Platelets and cancer… the plot doesn't always thicken

Philip Crispin[1,2] and Elizabeth E Gardiner[1]

[1]ACRF Dept. Cancer Biology and Therapeutics, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia; [2]Department of Clinical Haematology, The Canberra Hospital, Garran, Australia

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Platelets have critical roles in preventing blood loss following injury, promoting wound healing, and in fighting infection through innate immune defense strategies. Deficiencies in platelet number or function either as a result of disease, or as a consequence of therapy, or both, can lead to dramatic and potentially fatal consequences. With the advent of new therapeutics targeting pathways within haematological malignant cells that are also important for platelet function, monitoring the state of a patient's haemostasis system is now an important clinical consideration.

Thromboembolism is a well-established risk in cancer and justifiably the focus of much research and clinical attention to understand the mechanisms and reduce the significant morbidity and mortality associated with it. Platelet activation has been identified as a contributing factor to thromboembolism in malignancy. This may occur by direct interaction with tumour cells, particularly through tumour cell-expressed podoplanin activating platelets via C-type lectin-like receptor (CLEC)-2 [1]. Malignant cells can release adenosine diphosphate (ADP), directly activating platelets. Indirectly, podoplanin may be expressed by non-malignant cells, including endothelial cells, macrophages and fibroblasts in the tumour microenvironment and tumour cells may express or secrete tissue factor. The latter activates the coagulation system and indirectly activates platelets via thrombin.

In non-myeloid malignancies, bleeding is often associated with local tumour-associated factors, such as vascular invasion or tumour necrosis. Thrombocytopenia induced by treatment is also a recognised risk for bleeding. While typically due to myelosuppression, and therefore seen less commonly in therapy for non-haemopoietic malignancy, immune-mediated thrombocytopenia is also an infrequent complication of checkpoint inhibitor therapy [2]. Consideration of bleeding risk is therefore increasingly needed in oncological practice. In this edition of the Journal, Dmitrieva and colleagues explore the timing of bleeding risk and platelet dysfunction in lymphoproliferative disorders treated with the first-in-class Bruton's tyrosine kinase (BTK) inhibitor ibrutinib [3].

BTK is an important molecular player in immunoreceptor tyrosine-based activation motif (ITAM) signalling pathways that govern survival and activation signals in lymphocytes and other immune cells. BTK inhibition has advanced the therapy of chronic lymphocytic leukaemia (CLL) and other B cell lymphoproliferative disorders. Showing improved overall survival in relapsed and refractory CLL, even with the negative prognostic indicators of 17p deletion or TP53 mutation, IGHV status, del(11q) and fludarabine refractoriness [4], ibrutinib has an established place in CLL treatment. It is also of benefit in mantle cell lymphoma (MCL) [5], a lymphoma with poor prognosis, and Waldenström macroglobulinaemia [6]. Studies in other B cell lymphoproliferative disorders are in progress. Ibrutinib carries with it a clinically important risk of bleeding, particularly bruising and mucocutaneous haemorrhage characteristic of impaired primary haemostasis, which has been attributed to platelet dysfunction [7]. As ITAM signalling involving BTK is important for normal platelet function and underpins platelet responses to numerous agonists including collagen and von Willebrand Factor, inhibition of BTK disrupts these haemostatic pathways.

Understanding the functional impacts of ibrutinib is also proving critical. Advances in our understanding suggest the potential for repurposing as specific atherosclerosis inhibitor as low concentrations impede glycoprotein (GP) VI mediated platelet aggregation without impacting primary haemostasis [8, 9]. There are also clinical trials of BTK inhibitors underway in immune and inflammatory disorders, including immune thrombocytopenic purpura. Furthermore, comparisons with more specific BTK inhibitors suggest that the risk of bleeding may be decreased while maintaining on-target effects [10].

Dmitrieva and colleagues examined platelet dysfunction over time during ibrutinib treatment [3]. They found that platelet function decreased following initiation of ibrutinib, but then improved in concert with disease response. Importantly, platelet dysfunction was observed at baseline, prior to ibrutinib initiation, pointing to an underlying platelet function defect, an issue previously reported without ibrutinib treatment in CLL [11]. Many people with CLL and MCL have had prior alkylating agents and fludarabine, therapy-induced myeloid stem cell changes could be speculated to cause the platelet dysfunction. However, prior therapy was not required for, or predictive of measurable platelet impairment. Furthermore, the platelet function defects at baseline were different between the CLL and MCL cohorts.

