Received: 31 January 2022 | Revised: 13 September 2022 | Accepted: 22 December 2022

DOI: 10.1111/bph.16028

Themed Issue: Cancer Microenvironment and Pharmacological Interventions

THEMED ISSUE REVIEW

Stromal bone marrow fibroblasts and mesenchymal stem cells support acute myeloid leukaemia cells and promote therapy resistance

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Funding information
Research in our laboratory is funded by Tenovus Scotland (S20-04) and the British Society for Haematology (34847/ESR2017).

The bone marrow (BM) is the primary site of adult haematopoiesis, where stromal elements (e.g. fibroblasts and mesenchymal stem cells [MSCs]) work in concert to support blood cell development. However, the establishment of an abnormal clone can lead to a blood malignancy, such as acute myeloid leukaemia (AML). Despite our increased understanding of the pathophysiology of the disease, patient survival remains suboptimal, mainly driven by the development of therapy resistance. In this review, we highlight the importance of bone marrow fibroblasts and MSCs in health and acute myeloid leukaemia and their impact on patient prognosis. We discuss how stromal elements reduce the killing effects of therapies via a combination of contact-dependent (e.g. integrins) and contact-independent (i.e. secreted factors) mechanisms, accompanied by the establishment of an immunosuppressive microenvironment. Importantly, we underline the challenges of therapeutically targeting the bone marrow stroma to improve acute myeloid leukaemia patient outcomes, due to the inherent heterogeneity of stromal cell populations.

LINKED ARTICLES: This article is part of a themed issue on Cancer Microenvironment and Pharmacological Interventions. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v181.2/issuetoc

KEYWORDS
acute myeloid leukaemia, AML, bone marrow stroma, fibroblasts, mesenchymal stem cells, therapeutic targeting, therapy resistance

1 | INTRODUCTION

Acute myeloid leukaemia (AML) is an aggressive haematological malignancy characterised by the expansion of abnormal myeloid progenitors (myeloblasts) in the bone marrow (BM), with frequent involvement of the peripheral blood and extramedullary tissues (e.g. spleen and liver) (Estey, 2018). As a result of this disruption to normal haematopoiesis, there is insufficient production of healthy, mature blood cells, leading to bone marrow failure and ultimately death. Current treatment strategies are still based upon the intensive '7 + 3' regimen of **cytarabine** plus an anthracycline (e.g. **daunorubicin**); however, survival rates are poor (Estey, 2018). Relapsed or refractory disease occurs in the majority of patients due to therapy resistance. The resistant acute myeloid leukaemia

Abbreviations: α-SMA, alpha-smooth muscle actin; AML, acute myeloid leukaemia; BM, bone marrow BMME, bone marrow microenvironment; CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; HS(P)C, haematopoietic stem (and progenitor) cell; MSCs, mesenchymal stem cells.

blasts/clones may be pre-existing and present at diagnosis (intrinsic resistance), remaining dormant throughout the treatment period and underlying primary refractory disease (Horibata et al., 2019), or develop as a direct result of treatment under the influence of selective pressure exerted by cytotoxic chemotherapy (Caiado et al., 2019). These resistant acute myeloid leukaemia blasts may also directly arise via the interaction between acute myeloid leukaemia cells, surrounding cells and extracellular matrix (ECM) components present within the bone marrow microenvironment, termed acquired/extrinsic resistance (Zeng et al., 2017).

The tumour microenvironment comprises various tissue-resident/supporting cells (e.g. fibroblasts) and infiltrating cells of the immune system (e.g. monocytes/macrophages) that collectively contribute towards cancer progression and therapy resistance (Ireland & Mielgo, 2018). Specifically, cellular constituents of the tumour microenvironment are thought to undergo phenotypic and functional alterations, adopting cancer-promoting characteristics, such as the functional polarisation of macrophages towards an M2-like, tumour-promoting phenotype in the bone marrow microenvironment of acute myeloid leukaemia patients (Mao et al., 2021; Miari et al., 2021). In this review, we focus on subsets of fibroblasts and mesenchymal stem cells (MSCs), and their role as important stromal elements in a healthy versus the malignant bone marrow microenvironment. Although the general properties and heterogeneity of fibroblasts and MSCs have been extensively described elsewhere (Andrzejewska et al., 2019; Dominici et al., 2006; LeBleu & Neilson, 2020), we aim to address these properties in specific relation to the haematopoietic system and acute myeloid leukaemia. We present the importance of these stromal cell types in supporting haematopoiesis and the dysregulation of these processes that lead to disease establishment. Lastly, we address the current knowledge regarding the role of these cells in driving resistance to therapeutics and the impact on patient prognosis, while highlighting the potential for therapeutically targeting stroma-driven therapy resistance to improve clinical outcomes in acute myeloid leukaemia.

2 | DEVELOPMENTAL ORIGINS OF FIBROBLASTS AND MESENCHYMAL STEM CELLS (MSCs) AND THEIR ROLE IN THE HAEMATOPOIETIC STEM CELL NICHE

Haematopoiesis is an intricate process whereby haematopoietic stem cells are produced, differentiate into mature blood cells and are released into the circulation. Primitive haematopoietic stem cells, known as long-term haematopoietic stem cells (LT-HSCs), provide a lifelong supply of haematopoietic cells, due to their extensive self-renewal capacity, whereas haematopoietic stem and progenitor cells, on the next level of the haematopoietic hierarchy, give rise to the various blood cell lineages through differentiation and limited self-renewal (Höfer & Rodewald, 2018). Blood cell production begins early in the developing foetus, and throughout embryonic development haematopoiesis occurs in different organs of the embryo, until haematopoietic stem cells migrate to the bone marrow shortly after birth, establishing the major haematopoietic tissue of the adult (Laurenti & Göttgens, 2018; Morrison & Scadden, 2014). On average, an adult human produces approximately 500×10^9 blood cells daily; thus, the process of haematopoiesis requires stringent control (Anthony & Link, 2014). The bone marrow is the soft tissue located in central regions of long bones, such as the pelvis, femur and rib cage. Conditions within the bone marrow are optimal to support homing of haematopoietic stem cells/haematopoietic stem and progenitor cells, and their differentiation into multipotent progenitors, until their final maturation into fully functional blood cells (Anthony & Link, 2014; Laurenti & Göttgens, 2018). Importantly, bone marrow stromal elements provide essential mechanical support, in addition to a broad range of growth factors that support the haematopoietic system (Anthony & Link, 2014; Morrison & Scadden, 2014). Both fibroblasts and MSCs are abundant cells of the bone marrow stroma, with crucial roles in regulating normal haematopoiesis.

Fibroblasts are a diverse cell type located throughout the body with important structural/supportive roles in health and in processes such as wound healing. The developmental origin of fibroblasts can be traced back to gastrulation, when epiblasts undergo epithelial-to-mesenchymal transition (LeBleu & Neilson, 2020). The resulting primary mesenchyme subsequently gives rise to early mesenchymal cells, forming tissue-resident fibroblasts, bone and blood among other tissues. Under homeostasis conditions, fibroblasts are generally quiescent, spindle-shaped cells that are the main producers of extracellular matrix proteins (e.g. collagen and **fibronectin**) in connective tissues. Fibroblasts will only transiently acquire an activated phenotype during tissue remodelling and healing processes, following which they revert to an inactive state or undergo apoptosis (Gieniec et al., 2019). Depending on the tissue of origin and their activation/proliferation status, fibroblasts express a number of cell-surface markers, including Thy-1/CD90, podoplanin, **platelet-derived growth factor receptor alpha (PDGFRα)** and **PDGFRβ** and cytoplasmic alpha-smooth muscle actin (α-SMA) (LeBleu & Neilson, 2020).

Specifically in the bone marrow, fibroblasts provide mechanical/physical support to haematopoietic stem cells/haematopoietic stem and progenitor cells during their self-renewal and differentiation, through the ongoing synthesis, secretion and remodelling of extracellular matrix components into a highly organised meshwork (Lee-Thedieck et al., 2021) (Figure 1). An important extracellular matrix-to-haematopoietic stem cell/haematopoietic stem and progenitor cell interaction occurs between laminin and **integrin α6β1** on long-term -haematopoietic stem cells that is essential for the maintenance of long-term-haematopoietic stem cells, but not short-term repopulating haematopoietic stem cells (Notta et al., 2011). Additional key haematopoietic stem cell/haematopoietic stem and progenitor cell-to-extracellular matrix adhesive interactions are **integrin α4β1 (very late antigen-4 [VLA-4])**/fibronectin, cluster of differentiation 44 (CD44)/**hyaluronic acid (HA)** and **integrin α2β1**/**collagen** (Lee-Thedieck et al., 2021; Weinstein et al., 1989) (Figure 1). Additionally, extracellular matrix molecules sequester various growth factors and cytokines, increasing local bioavailability to haematopoietic stem cells/haematopoietic stem and progenitor cells (Huang et al., 2021).

Along with fibroblasts, MSCs are found in abundance in bone marrow niches. Reports place these cells in the mesoderm, from where they travel throughout the developing embryo to form connective tissues

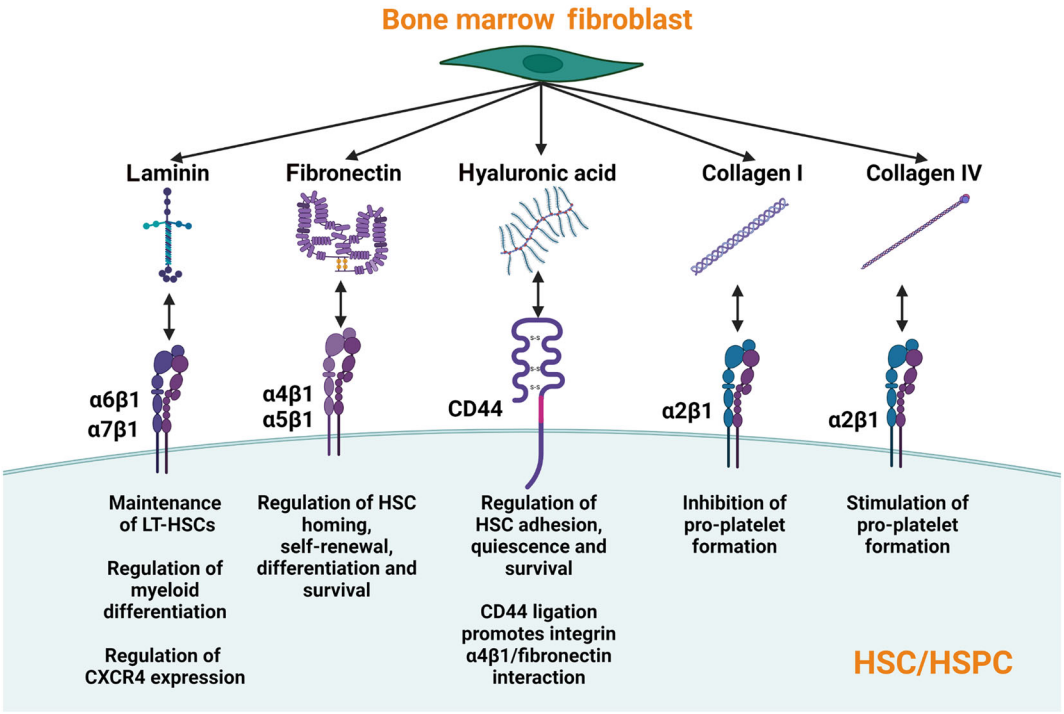


FIGURE 1 The continuous secretion and remodelling of extracellular matrix (ECM) by bone marrow fibroblasts regulates haematopoietic stem cells (HSCs)/haematopoietic stem and progenitor cells (HSPCs). Crucial interactions between ECM components and HSCs/HSPCs regulate numerous functions of the haematopoietic system. HSC/HSPC-to-ECM interactions, mainly mediated via heterodimeric integrin receptors, play a key role in controlling homing, self-renewal, differentiation and proliferation pathways in haematopoietic cells. Key adhesive interactions are displayed in the figure. CD44, cluster of differentiation 44; CXCR4, C–X–C motif chemokine receptor 4; long-term (LT)-HSCs, long-term haematopoietic stem cells. Created with BioRender.com

(Andrzejewska et al., 2019; Brown et al., 2019). In the adult, MSCs can be sourced from connective tissues, including bones, tendons and muscles, with the bone marrow contributing as a major source of MSCs. A defining set of criteria was published by the International Society for Cellular Therapy (ISCT), whereby MSCs are defined via three features. Specifically, MSCs are defined as multipotent cells able to differentiate into osteoblasts, chondrocytes and adipocytes in *ex vivo* studies and are highly adherent to plastic and express characteristic, non-haematopoietic cell-surface markers (**CD73**$^{+}$, **CD90**$^{+}$, **CD105**$^{+}$ and **CD11b**$^{-}$, **CD14**$^{-}$, **CD19**$^{-}$, CD34^{-}, **protein tyrosine phosphatase receptor type C [PTPRC/CD45]**$^{-}$, CD79a^{-} and human leukocyte antigen [HLA] class II^{-}) (Dominici et al., 2006). Since then, additional markers such as CD44, **vascular cell adhesion molecule-1 (VCAM-1/CD106)**, **melanoma cell adhesion molecule (MCAM/CD146)** and **nerve growth factor receptor (NGFR/CD271)** have also been utilised to describe bone marrow-MSCs, and these display more variable expression between donors (Andrzejewska et al., 2019). One of the most important properties of MSCs is their applicability in regenerative medicine, such as in the prevention of graft-versus-host disease (Brown et al., 2019; Tisato et al., 2007), attributed to the ability of MSCs to adopt an immune-suppressive phenotype in the presence of inflammatory mediators, where they express **intercellular adhesion molecule-1 (ICAM-1/CD54)**, VCAM-1, human leukocyte antigen (HLA)-ABC, HLA-DR (major histocompatibility complex-II [MHC-II]) and **programmed cell death 1 ligand 1 (PD-L1/CD274)** (Dal Collo et al., 2020).

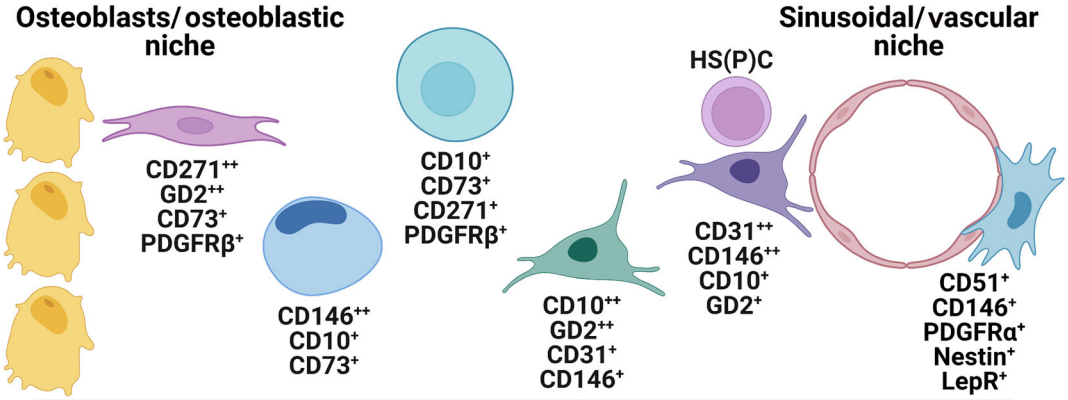
Although the precise role of bone marrow-MSCs remains to be elucidated, it is believed that MSCs are the precursors of cells that ultimately shape the haematopoietic stem cell/haematopoietic stem and progenitor cell-supportive bone marrow microenvironment, via secretion of multiple cytokines, chemokines and growth/differentiation factors (e.g. **interleukin-6 [IL-6]**, **interleukin-8 [IL-8]**, **C–C motif chemokine ligand 2 [CCL2]** and stem cell factor [SCF]) (Haynesworth et al., 1996; Leuning et al., 2018) and depending on donor age, and account for approximately 0.01% of total bone marrow nucleated cells (Rebolj et al., 2018). Expression of ephrin receptors and ephrin ligands by bone marrow-MSCs and haematopoietic stem cells regulate haematopoietic stem cell adhesion to stroma, thus controlling homing/mobilisation of haematopoietic stem cells in the bone marrow, while also impacting MSC differentiation (Nguyen et al., 2016; Ting et al., 2010).

3 | CHARACTERISATION OF DISTINCT FIBROBLAST AND MESENCHYMAL STEM CELL (MSC) POPULATIONS UNDER STEADY STATE

Simple observation of bone marrow-MSCs by light microscopy allows the identification of three morphologically distinct populations:- one consisting of spindle-shaped fibroblast-like cells (cycling cells), another consisting of large, flat, granular cells (cycling cells) and a third,

consisting of population of small, circular cells with high capacity for self-renewal (quiescent cells) (Colter et al., 2000, 2001). Molecular and transcriptomic analyses have confirmed the morphological separation of these cells and have identified many similarities between fibroblasts and MSCs, with regard to molecular markers, phenotype and function, blurring our perception of fibroblasts and MSCs as distinct cell types (Adamo et al., 2020; Baryawno et al., 2019; Ichim et al., 2018). Using immunohistochemistry and flow cytometry, numerous, distinct MSC populations in specific bone marrow locations have been described, displaying expression of unique combinations of surface markers that often reflect their functional properties and differentiation propensities (Fountaine et al., 2019; Fukiage et al., 2008; Muñiz et al., 2015; Pinho et al., 2013; Rasini et al., 2013; Schäfer et al., 2019) (Figure 2). Interestingly, the concept of MSC polarisation was introduced by Waterman et al. (2010), who demonstrated the ability of MSCs to adopt either a pro-inflammatory MSC1 or anti-inflammatory MSC2 phenotype in response to **toll-like receptor-4 (TLR4)** and **TLR3** activation, respectively.

Much of our understanding of bone marrow stroma arises from single-cell RNA-sequencing (scRNA-seq) studies in murine models, even though murine stromal cells are inherently different to human cells (Bernardo & Fibbe, 2013; Buechler et al., 2021). An elegant study



Mesenchymal stem cells		
Marker	*Localisation*	*Function/characteristics*
CD271, GD2, CD73, PDGFRβ	Osteoblastic niche	High expression of CD271 and GD2, propensity for osteogenic differentiation
$CD146^{++}CD10^{+}CD73^{+}$ population	Central bone marrow	Propensity for adipogenic differentiation
CD10, CD73, CD271, PDGFRβ	Central bone marrow	Specific function currently unknown
CD10, GD2, CD31, CD146	Central bone marrow	High expression of CD10 and GD2, specific function currently unknown
CD31, CD146, CD10, GD2	Vascular/sinusoidal niche	High expression of CD31 and CD146, HSC/HSPC support
CD51, CD146, PDGFRα, Nestin, LepR	Vascular/sinusoidal niche	Expression of HSC/HSPC-supportive genes (e.g., CXCL12)
Additional MSC markers		
CD90	Undefined	Regulation of MSC differentiation
$CD13^{high}CD105^{+}CD45^{-}$	Undefined	Minor subset of BM-MSCs, specific function currently unknown

FIGURE 2 The spatial distribution and identity of distinct mesenchymal stem cell (MSC) populations in the human bone marrow (BM). The human BM contains numerous, distinct populations of MSCs displaying expression of subset-specific combinations of cell-surface markers. These differences are reflected in their spatiotemporal organisation within the BM. Haematopoiesis-supporting MSCs are predominantly localised in the sinusoidal/vascular niche, regulating haematopoietic stem cell (HSC)/haematopoietic stem and progenitor cell (HSPC) quiescence and differentiation. Many other populations have been identified in the human BM; however, their specific function in the haematopoietic microenvironment remains unclear. In this figure, we highlight MSC populations based on the most frequently reported cell-surface markers. CD, cluster of differentiation; CXCL12, C–X–C motif chemokine ligand 12; GD2, disialoganglioside GD2; HS(P)C, haematopoietic stem (and progenitor) cell; LepR, leptin receptor; PDGFRα, platelet-derived growth factor receptor-α; PDGFRβ, platelet-derived growth factor receptor-β. Created with BioRender.com

by Baryawno et al. (2019) mapped the distinct populations of murine bone marrow stromal cells, identifying 17 distinct cell clusters, which included one MSC population (leptin receptor$^+$ [LepR$^+$]), five fibroblast populations and two clusters expressing fibroblast, chondrocyte and osteoblast markers. This was shortly followed by a study by Wolock et al. (2019), who focussed on bone marrow-MSCs, rather than bulk bone marrow stroma (gating on CD45$^-$Ter119$^-$CD31$^-$**CD51**$^+$Sca1$^+$), characterising seven cell populations that emerge as a result of a branching differentiation process of MSCs into adipocytes, osteoblasts and chondrocytes. Therefore, these data confirm the presence of a common progenitor for MSC-derived cells. Each cell cluster was characterised by a distinct expression of genes involved in cellular processes, including haematopoietic stem cell maintenance/support and cell adhesion or differentiation pathways (Wolock et al., 2019). Notably, genes associated with haematopoietic support were enriched in a single-cell population consisting of **C–X–C motif chemokine ligand 12 (CXCL12)**-abundant reticular cells. Similarly, Buechler et al. (2021) illustrated the compartmentalisation of the murine fibroblast lineage into universal, specialised and activated subtypes, whereby a universal/common ancestor fibroblast population gives rise to tissue-specific specialised subtypes (steady state) that may acquire an activated phenotype during disease/infection. Based on these studies, scRNA-seq of human bone marrow stroma (CD45$^{low/-}$CD235a$^-$**CD271**$^+$) identified six stromal cell types across nine clusters, which included pre-fibroblasts and multipotent stromal stem cells (Li et al., 2022).

4 | STROMAL CELL LINES TO MODEL THE BONE MARROW MICROENVIRONMENT *IN VITRO*

Due to the inevitable variation of primary bone marrow-MSCs, potential limitations with regard to sourcing adequate cell numbers (Brown et al., 2019) and the loss of colony-forming efficiency and differentiation ability over time (DiGirolamo et al., 1999), stromal cell lines may be used as suitable alternatives to primary MSCs. In 1995, Roecklein and Torok-Storb immortalised healthy bone marrow-derived stromal cells using the human papilloma virus genes E6 and E7, establishing a panel of 27 distinct cell lines (HS-1 to HS-27). Of these, the fibroblast-like HS-5 (RRID:CVCL_3720) are more frequently used in experiments requiring modelling of the bone marrow microenvironment or *in vitro* support of primary haematopoietic cells, compared to the epithelioid HS-27 (RRID:CVCL_0E34), due to their plethora of secreted factors (Garrido et al., 2001; Long et al., 2015, 2017; Macanas-Pirard, Broekhuizen, et al., 2017; Roecklein & Torok-Storb, 1995). Both HS-5 and HS-27A (RRID:CVCL_3719, subclone of HS-27) cell lines can be stably transfected to express fluorescent proteins without any significant impact on secretome profile, allowing purification of cell populations following co-cultures for downstream processing and analysis (Long et al., 2015). Additionally, the murine MS-5 bone marrow cell line (RRID:CVCL_2128) is used, to a lesser extent, as a stromal support for haematopoietic cells (Konopleva et al., 2002).

A comparison of primary MSCs with HS-5 and HS-27A cell lines showed that both cell lines express the typical MSC cell-surface markers as defined by the International Society for Cellular Therapy, with HS-5 cells displaying higher expression of CD73 and lower expression of CD90 compared to primary MSCs, whereas HS-27A cells express all MSC markers at much higher levels compared to both primary MSCs and HS-5 cells (Adamo et al., 2020). Additionally, expression of MSC immunomodulatory markers was present on the stromal cell lines upon exposure to inflammatory stimuli, with the exception of CD106/VCAM-1 that was not observed on HS-5 cells. Despite these findings, principal component analysis demonstrated a significant overlap of MSCs only with HS-5 cells based on the most significant differentially expressed stromal genes (Adamo et al., 2020). Thus, HS-5 cells are often considered as either an MSC or a fibroblast cell line.

5 | REPROGRAMMING OF BONE MARROW STROMA BY ACUTE MYELOID LEUKAEMIA (AML) CELLS

Having discussed how fibroblasts and mesenchymal stem cells (MSCs) in the bone marrow stroma support normal haematopoiesis, it is also important to note the bidirectional communication between acute myeloid leukaemia and stroma in the context of leukaemogenesis. In a seminal study to assess the impact of acute myeloid leukaemia blasts on fibroblasts, reduced proliferation of fibroblasts was observed when HFL1 fibroblasts (RRID:CVCL_0298) were cultivated with primary acute myeloid leukaemia patient blasts in direct contact or via transwell inserts (Glenjen et al., 2004). On the contrary, acute myeloid leukaemia/HS-27 transwell co-culture enhanced proliferation of the low-proliferative HS-27 cell line, with no apparent effect via direct co-culture (Glenjen et al., 2004). Thus, the effects of the bidirectional cell-to-cell communication vary greatly among cell types and are most likely context dependent. Further dysregulation of acute myeloid leukaemia bone marrow-derived fibroblasts was demonstrated by their diminished haematopoiesis-supporting capacity, when compared to fibroblasts from healthy bone marrow, correlating with the down-regulation of genes involved in haematopoietic support (e.g. **Dickkopf WNT signalling pathway inhibitor 1 [DKK1]**) (Li, Dürig, et al., 2015). In addition, stromal HS-5 cell lines displayed higher mRNA and protein expression of **IL-6** following co-culture with acute myeloid leukaemia cell lines (Hou et al., 2020). In a murine MLL-AF9 acute myeloid leukaemia model, the presence of leukaemic cells induced alterations in bone marrow fibroblasts, increasing MSC-like fibroblasts, while decreasing fibroblasts associated with healthy bone formation, confirming the reprogramming of stromal elements in the presence of leukaemic cells (Baryawno et al., 2019).

Primary bone marrow-MSCs isolated from leukaemic patients, or bone marrow-MSCs co-cultured with acute myeloid leukaemia cells,

displayed enrichment of NF-κB-target genes (e.g. *IL-6*, *IL-8* and ***CCL2***) that have been shown to promote therapy resistance in acute myeloid leukaemia cells (Abdul-Aziz et al., 2017; Jacamo et al., 2014). However, despite these changes, acute myeloid leukaemia-MSCs were found to retain the expression of typical MSC markers (CD44, CD73, CD90 and CD105), along with their capacity to activate regulatory T-cells (Corradi et al., 2018). Reports regarding the differentiation capacity of acute myeloid leukaemia bone marrow-MSCs are conflicting, with some evidence suggesting no alterations in osteogenic and adipogenic differentiation capacity (Corradi et al., 2018), whereas other studies observed impaired osteogenic differentiation (Geyh et al., 2018), osteogenic-biased (Battula et al., 2017) or adipogenic-biased (Azadniv et al., 2020) differentiation. This discrepancy may be related to the underlying pathology of acute myeloid leukaemia, where high-risk acute myeloid leukaemia (e.g. $TP53^{mut}$ and *FLT3*-ITD^{+}) has been linked to reduced bone marrow-MSC differentiation capacity (de la Guardia et al., 2017). **Focal adhesion kinase (FAK)** is a non-receptor tyrosine kinase responsible for signal transduction downstream of integrin ligation by activation of growth factor receptors and is indicative of cell-to-extracellular matrix interactions at plasma membrane regions known as focal adhesions (Zhou et al., 2019). In FAK^{+} acute myeloid leukaemia, bone marrow-MSCs expressed higher levels of IL-6, IL-8, CXCL12 and **angiopoietin-1** compared to FAK^{-} acute myeloid leukaemia, suggesting that different molecular abnormalities in acute myeloid leukaemia may have different effects on bone marrow stroma (Despeaux et al., 2011). Importantly, MSCs isolated from a leukaemic bone marrow are genetically stable, lacking the presence of acute myeloid leukaemia-related chromosomal/molecular abnormalities (Corradi et al., 2018; de la Guardia et al., 2017).

6 | CANCER-ASSOCIATED FIBROBLASTS (CAFs) IN SOLID TUMOURS AND BLOOD CANCERS: ROLE IN THERAPY RESISTANCE

As mentioned previously, the tumour microenvironment consists of tissue-resident cells that coordinate their functions to support tumour growth and dissemination, among which are CAFs that have mainly been studied in the context of solid tumours. These cells may arise from local resident fibroblasts, epithelial-to-mesenchymal transition or endothelial mesenchymal transition (EndMT), or by recruitment of bone marrow-MSCs, upon the influence of tumour secreted factors (Liu et al., 2019). The multiple origins of CAFs are thought to underlie their broad heterogeneity (Liu et al., 2019). A novel origin of haematopoietic stem cell-derived CAFs was proposed through the identification of CD45^{+} CAFs in the stroma of murine models of lung cancer (McDonald et al., 2015). The characterisation of CAFs is based upon the expression of cell-surface markers, which include **fibroblast activation protein (FAP)** and fibroblast-specific protein-1 (FSP-1), and cytoplasmic markers, such as alpha-smooth muscle actin (α-SMA), and the release of tumour-promoting (e.g. **IL-6**), pro-angiogenic (e.g. **vascular endothelial growth factor [VEGF]** and angiopoietin-1) and metastasis-promoting/extracellular matrix remodelling secreted factors (e.g. **matrix metalloproteinases 2 (MMP2)** and **MMP9** and **transforming growth factor-β [TGF-β]**) (Liu et al., 2019). No single marker is exclusively expressed by CAFs, rendering identification of pure CAF populations based solely on cell-surface phenotyping challenging.

Research into solid tumours has highlighted the key role of CAFs in reducing the response of the malignant cells to administered therapies (Goulet et al., 2019; Su et al., 2018; Zhang et al., 2019). For example, a novel population of CD10^{+}GPR77^{+} CAFs was described to promote chemotherapy resistance and cancer stemness in breast cancer by the release of IL-6 and IL-8 (Su et al., 2018). In addition to the tumour-supportive role of CAFs, certain subtypes also bear important immunomodulatory functions, as seen in a murine model of hepatoma, in which FAP^{+} CAFs displayed an inflammatory phenotype, determined based upon the expression of CCL2, IL-6, **C–X–C motif chemokine ligand 2 (CXCL2)** and CXCL12 (Yang et al., 2016). Moreover, in colorectal carcinoma, CAF-derived IL-8 promoted directed chemotaxis of monocytes into the tumour microenvironment. In turn, CAF-derived IL-6 polarised monocytes towards an M2-like macrophage phenotype, accompanied by reduced cytotoxicity of natural killer cells (Zhang et al., 2019).

Although studies in solid tumours have provided evidence of CAF involvement in therapy resistance, less is known regarding CAFs in blood cancers. One of the first reports confirming the presence of CAFs in the bone marrow of acute myeloid leukaemia patients came from a study by Zhai et al. (2016), in which they described the presence of α-SMA^{+}, FAP^{+} and FSP1^{+} stromal cells in acute myeloid leukaemia bone marrow samples. The presence of these cells was accompanied by enhanced deposition of reticulin fibres, fibrils of type III collagen surrounding a core of type I collagen. A novel FAP^{+} population of bone marrow-MSCs, most likely resulting by TGF-β-mediated transformation of bone marrow-resident MSCs into CAF-like cells, was also described in newly diagnosed acute myeloid leukaemia patients and directly correlated with the proportion of myeloblasts in the bone marrow (Mei et al., 2021). Because the current knowledge on acute myeloid leukaemia-CAFs is limited, much of our understanding is supported by studies in other haematological malignancies. In multiple myeloma (MM), the presence of FSP-1^{+}, FAP^{+} and α-SMA^{+} bone marrow fibroblasts induces transformation of bone marrow-MSCs and bone marrow-endothelial cells into CAF-like cells (Frassanito et al., 2014). These CAF-like cells prevented spontaneous apoptosis of CD138^{+} plasma cells and stimulated proliferation of multiple myeloma cells. Interestingly, MSCs in chronic lymphocytic leukaemia (CLL) acquired a CAF-like transcriptional profile following the uptake of functional micro-RNAs and proteins contained in chronic lymphocytic leukaemia-secreted exosomes (Paggetti et al., 2015). Further supporting the ability of bone marrow-MSCs to differentiate into CAFs is a study in B-cell acute lymphoblastic leukaemia (B-ALL), demonstrating that B-acute lymphoblastic leukaemia cells increased expression of FAP and α-SMA on bone marrow-MSCs under co-culture conditions (Pan et al., 2020). In turn, these CAF-like

MSCs protected B-acute lymphoblastic leukaemia cells against daunorubicin-induced apoptosis (Pan et al., 2020).

7 | EXPLOITATION OF THE SUPPORTIVE STROMAL MICROENVIRONMENT BY ACUTE MYELOID LEUKAEMIA CELLS TO DRIVE THERAPY RESISTANCE

Numerous studies to date have investigated the role of bone marrow fibroblasts and MSCs in acute myeloid leukaemia therapy resistance, with efforts only recently aiming to identify phenotypic/functional alterations in these cells that may characterise them as cancer-associated fibroblasts (CAFs). In a seminal study conducted by Garrido et al. (2001), direct co-culture of acute myeloid leukaemia blasts with HS-5 cells conferred protection against cytarabine- and daunorubicin-induced apoptosis, two commonly used conventional induction acute myeloid leukaemia chemotherapeutic agents. Although no induction of **pro-survival B-cell lymphoma-2 (BCL-2) proteins** was recorded in their findings, Konopleva et al. (2002) conducted similar studies utilising murine MS-5 cells. These authors reported up-regulation of the anti-apoptotic protein **BCL-2** in HL-60 acute myeloid leukaemia cell lines (RRID:CVCL_0002), as well as BCL-2 and **B-cell lymphoma-extra large (BCL-xL)** up-regulation in primary acute myeloid leukaemia samples in their stromal co-cultures. Notably, BCL-2 up-regulation was more prominent in samples obtained from patients that responded poorly to induction chemotherapy, whereas samples from treatment responders did not display significant induction of BCL-2, in the presence of cytarabine when co-cultured with the MS-5 cells. These data indicate an anti-apoptotic priming of acute myeloid leukaemia blasts that involves BCL-2 as a major pro-survival signalling component and may explain the discrepancy between the two publications regarding BCL-2 induction, although the use of different stromal cell lines could very likely be a contributing factor also. Both research groups report enhanced protection mediated by direct co-culture conditions, when compared to secreted factors (Garrido et al., 2001; Konopleva et al., 2002). Moreover, the reduced sensitivity to treatment was driven by anti-apoptotic responses, with no indication of the stromal cells inducing a cell cycle arrest in acute myeloid leukaemia cells (Garrido et al., 2001; Konopleva et al., 2002).

Over many years of research, integrins have been identified as crucial regulators of contact-dependent therapy resistance in acute myeloid leukaemia, mediating cell-to-extracellular matrix and cell-to-cell interactions. The high expression of integrin α4β1/VLA-4 on acute myeloid leukaemia cells and its interaction with fibronectin induces **FAK/protein kinase 2** (PKB/AKT)/BCL-2 signalling that provides resistance to etoposide and **mitoxantrone** (Hazlehurst et al., 2001; Matsunaga et al., 2003). Additionally, VLA-4 interacts with **VCAM-1** expressed on bone marrow-MSCs, driving interleukin-1α (IL-1α) expression in the leukaemic cells that, in turn, activate nuclear factor kappa-light-chain enhancer of activated B cells (**NF-κB**)-mediated gene expression of tumour-promoting factors by the stroma (e.g. CCL2 and IL-6) (Jacamo et al., 2014). Furthermore, CD44/HA ligation promotes VLA-4/VCAM-1 adhesion (Gutjahr et al., 2021). In a similar manner, **αvβ3 integrin** adheres to stroma-secreted osteopontin, an interaction that induces the phosphoinositide 3-kinase (PI3K)/AKT/**glycogen synthase kinase-3 beta (GSK3β)**/β-catenin axis in acute myeloid leukaemia cells and reduces the sensitivity of MV4-11 acute myeloid leukaemia cells to the targeted therapy **sorafenib** (Yi et al., 2016). These findings suggest that stroma-driven therapy resistance is not restricted to conventional chemotherapy agents but has the capacity to protect against the killing effects of novel, targeted compounds.

Acute myeloid leukaemia cell lines, primary acute myeloid leukaemia blasts and $CD34^{+}CD38^{-}$ leukaemic stem cell-enriched populations, cultured in direct contact with HS-5 cells, displayed reduced sensitivity to cytarabine, daunorubicin and **ABT-737** (BCL-2/BCL-xL/**BCL-W** inhibitor) (O'Reilly et al., 2018). These protective effects were mediated by an up-regulation of the anti-apoptotic protein **myeloid cell leukaemia 1 (MCL-1)**, a protein critical for acute myeloid leukaemia cell survival (Glaser et al., 2012). Subsequently, O'Reilly et al. (2018) demonstrated that inhibition of **CDK9**, resulting in reduced CDK9-mediated transcription of MCL-1, or direct targeting of MCL-1, successfully re-sensitised acute myeloid leukaemia cells to ABT-737 in the presence of stroma. These findings were subsequently confirmed by other studies, reporting that selective MCL-1 inhibition can effectively be used to treat **venetoclax**-resistant disease, and highlight the importance of combination therapy to circumvent stroma-mediated resistance in acute myeloid leukaemia (Anstee et al., 2019; Carter et al., 2022; Moujalled et al., 2019; Ramsey et al., 2018).

In a study focussing on paediatric acute myeloid leukaemia, HS-5 and HS-27A-mediated resistance to etoposide was also found to require direct cell-to-cell contact in NB4 (RRID:CVCL_0005) and THP-1 (RRID:CVCL_0006) paediatric acute myeloid leukaemia cell lines (Long et al., 2015). On the contrary, stroma-induced resistance to cytarabine and mitoxantrone were mediated by a combination of both contact-based mechanisms and soluble secreted factors (Long et al., 2015). This study provided evidence that mechanisms of resistance may differ depending upon the therapeutic compound, and stromal or acute myeloid leukaemia cells under investigation and highlights the importance of stromal secreted factors in addition to direct cell-to-cell contact in driving therapy resistance. In a subsequent study, both HS-5 and HS-27A cells induced extracellular-regulated kinase 1/2 (ERK1/2) phosphorylation (pERK1/2) in paediatric acute myeloid leukaemia samples, whereas only HS-5 cells activated signal transducer and activator of transcription 3 and 5 (**STAT3** and STAT5) in leukaemic samples, indicating an secreted factor-driven mechanism of therapy resistance, due to the significantly more enriched secretome of HS-5 cells compared to HS-27A cells (Long et al., 2015, 2017; Roecklein & Torok-Storb, 1995). Moreover, the authors showed that treatment with the **mitogen-activated protein kinase kinase (MAPKK or MEK)** inhibitor, **selumetinib**, reversed stroma-induced pERK1/2 signalling, but was only able to re-sensitise acute myeloid leukaemia cells to mitoxantrone and etoposide during HS-27A co-culture, but not HS-5 co-culture (Long et al., 2017). These data further corroborate the need for combination treatments, due to the

activation of multiple anti-apoptotic pathways in the leukaemic cells that are not targeted by conventional chemotherapeutics. In this specific set of experiments, acute myeloid leukaemia cells displayed higher proliferation rates in co-culture compared to monoculture, in agreement with a previous study describing higher proliferation of acute myeloid leukaemia cells in the presence of HS-27A and HFL1 fibroblast-secreted factors (Ryningen et al., 2005). These data, however, contradict studies by other groups indicating that stroma promotes a more quiescent phenotype in acute myeloid leukaemia cells, by induction of a G0/G1 cell cycle arrest (e.g. CD44/PI3K/AKT/$p27^{Kip1}$) (Chen et al., 2015; Macanas-Pirard, Broekhuizen, et al., 2017).

Further confirming that therapy resistance in acute myeloid leukaemia can indeed be mediated by stromal cell-derived secreted factors, supernatants from bone marrow-MSCs and HS-5 cells activated the AKT/mammalian target of rapamycin (mTOR) pathway in U937 (RRID:CVCL_0007) and THP-1 acute myeloid leukaemia cell lines, and primary acute myeloid leukaemia cells, which correlated with protection against cytarabine-induced apoptosis (Macanas-Pirard, Broekhuizen, et al., 2017). Additionally, the authors observed down-regulation of the cytarabine nucleoside transporter **ENT1** on the surface of acute myeloid leukaemia cells, contributing to reduced uptake of the chemotherapy agent (Macanas-Pirard, Broekhuizen, et al., 2017). Importantly, bone marrow-MSC supernatants obtained from patients in remission following induction chemotherapy maintained their ability to protect acute myeloid leukaemia blasts against the killing effects of cytarabine (Macanas-Pirard, Broekhuizen, et al., 2017). These findings indicate that cytotoxic treatment does not affect the chemoprotective capacity of stromal cells (Macanas-Pirard, Broekhuizen, et al., 2017).

With the advent of technological advancements, efforts have been made to further elucidate the impact of stromal cells on proteome reprogramming in acute myeloid leukaemia cells. Using reverse-phase protein array, a proteomic-based approach, Zeng et al. (2017) assessed levels of proteins from major cell survival pathways (e.g. **PI3K**/AKT/**mTOR**, MEK/ERK and p53 family) in 20 primary acute myeloid leukaemia samples and 2 acute myeloid leukaemia cell lines treated in monoculture or co-culture with MS-5 cells or primary healthy or acute myeloid leukaemia bone marrow-MSCs. Across the primary samples and cell lines, treatment with **temsirolimus** (mTOR inhibitor), ABT-737 (BCL-2/BCL-xL inhibitor) or nutlin-3a (mouse double minute 2 homologue/**MDM2** inhibitor) resulted in changes in the acute myeloid leukaemia protein profile, which were further altered in the presence of stroma. Overall, stromal cells promoted therapy resistance by stimulating anti-apoptotic signalling, which was partly overcome by combination treatments of temsirolimus + ABT-737 and to a lesser extent by temsirolimus + nutlin-3a, most likely due to the absence of *TP53* mutations in the acute myeloid leukaemia samples utilised (Zeng et al., 2017). In a similar study, Long et al. (2015) identified differentially expressed genes in THP-1 and NB-4 acute myeloid leukaemia cell lines following their co-culture with HS-5 and HS-27A cells. Among the identified genes, spleen tyrosine kinase was found to contribute to mitoxantrone resistance, following up-regulation by stroma-derived cysteine-rich angiogenic inducer 61 (CYR61), a secreted extracellular matrix protein involved in cell-to-cell communication and signalling via sustained ERK1/2 activation following integrin ligation in acute myeloid leukaemia cells (Long et al., 2017). The secretion of pro-tumourigenic, immunosuppressive cytokines and chemokines by bone marrow stromal cells in acute myeloid leukaemia and their ability to activate many of the pro-survival signalling pathways discussed make them ideal candidates for further research.

8 | CYTOKINES, CHEMOKINES AND GROWTH FACTORS IN THE ACUTE MYELOID LEUKAEMIA STROMA

The investigation of secreted factors in acute myeloid leukaemia plasma stemmed from evidence suggesting that cytokines/chemokines may have a central role in leukaemia progression. In a screen of 42 acute myeloid leukaemia patient peripheral blood plasma samples, **tumour necrosis factor-α (TNF-α)**, IL-6 and **interleukin-10 (IL-10)** were significantly enriched compared to control peripheral blood samples across all age groups (Sanchez-Correa et al., 2013). Utilising a multiplex cytokine array, Stevens et al. (2017) screened a total of 41 secreted factors in paediatric acute myeloid leukaemia bone marrow plasma samples, obtained at diagnosis, and identified IL-6, IL-8 and IL-10 to be significantly up-regulated cytokines in a subset of acute myeloid leukaemia patient bone marrow compared to healthy bone marrow, supplementing previous findings (Sanchez-Correa et al., 2013). Below, we discuss several of the most prominent cytokines in acute myeloid leukaemia secreted by bone marrow stromal fibroblasts/MSCs.

8.1 | IL-6

IL-6 is a multi-functional, pleiotropic cytokine involved in numerous cellular processes and forms the founding member of the IL-6 cytokine family. Briefly, IL-6 signalling occurs in the presence of two subunits, **IL-6 receptor alpha (IL-6Rα, binding subunit)** and **glycoprotein 130 (gp130)/IL-6 receptor beta (IL-6Rβ, signal transduction subunit)**, both of which exist in membrane bound and soluble forms (Murakami et al., 2019). For IL-6 signalling to occur, IL-6Rα interacts with gp130, inducing gp130 homodimerisation and downstream intracellular signalling via **Janus kinases** (JAK1, JAK2 and JAK3). Phosphorylation of the transcription factor STAT3 at select tyrosine residues (e.g. Tyr705) leads to STAT3 dimerisation, nuclear translocation and regulation of gene expression, such as MCL-1 (Shastri et al., 2018).

In the first study to assess expression of IL-6, IL-6Rα and gp130 in leukaemic cells, all patient samples displayed expression of both receptors, with M2 and M5 acute myeloid leukaemia patients (French–American–British classification system, now replaced by

European Leukemia Network classification) (Döhner et al., 2017) also demonstrating high expression of IL-6 (Inoue et al., 1994). The authors identified a correlation between the levels of IL-6Rα and gp130 expression with the responsiveness of acute myeloid leukaemia cells to exogenous IL-6 (Inoue et al., 1994). Subsequent studies by Stevens et al. (2017) showed that recombinant IL-6 and soluble IL-6Rα treatment protected primary paediatric acute myeloid leukaemia cells and cell lines against mitoxantrone-induced apoptosis, but no protective effects were observed against the cytotoxic effects of cytarabine and etoposide (Stevens et al., 2017). This study further confirms previous observations that cytarabine and etoposide resistance most likely requires direct cell-to-cell contact between acute myeloid leukaemia and stroma (Long et al., 2015, 2017) and was the first to provide evidence of IL-6 driving therapy resistance in acute myeloid leukaemia. When assessing the IL-6 target STAT3, no difference in constitutive Tyr705–STAT3 phosphorylation was observed between leukaemic stem cell-enriched and non-leukaemic stem cell paediatric acute myeloid leukaemia cell populations, but leukaemic stem cells were more responsive to exogenous IL-6. Thus, IL-6 may promote therapy resistance and paediatric acute myeloid leukaemia relapse by activation of pro-survival signalling, via the pTyr705–STAT3 signalling axis in leukaemic stem cells (Stevens et al., 2017), contrary to previous findings correlating stroma-induced pSTAT3 with excellent survival rates (Long et al., 2017). These mechanisms have been explored in depth in more recent studies.

Conditioned media from HS-5 cells, which secrete high concentrations of IL-6 (Hou et al., 2020; Roecklein & Torok-Storb, 1995), induced phosphorylation of STAT3 at Tyr705 in a panel of acute myeloid leukaemia cell lines, including Kasumi-1 (RRID:CVCL_0589) (Long et al., 2015), U937 and HL-60 (Hou et al., 2020). In response to HS-5 co-culture, acute myeloid leukaemia cells induced upregulation of both IL-6 receptor subunits, indicating that acute myeloid leukaemia cells indeed respond to stroma-derived IL-6 (Hou et al., 2020). Interestingly, IL-6 has been linked to therapy resistance in acute myeloid leukaemia, via induction of oxidative phosphorylation (OXPHOS) (Hou et al., 2020). Phosphorylation of STAT3 at Ser727 is a marker of activation of mitochondrial STAT3, which is involved in metabolic regulation. Transwell co-culture of acute myeloid leukaemia cell lines with HS-5 cells led to enhanced oxidative phosphorylation with a concomitant increase in pSer727–STAT3 and subsequent resistance to cytarabine and daunorubicin (Hou et al., 2020). Treatment with the STAT3 phosphorylation inhibitor, **TTI-101 (C188-9)** or co-culture with IL-$6^{-/-}$ HS-5 cells, prevented IL-6-mediated STAT3 activation, reduced cellular metabolism and partially re-sensitised acute myeloid leukaemia cells to the killing effects of chemotherapy (Hou et al., 2020). These findings confirm previous studies describing a population of chemo-resistant leukaemic stem cells with a high- oxidative phosphorylation profile, which can be further enhanced in the presence of bone marrow-MSCs, and a highly active **glutathione** antioxidant system to protect against reactive oxygen species-induced cellular damage (Farge et al., 2017; Forte et al., 2020). Active transfer of mitochondria, via mitochondrial nanotunnels, from MS-5, HS-5 and primary bone marrow-MSCs to acute myeloid leukaemia cells has also been described to promote oxidative phosphorylation in leukaemic cells and is enhanced in the presence of cytarabine, underlying therapy resistance (Moschoi et al., 2016).

Taken together, these data suggest that IL-6 mediates therapy resistance in acute myeloid leukaemia through a combination of pro-survival signalling and regulation of acute myeloid leukaemia cell metabolism, driven by STAT3 and its target MCL-1, suggesting the potential of IL-6 or metabolic targeting for the treatment of acute myeloid leukaemia (Carter et al., 2022; Yan et al., 2021) (Figure 3).

8.2 | IL-8

IL-8/CXCL8 is a chemokine of the CXC family that is secreted by fibroblasts, macrophages and epithelial and endothelial cells in response to inflammatory stimuli (Liu et al., 2016). IL-8 signalling is mediated via two G-protein coupled receptors, **C–X–C motif chemokine receptor 1 (CXCR1)** and **C–X–C motif chemokine receptor 2 (CXCR2)**, with receptor engagement mainly activating the PI3K/AKT and MAPK signalling pathways (Liu et al., 2016). Co-culture of acute myeloid leukaemia blasts with HFL1 and HS-27A stromal cell lines promoted IL-8 secretion and chemotherapy resistance (Ryningen et al., 2005). Although IL-8 was secreted by both the leukaemic cells and the fibroblast cell lines, the authors stated that the increased chemokine levels observed were derived from the acute myeloid leukaemia blasts. Furthermore, higher bone marrow plasma IL-8 levels have been observed in acute myeloid leukaemia patients, as compared to plasma from healthy bone marrow, and the authors identified IL-8 as a central component of the altered cytokine/chemokine signalling network in the acute myeloid leukaemia bone marrow (Çelik et al., 2020). Other studies have described increased mRNA and protein levels of IL-8 in bone marrow-MSCs following their co-culture with acute myeloid leukaemia cells, confirming stroma as an additional source of IL-8 (Abdul-Aziz et al., 2017). In a different study, exosomes secreted by KG-1a (RRID:CVCL_1824) acute myeloid leukaemia cell lines promoted transcription and secretion of IL-8 by HS-5 cells and in a time- and dose-dependent manner protected the leukaemic cells against etoposide-induced apoptosis (Chen et al., 2019). Combination treatment of cytarabine plus the novel IL-8 small-molecule inhibitor NCI34255 re-sensitised acute myeloid leukaemia cell lines to cytarabine-induced apoptosis, confirming the pro-survival effects of IL-8, which were found to be mediated via AKT signalling (Vijay et al., 2019). Further experiments with the CXCR2 inhibitor, SB332235, were shown to limit the proliferative capacity of acute myeloid leukaemia cell lines and primary acute myeloid leukaemia samples, by induction of a G0 cell cycle arrest (Schinke et al., 2015). These data suggest that IL-8 signalling is an attractive therapeutic candidate.

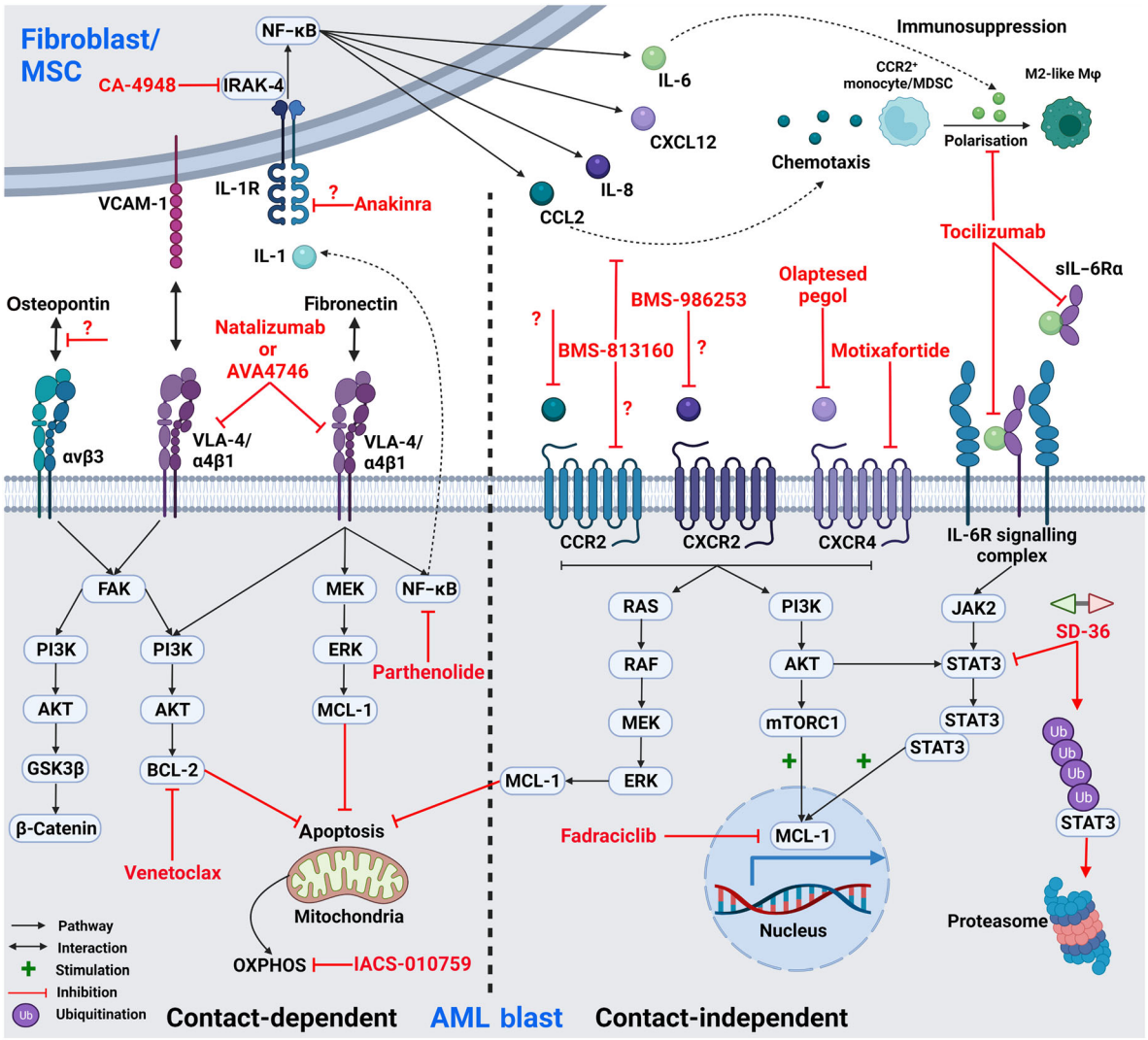


FIGURE 3 The contact-dependent and contact-independent mechanisms of fibroblast/mesenchymal stem cell (MSC)-driven therapy resistance as therapeutic targets for acute myeloid leukaemia (AML). Bone marrow fibroblasts and MSCs activate numerous pro-survival signalling pathways in AML cells that have the potential to be exploited therapeutically. Contact-dependent therapy resistance is mainly driven by integrin adhesive interactions with stromal cells (e.g. very late antigen-4 [VLA-4]/vascular cell adhesion molecule-1 [VCAM-1]) and extracellular matrix (ECM) components (e.g. VLA-4/fibronectin and αvβ3/osteopontin) (left), whereas cytokines and chemokines also up-regulate anti-apoptotic pathways by interacting with their cognate receptors on the surface of AML cells (C–C motif chemokine ligand 2 [CCL2]/C–C motif chemokine receptor 2 [CCR2], interleukin-8 [IL-8]/C–X–C motif chemokine receptor 2 (CXCR2), C–X–C motif chemokine ligand 12 [CXCL12]/C–X–C motif chemokine receptor 4 [CXCR4] and interleukin-6 [IL-6]/IL-6 receptor alpha [IL-6Rα]/gp130). The significant level of overlap between signalling pathways suggests that multi-targeted therapies/combination treatments will most likely provide maximal therapeutic benefit in AML patients. