IMMUNOTHERAPY

Checkpoint inhibition in the bone marrow

The coupling of blood platelets bearing anti-programmed cell death protein 1 antibodies to haematopoietic stem cells enables delivery of checkpoint-blockade therapy to bone marrow to promote T-cell-mediated control of leukaemia in mice.

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Acute myeloid leukaemia (AML) is a malignancy of clonally expanding myeloid progenitor cells in the bone marrow and blood. The mainstay treatment of AML, based mainly on chemotherapeutic drugs with or without haematopoietic stem cell (HSC) transplantation, has remained largely unchanged over several decades and often fails in patients, making AML the leading cause of leukaemia-related deaths in Western countries. The success of immunotherapies for melanoma and other solid tumours owing to the development of immune checkpoint inhibitors (such as antibodies to programmed cell death protein 1 (PD-1)) — a breakthrough in cancer therapeutics, as acknowledged by the 2018 Nobel Prize in Medicine — has prompted the application of checkpoint inhibitors to AML and other haematological malignancies. Encouraging data from preclinical models suggest a therapeutic role for immune checkpoint blockade in AML[1], but the first clinical trials using checkpoint inhibitors as single-agent therapy in this disease have shown only modest clinical efficacy, and it seems unlikely that any of these compounds, when used as monotherapy, will cure the disease[2]. Therefore, these therapeutic modalities require optimization to improve clinical efficacy, and potential combination strategies that rely on the mechanistic understanding of tumour immune control and escape mechanisms should be explored. Reporting in *Nature Biomedical Engineering*, Zhen Gu and colleagues now show effective control of the expansion of AML cells in mice following treatment with engineered cell constructs consisting of HSCs (S), platelets (P) and anti-PD-1 antibodies (aPD-1)[3]. The S–P–aPD-1 construct enables targeted delivery of the immune checkpoint inhibitor aPD-1 into the bone marrow[3], effectively targeting the main site of leukaemia clonal expansion.

To generate the constructs, Gu and co-authors decorated murine blood-derived platelets with aPD-1 antibodies, and then conjugated these to murine bone-marrow-derived HSCs. The integrity of the construct was monitored by imaging via confocal microscopy and scanning electron microscopy. Using fluorescently labelled aPD-1 in the S–P–aPD-1 constructs, the researchers demonstrated a significant extension of the aPD-1 antibodies' half-life, as well as enhanced accumulation of the antibodies in the bone marrow of mice following administration of the construct when compared with soluble aPD-1 or platelet-conjugated aPD-1. Increased delivery of aPD-1 to the bone marrow could be attributed to the presence of HSCs in the constructs, providing a rational basis for their superior migration and homing capacity to this tissue as a result of the cells' endogenous expression of bone-marrow-homing factors. The authors further hypothesize that, after entering bone marrow tissue, platelets are activated and release platelet-derived microparticles (PMPs) containing aPD-1, which then blocks the inhibitory receptor PD-1 on local effector T cells (Fig. 1). However, microscopy images at higher resolution would be needed for the quantification of PMPs in the bone marrow of treated mice and for confirming the presence of PMPs in the tissue.

To monitor the treatment efficacy of the S–P–aPD-1 constructs, mice inoculated with a luciferase-tagged C1498 AML cell line were distributed among the following treatment arms: infusion of HSCs, platelets or free aPD-1 as monotherapies, or of the constructs S–aPD-1, P–aPD-1, P–aPD-1 plus co-injection of HSCs, and S–P–aPD-1. The therapeutic agents were administered 1 week after injection of the AML cell line. Nearly 90% of mice treated with the S–P–aPD-1 construct were still alive 80 days after treatment, whereas animals in all other treatment groups did not survive longer than 40 days. Notably, mice treated with S–P–aPD-1 showed only barely detectable tumour by bioluminescence measurements just 3 weeks after initiation of the treatment. Using flow cytometry and histopathology analyses, Gu and colleagues also showed that treatment with S–P–aPD-1 resulted in negligible amounts of leukaemia cells in the main organs, including the bone marrow, liver and spleen, which displayed normal tissue morphology.

To confirm that the success of S–P–aPD-1 treatment resulted from activation of an antitumour immune response, Gu and co-authors show that S–P–aPD-1-treated mice had a higher number of T cells in the blood and bone marrow, increased percentage of $CD8^+$ effector T cells, and enhanced expression of the activation markers CD69 and CD25 and effector molecules interferon and granzyme B. In addition, cytokine quantification in the plasma of these mice revealed an increase of pro-inflammatory factors, supporting the idea of a boosted immune response. When mice that controlled leukaemia growth as a result of S–P–aPD-1 treatment received a second injection of C1498 AML cells, the mice remained leukaemia-free for several weeks. The dependency of S–P–aPD-1 treatment on T cells was proven by using $Rag1^{-/-}$ mice, which lack B cells and T cells, as well as mice in which $CD8^+$ T cells were depleted by anti-CD8 antibody injections, as in both animal groups S–P–aPD-1 treatment had no effect on leukaemia development or on the survival of the mice when compared with control mice receiving free aPD-1 antibodies. Lack of treatment response to S–P–aPD-1 was also demonstrated in $PD\text{-}1^{-/-}$ mice.