The mechanisms for platelet dysfunction in CLL have not been well elucidated and no data exist for this effect in MCL. Pulte and colleagues [12] found high expression of CD39, an ecto-nucleotidase metabolising ATP and ADP to AMP, on CLL cells and that their addition to platelet-rich plasma (PRP) inhibited ADP-induced platelet aggregation. There are however grounds to question the role of CD39 in the pathogenesis of the specific findings reported by Dmitrieva, predominantly because CLL cells would not be present to inhibit ADP in PRP during the aggregation studies. Mantle zone lymphocytes do express CD39 [13], but the lymphocyte numbers did not exhibit the same change seen in CLL and the pattern of impairment is not suggestive of ADP inhibition. The mechanisms for platelet impairment in CLL and MCL, which are likely to be different, remain uncertain.

As with the pro-coagulant interactions between malignant cells and platelets, it is possible that negative regulation via platelet surface receptors, by direct or indirect means, may be implicated in the reduced responses to agonists. The absence of CLL or MCL cells within the PRP during testing suggests that any such effect would need to be plasma-based, have permanently changed platelet receptors or structures, or involve signals already within the platelet.

Thinking about other potential mechanisms, platelets are known to absorb RNA from their environment. Tumour-educated platelets have been proposed as a source of RNA for liquid biopsies due to the enhanced concentration of tumour-derived RNA within them. It is known that platelet-transported RNA can be transferred to tumour cells to affect tumour growth [14]. It is also known that plasma microRNA changes are associated with levels of platelet activation [15], implying that plasma derived RNA is not simply passively transported within platelet vesicles. It is not known what role tumour RNA has, if any, on the function of platelets. It seems both plausible and probable that tumour-derived mRNA and miRNA within the platelet will influence protein production and/or expression. This may be a potential mechanism by which down-regulation of platelet activation pathways might occur.

An alternative explanation may be that platelets are impaired from the time of production. Megakaryocyte development and maturation in the bone marrow occurs within a specific microenvironment. In this niche, cell adhesion, levels of cytokines and growth factors, and physical properties all impact on the development of megakaryocytes and thrombopoiesis. In the laboratory, megakaryocyte maturation is known to be more physiological when cultured in stiff methylcellulose gels rather than liquid media, leading to enhanced proplatelet formation [16]. This highlights the importance of the physical environment on megakaryopoiesis. In order to produce platelets, megakaryocytes also need to extend proplatelets into marrow sinusoids, so maintaining proximity to the vasculature is a requirement of platelet production. This may be difficult with marrow infiltration in malignancy. The marrow is particularly crowded in CLL. While nodular infiltrates may be seen in early disease, diffuse interstitial infiltrates are typically seen in more advanced disease. By contrast in MCL, marrow involvement, although common, typically involves more focal infiltrates or lower degrees of interstitial infiltration with less widespread architectural disruption than in CLL (Figure 1).

This is reflected in the lower platelet counts in CLL, but poor megakaryocyte localisation and disruption of the microenvironment could conceivably have an impact on the functional state of the platelets produced. In support of this, low levels of platelet adheso-signalling receptors GPIbα and GPVI were detected on circulating platelets from a small cohort of untreated, refractory CLL patients [17].

The improvement in platelet function over time while on ibrutinib has been mirrored in a small study with the phosphoinositide 3-kinase inhibitor idelalisib [18]. Idelalisib does not appear to affect platelet function, however disease reduction improved baseline impaired platelet aggregation. This points to the causative role of the underlying lymphoproliferative disorder in the platelet function defect. Despite many advances and the understanding of the critical linkage between the cancer cell, haemostasis, tumour microenvironment and tumour growth and progression, there remain considerable gaps in our understanding of tumour cell – haemostasis interactions.

For the clinician, the risk of bleeding with ibrutinib needs to be considered when making treatment decisions in B cell malignancies. In the oncology clinic, platelet count remains most predictive of bleeding in CLL and MCL, but platelet function is not routinely measured in clinical practice and is frequently not considered. Patients are often elderly, increasing the risk of haemorrhage, and often have comorbidities requiring anti-platelet or anticoagulant therapy [19]. Ibrutinib itself increases the risk of atrial fibrillation [20] and therefore a proportion of patients on treatment may be expected to require anticoagulation for stroke prevention. For the population being treated, additional platelet inhibition may carry risks and there are no data to support the withdrawal of concurrent antiplatelet therapy prescribed for coexisting cardiovascular disease. A better appreciation of the nature and extent of impaired haemostasis may be helpful in informing treatment decisions.

More generally, elucidating the underlying platelet dysfunction in lymphoproliferative disorders may improve the management of these conditions. Our current approach to venous thromboembolism prophylaxis in malignancy is largely identical across tumour types, although the risk of thrombosis varies. A better understanding of functional haemostasis within individuals may help to personalise the pharmacological approach. Tests of platelet function, thrombin generation or whole blood assays as well as monitoring surface levels of key platelet receptors may have a future role in individualising patient care, but for the present time further research is required on both the pro-thrombotic and inhibitory effects of malignancy on platelets and haemostasis.

