, evolution, relapse and metastasis. However, currently in Australasia low cell number or single cell epigenomics analyses are not well established, and therefore robust SCM investigation is not yet possible.

Previously, we have established analytical tools and methods for large-scale methylation analysis in bulk cells[1-3]. We are now establishing an experimental and analytical approach for the interrogation of single-cell DNA methylation patterns, specifically using single-cell reduced representation bisulfite sequencing (sc-RRBS) and whole genome bisulfite sequencing (sc-WGBS)[4]. Several single cell libraries have been prepared, yet alignment to the human bisulfite converted genome remains low. We are currently developing unique individual protocols and troubleshooting steps to optimise the progressive steps of the sc-RRBS protocol including; lysis, MSPI digestion, end repair/dA-tailing, adapter ligation, bisulfite conversion and PCR amplification. Streamlining this protocol will open new avenues to perform DNA methylation assessment on rare cell populations, such as circulating tumour cells and provide critical information on the phenotype of individual cells to assist clinicians in prediction of the reoccurrence or metastasis of disease.

1. Chatterjee A, Stockwell PA, Ahn A, Rodger EJ, Leichter AL, Eccles MR: Genome-wide methylation sequencing of paired primary and metastatic cell lines identifies common DNA methylation changes and a role for EBF3 as a candidate epigenetic driver of melanoma metastasis. *Oncotarget* 2016.
2. Stockwell PA, Chatterjee A, Rodger EJ, Morison IM: DMAP: differential methylation analysis package for RRBS and WGBS data. *Bioinformatics* 2014, 30(13):1814-1822.

M17: Stratification of melanoma patients according to PD-L1 expression, CpG methylation and presence of tumour-infiltrating lymphocytes

Ahn, A.[1,2], Rodger, E.J.[1,2], Eccles M.R.[1,2], Chatterjee A.[1,2]

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Immune checkpoint inhibitors have revolutionised the treatment of melanoma, but only a subset of patients respond. The tumour microenvironment, namely the interaction between immune cells and tumour cells, plays a crucial role in the treatment outcome of patients. In order to better predict response to immunotherapy a tumour stratification framework has been proposed on the basis of PD-L1 expression and the presence of tumour-infiltrating lymphocytes (TILs). Here we use RNA-Seq data from the Cancer Genome Atlas (TCGA) to categorise 469 melanoma patients according to PD-L1 mRNA expression and the presence of CD8+ TILs. The cell-type enrichment webtool *xCell* was used to categorise patients into 4 groups: $TIL^+/PD\text{-}L1^+$ (44.3%), $TIL^-/PD\text{-}L1^+$ (5.5%), $TIL^+/PD\text{-}L1^-$ (18.3%) and $TIL^-/PD\text{-}L1^-$ (31.8%). Two independent deconvolution methods (*CiberSort* and *MCPcounter*) were used to validate the grouping of TIL^+ and TIL^-. Further, to compare this expression-based classification with an independent method, we compared these results with a CpG methylation based TIL signature (meTILs). Better understanding of the molecular subgroups of melanoma patients holds critical implications for a patient's response to immunotherapy. As shown in other studies, the $TIL^+/PD\text{-}L1^+$ patient group had a favourable prognosis with a longer overall survival. In addition, the $TIL^+/PD\text{-}L1^+$ patient group had an upregulation of genes related to numerous immune pathways including the viral defensive pathway and cytokine expression whereas the $TIL^-/PD\text{-}L1^-$ group had a downregulation of these immune signatures. Overall, we demonstrate an effective method to use RNA-Seq and DNA methylation data for stratification of melanoma patients that could potentially be used to inform prognosis and predict response to immunotherapy

M18: Epigenetic drivers of melanoma metastasis identified by genome-wide reduced-representation bisulphite sequencing (RRBS) analysis

Eccles, M.R.[1], Chatterjee, A.[1], Rodger, E.J.[1], Ahn, A.[1], Leichter, A.[1], Motwani, J.[1], Stockwell, P.[2], Parry, M.[3]
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As cancer metastasis is a major cause of cancer-related mortality, understanding the causes and effects of genomic changes in this process will help in identifying metastasis-related treatment targets. We have focused on investigating genomic changes in melanoma metastasis, because melanoma is one of the most aggressive and invasive of human cancers. While epigenetic alterations are hypothesized to play a facilitatory role in metastasis, for example in the promotion of epithelial to mesenchyme transition (EMT), which is increasingly supported by accumulating evidence, it is not clear whether epigenetic changes are necessary or sufficient to *drive* metastasis. We have addressed this question by using an approach involving RRBS mapping of genome-wide DNA methylation changes in order to identify shared methylation changes across a series of primary and metastatic matched melanoma samples. Using this approach we identified 75 common (10 hyper- and 65 hypomethylated) genomic regions associated with 68 genes showing significant methylation differences between metastatic and primary tumours, which were shared amongst all three unrelated metastatic melanoma cell lines. One epigenetic change involved elevated *Early B Cell Factor 3* (*EBF3*) mRNA levels and concomitant promoter hypermethylation in the three metastatic melanoma cell lines. RNAi-mediated knockdown of *EBF3* in melanoma cell lines demonstrated an oncogenic role for *EBF3* expression in promoting aggressive melanoma behaviour. We investigated collections of additional melanomas, and identified similar significant promoter hypermethylation and significantly elevated *EBF3* mRNA levels in metastatic versus primary melanomas in two publicly available independent melanoma cohorts (n=40 and 458 melanomas, respectively). Moreover *EBF3* has recently been identified as a top hit in GWAS screening of melanoma susceptibility loci. Including ours, several studies now suggest that epigenetic alterations have the potential to be oncogenic “drivers” of cancer aggressiveness, and are commonly recruited during the process of metastatic progression in tumour cells.

M19: Characterizing the therapeutic potential of crizotinib in cutaneous malignant melanoma

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Despite the success seen with BRAF and MEK inhibitors against cutaneous malignant melanoma, the emergence of drug resistance is almost inevitable[1]. One of the resistance mechanisms involves the HGF/c-MET cross-talk between melanoma and fibroblasts in the tumour microenvironment. Fibroblast-derived HGF activates c-MET signalling in melanoma cells, thereby providing an alternative pathway for proliferation and survival in the presence of BRAF and MEK inhibition[2].

SRB proliferation assays and Western blotting were used to characterize melanoma sensitivity to ALK/c-MET inhibitor crizotinib, both as monotherapy as well as in combinations with BRAF inhibitor, vemurafenib and MEK inhibitor, CI-1040. Melanoma lines and fibroblasts were co-cultured and treated with combination regimens of the aforementioned drugs. Crizotinib demonstrated a strong inhibitory effect on the growth of melanoma cells at submicromolar concentrations in virtually all cell lines irrespective of genotype. Importantly, combinations of crizotinib with vemurafenib and CI-1040 exhibited synergistic effects when compared to monotherapy. Melanoma cell lines exhibit increased proliferation and resistance to vemurafenib and CI-1040 treatment when co-cultured with fibroblast lines. This drug protective effect was reduced when treatment was combined with crizotinib.

The HGF/c-MET crosstalk between melanoma cells and fibroblasts creates a niche that influences intrinsic resistance to BRAF and MEK inhibition. Additionally, c-MET signalling offers an alternative route for cell-survival by bypassing BRAF. Combined c-MET and MEK inhibition demonstrates superior anticancer effects compared to monotherapy. Therefore, targeting the HGF/c-MET axis in an adjuvant setting offers an attractive strategy to combat resistance to BRAF and MEK inhibitors.

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