Despite the excitement over the clinical success of immune checkpoint inhibitors in several cancers, it is currently not possible to reliably predict which patients will respond to this therapeutic modality and which will be refractory. A required prerequisite for treatment success seems to be the presence of a functional T-cell population. Contrary to popular belief, in patients with AML, T cells are preserved and may even be increased, both in peripheral blood and in the bone marrow[4]. Furthermore, T cells in the bone marrow of AML patients express activation markers (such as CD25, CD69 and OX40) at a significantly higher level

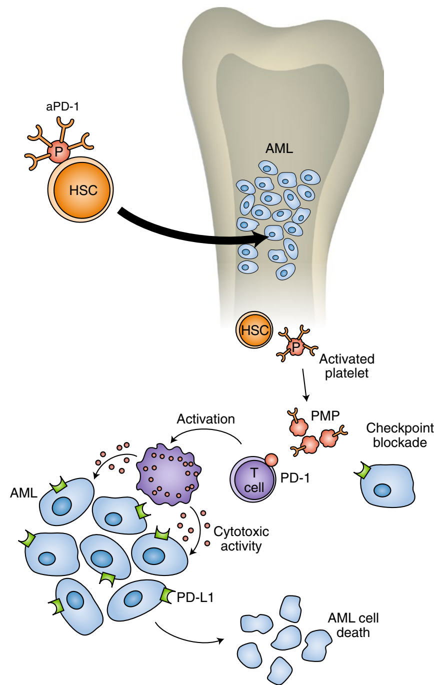

Fig. 1 | Re-activation of AML-targeting T cells via delivery of aPD-1 to the bone marrow of leukaemic mice by means of a platelet–HSC construct. Anti-PD-1 antibodies (aPD-1) are coupled to murine platelets (P), which are then conjugated to haematopoietic stem cells (HSCs) (S) from mice. Once injected into AML-bearing mice, the S–P–aPD-1 construct accumulates in the bone marrow, owing to HSC migration and homing into this tissue. After entering the bone marrow, HSC-coupled platelets are activated, shedding aPD-1-containing PMPs. Binding of aPD-1 to PD-1 expressed on T cells blocks the PD-1/PD-L1 immune checkpoint and leads to T-cell activation and to targeted eradication of AML cells in mice.

than seen in healthy donors, suggesting that the bone marrow in AML is an inflamed microenvironment[5]. A second requirement for effective immune checkpoint blockade is the expression of inhibitory receptors and their ligands on the tumour and its immune microenvironment. Malignant cells in haematopoietic cancers are in constant interaction with immune cells at the site of tumour development, which might explain the increased overall expression of inhibitory surface molecules on the $CD4^+$ and $CD8^+$ T cells of AML patients when compared with controls[6]. Also, AML cells express several immune checkpoint molecules, making them potential direct targets for these therapies[7].

An additional requirement for successful therapy by checkpoint blockade is the presence of tumour neo-antigens. In comparison to other cancer types, AML presents with a low mutational burden and therefore might respond poorly to immune checkpoint inhibitors[8]. Nevertheless, recent findings revealed that besides mutational load, the underlying genetic heterogeneity of a particular tumour, as well as the T-cell receptor repertoire of the patients, are key determinants for immune recognition and antitumour immunity and, therefore, for the response to immune checkpoint blockade[9]. AML is thought to progress via a successive accumulation of genetic and epigenetic mutations that leads to clonal evolution and diversification[10]; as a result, AML is composed of heterogeneous populations of malignant cells, a potential advantage for therapies based on immune checkpoint blockade. Nevertheless, although clonally expanded T-cell populations can be found in AML patients, AML-specific T-cell receptors may be of low affinity and poorly capable of targeting AML myeloblasts owing to tolerance mechanisms during T-cell development in the thymus, potentially limiting the success of immune checkpoint blockade[11].

Given that clinical trials using immune checkpoint inhibitors as monotherapy for AML showed only modest efficacy[2], rationally designed combination approaches are currently being tested. Hypomethylating agents such as azacitidine enhance antitumour immune responses, but this effect can be limited by an increased expression of immune checkpoint proteins[6,12]; this limitation could potentially be counteracted with checkpoint blockade. The combination of allogeneic stem cell transplantation with checkpoint blockade in the context of AML is also under investigation, although a pertinent concern with this approach is the risk of inciting graft-versus-host disease due to non-antigen-specific T-cell stimulation[2].

Although immune checkpoint blockade can induce significant antitumour benefits, unique adverse effects can occur through non-specific immunologic activation. On the one hand, compared with the currently used systemic administration of checkpoint antibodies in cancer patients, the targeted delivery of these agents to the bone marrow by S–P–aPD-1 constructs might limit at least some of these adverse effects. On the other hand, using activated platelets as carriers of the antibodies might also increase the risk of such adverse effects, as platelets will also deliver other factors (such as cytokines) to the bone marrow or to other lymphatic tissues, which may result in severe systemic toxicity. A complete unknown of this therapeutic approach are the long-term effects in patients, as the half-life of these cellular constructs and the persistence of response to a clonally evolving tumour remain unclear. Even though the challenge of individualized manufacturing of such therapeutic agents might be yet another downside to this therapeutic approach, there is hope that its further development might lead to promising therapeutic options for patients that suffer from incurable AML.

The preclinical data presented by Gu and colleagues suggests a superior activity of immune checkpoint inhibitors in AML when coupled to HSC-fused platelets. The beauty of this approach is its potential application to any therapeutic antibody that requires efficient delivery and release in the bone marrow of patients, not only in the context of AML, but also with other diseases occurring in this tissue. Rationally designed combinations of antibodies within the same construct, such as combined targeting of several immune checkpoint molecules, provides great potential for new therapeutic armouries. Along this line, improvement of the efficacy of T-cell engager antibodies[13] by their specific delivery to the bone marrow, or their combination with checkpoint-targeting antibodies within the same construct, are among the potential applications these constructs might bear. ❐

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