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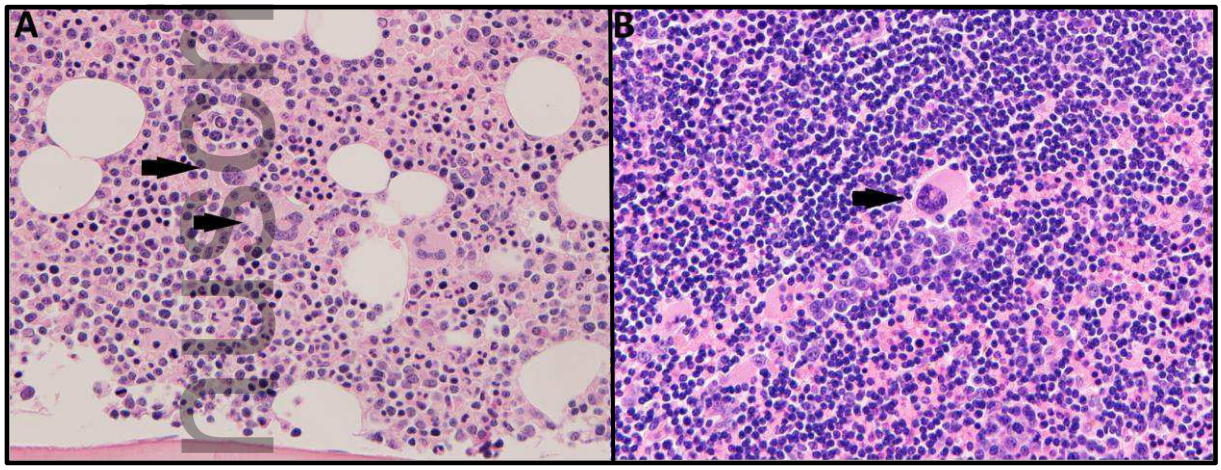
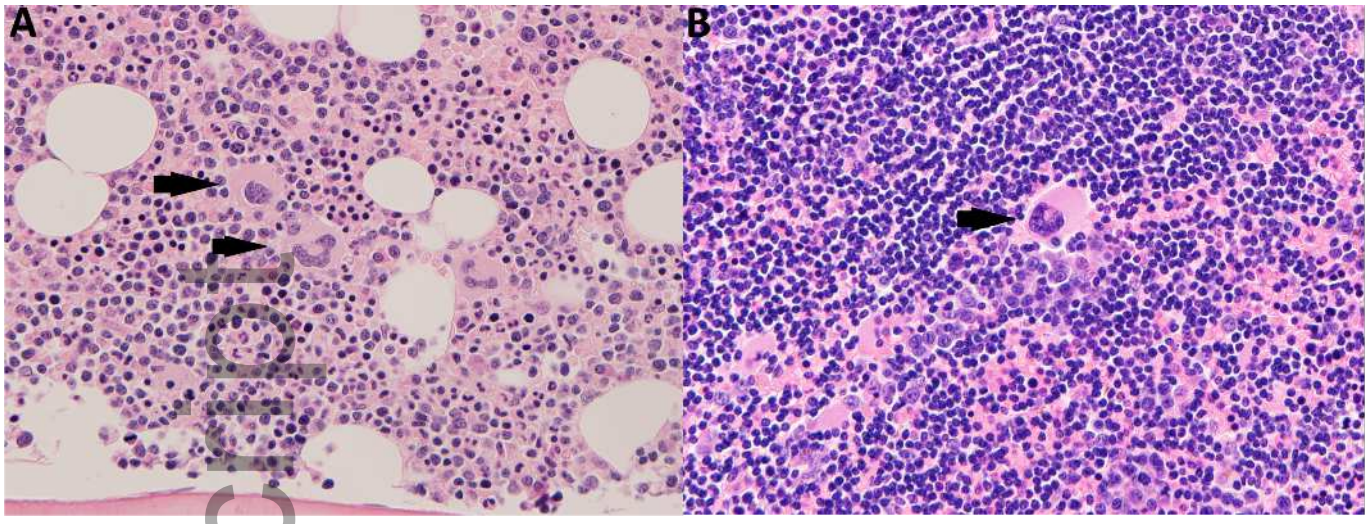
Figure Legend

Figure 1 **Megakaryocytes in lymphoproliferative marrow infiltrates**. The marrow in mantle cell lymphoma (A) shows megakaryocytes (black arrows) alongside an infiltrate of small to intermediate sized lymphoma cells and normal haemopoiesis. By contrast, the infiltrated marrow in chronic lymphocytic leukaemia (B) is typically densely packed, with the megakaryocytes surrounded by the smaller densely packed leukaemic lymphocytes.





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