Project Description

Title

The effect of propranolol on the metastatic niche in melanoma: A randomized controlled trial.

Short Title

The MELPROP Trial

Note: this ethics application addresses Aim 4 only of the original (attached) Grant Proposal *Molecular characterisation and targeting vessels of the metastatic niche in melanoma*.

The lethal aspect of solid cancers such as melanoma relies on the ability of the tumour to disseminate to secondary organs. This metastatic potential is primarily under the governance of the aberrant growth of both blood and lymphatic vessels within the tumour site and at the future site of metastasis. Every year over 10,000 patients are diagnosed with melanoma. The current paradigm for these patients with locally invasive disease comprises improved staging through sentinel node biopsy and surveillance to detect tumour recurrence in the form of regional or distant metastasis. There is no intervention that would reduce their risk of progression and this research project is designed at filling this gap.

Recent discoveries from our team have redefined some aspects of cancer biology:

1) Identification and characterisation of a novel vessel-resident endothelial progenitor (endovascular progenitors, EVPs) that is required to establish the neo-formed vasculature at the tumour site (1)

2) Discovery of 2 genetic pathways (SOX18 and RBPJ transcription factors) that are essential for EVPs to differentiate into blood vessels. Genetic disruption of either SOX18 or RBPJ in the host results in a dramatic reduction in metastatic potential of tumours and significantly increased survival and is accompanied by a loss of activity of endothelial progenitors.

3) Discovery and validation of an FDA approved compound that disrupts SOX18/RBPJ interaction promising rapid clinical translation through drug repurposing. This compound is the R(+)-enantiomer of the drug, Propranolol. Propranolol is a beta-adrenergic blocking agent which has been used clinically for decades to treat high blood pressure, irregular heartbeat and severe chest pain but also benign conditions such as migraine or social anxiety. Propranolol is composed of both R and S enantiomers that are mixed in equal quantities in the currently available form. The S(-)-enantiomer of Propranolol has the beta-adrenergic activity (and subsequent potential toxicity) but does *not* have the anti-vascular activity (19,20,21). The R-enantiomer (R-Propranolol) inhibits Sox18/RBPJ interaction but does not act on adrenergic receptors.

Excitingly, upon primary tumour formation, we have identified the activation of EVPs at distant sites in the metastatic niche (e.g. the lungs). In this project, we propose to investigate the functional importance of pharmacologically disrupting SOX18 and RBPJ pathways in the vascularisation of the metastatic niche in melanoma patients.

Background

Over-production of new blood vessels is a feature of cancer progression: solid tumours induce the growth of blood vessels. The role of blood vessels in supplying nutrients and in permitting tumour growth has been well documented and forms the basis for a number of anti-tumour therapies (2). Similarly, the metastatic process starts with an increase in blood and lymphatic vasculature at the site of future metastasis forming the metastatic niche (3, 4). This process has been shown essential in numerous models of solid cancer metastasis. Currently novel anti-angiogenic molecules have been used with moderate success against certain types of solid tumours (5). For decades, identifying, characterizing and targeting the cellular and molecular pathways responsible for blood vessel remodelling in disease has become an essential adjunct to tailor personalized medicine. Recently the research team has made the seminal discovery that during tumour-induced angiogenesis a vessel-resident progenitor contributes to *de novo* blood vessel formation (6).

A landmark finding in 1997 suggested that endothelial progenitor cells (EPCs) had the capacity to generate new blood vessels, a process called neo-vasculogenesis (7), in a variety of situations including tumours. This finding stimulated numerous studies on the role of these cells but was greatly hampered by the lack of a functional definition. Therefore, many studies revolved around cells that, despite being called EPCs, were hematopoietic in nature and able to promote angiogenesis without themselves participating in vessel formation (CIA)(8). We have recently published and characterised a stem/progenitor cell among endothelial cells based on functional and fate tracing studies *in vivo* in both mice and humans. These cells called endovascular progenitors (EVPs) reside in blood vessels, are slow cycling and can be activated to give rise to transit amplifying (TA) and differentiated cells (D) (6). We have made very similar observations in the human term placenta (9). We have also demonstrated the importance of SOX18 in the activation and progression of EVP cells towards TA and D in the context of skin wounds (6). Also we have published the role of NOTCH signalling in self-renewal of EVPs (10). Our work among a handful of other groups has consequently contributed to an international consensus on the definition of endothelial progenitors (8).

The research team has previously gathered evidence that EVPs also exist in tumours and are essential in tumour vascularization (32). On this basis we propose to inhibit the pathways that were identified as important for EVP function in tumours and apply our findings to the metastatic niche.

Hypothesis

Molecular disruption of SOX18 or RBPJ in the pre-metastatic niche results in defective vascularisation and reduces the spread of tumour.

Aim

To evaluate the effect of Propranolol, that contains a pharmacological inhibitor of SOX18/RBPJ, on sentinel node vascularisation in patients with stage T1b – T4b, N0, M0 melanoma (2018 AJCC classification)

Trial Design

Rationale: A recent study has reported the use of propranolol as an adjuvant therapy in a cohort of stage IB and stage II melanoma patients. This study showed a major benefit in progression free survival (hazard ration=0.18) in those taking propranolol (22). The study was not randomized nor was treatment attribution blinded and patients could choose to take propranolol (80mg per day, a standard dose) or not. As this is likely to represent an exciting strategy for adjuvant treatment of high-risk locally invasive melanomas, we propose to conduct an investigative randomized trial to establish the effect of Propranolol on the sentinel node vasculature of the metastatic niche by inhibiting SOX18/RBPJ.

Eligible participants: patients will be recruited from the Melanoma Clinic of the Princess Alexandra Hospital (PAH), a referral centre in Queensland for sentinel node biopsy. Previous studies conducted by the research team have shown that about 90 sentinel node procedures are performed each year at the PAH for locally invasive melanomas corresponding to the stages T1b-T4b, N0, M0. Patients newly diagnosed with a primary cutaneous melanoma that is ulcerated and/or thicker than 1mm and who elect to undergo a sentinel node biopsy procedure will be considered. Patients will be excluded if they (1) have clinical or imaging signs of regional or distant disease at diagnosis or (2) are currently prescribed any beta-blockers such as Propranolol or (3) have a contra-indication to beta-blockers (4) are pregnant or (5) are asthmatic.

Study Design: a randomized placebo controlled trial with a self-control design.

Intervention: Consenting patients will be randomized 1:1 to receive propranolol 40mg twice a day, or a placebo, during the time leading up to the sentinel node biopsy (usually 2-3 weeks). Patients and the surgical team will be blinded to the treatment regimen. Patients will be stratified based on sex (2 males for 1 female reflecting the population data diagnosed at this stage) and tumour thickness (<2mm or >2mm reflecting the 2018 T stage classification).

Once collected the sentinel node will undergo pathology review. A small 1mm punch biopsy will be performed on the fresh tissue for gene expression studies. After pathological examination, paraffin embedded sections will be obtained and stained for CD31, D2-40 and

Ki67. In addition, expression of Sox18, VCAM, IL33, Notch1, VEGFR2, considered as targets of Sox18 and RBPJ will be evaluated at RNA and protein level.

Outcome Measures: the primary outcome measure will be signs of activation of genes induced by Sox18/RBPJ activity: IL33 and VCAM reflecting Sox18 re-expression and RBPJ activity. This will be measured as surface of staining. Secondary outcome measures will be the number and proportion of staining of VCAM and IL33 in CD31+ endothelial cells; surface of CD31+ vessels (blood vascularisation) and surface of D2-40+ vessels (lymphatic vascularisation).

Analysis: primary and secondary outcomes will be compared between groups treated, or not treated, with Propranolol. This will reflect on the ability of propranolol to inhibit Sox18 function.

Subgroup analysis: subgroup analysis will be performed on sentinel nodes that are devoid of any tumour deposit (negative SLNB: 85% of cases) to ensure that vascular changes reflect a metastatic niche phenomenon and are not the results of direct tumour deposits in lymph nodes.

Power calculation: Given our pre-clinical findings, we expect a 50% decrease in expression of Sox18 target genes. Groups of 12 patients (3 per stratum, 2 males 1 female) ensure 80% power to detect a significant difference at p=0.05 level.

Expected results: This trial will validate in vivo in patients a novel molecular mechanism of action of propranolol through Sox18/RBPJ inhibition. It will also establish the grounds for a randomized trial testing the efficacy of propranolol as adjuvant therapy for disease free survival. However this is a separate project beyond the aims of the current proposal.

Timeline: This is a 3-year project.

Significance and innovation

The incidence of melanoma in Australia has been increasing and this is mostly true for thin melanomas (26, 27)*. This translates into a lifetime risk of one in every 16 Australians being diagnosed with an invasive melanoma. Of all cancers in Australia, **cutaneous melanoma ranks fourth in incidence** (after colorectal, breast and prostate cancer). In a recent European study, melanoma had the highest mortality related cost among all cancers examined due to lost productive years in young adults (€312,798 per death)(28). In particular, early locally invasive melanomas contribute a large proportion of years of life with disability and years of life lost in the disease (29).

Although patients diagnosed with thin melanomas (<1mm thickness) enjoy a high survival of 96% at 20 years (30), those individuals with thicker locally invasive melanoma (>1mm, stage II) or with ulceration (IB or II) of their tumour are at much higher risk of recurrence and death (70.8% melanoma specific survival at 10 years) (31). These high-risk individuals are often offered sentinel node biopsy as an additional means to evaluate their prognosis. However,

there are currently no adjuvant strategies to try to increase disease free survival in patients with locally advanced disease. Recently developed therapies for advanced disseminated melanoma, such as immune checkpoint inhibitors and Braf and MAP kinase pathway inhibitors, would be too expensive and too toxic to employ long term in this large population where 70% are long-term survivors.

We here propose a novel adjuvant strategy to reduce chances of tumour dissemination by altering the vascularization of the metastatic niche through disrupting biological function of recently uncovered endothelial progenitors. Innovative genetic models and small molecule screens ensure the high quality, visibility and translatability of this work. Importantly, the use of Propranolol, a drug with decades of safety data, ensures the immediate translation of these findings in a repurposing drug strategy.

Overall, we believe strongly that this work will change the way that high risk locally invasive melanomas are managed and is likely to result in high impact publications and international presentations.

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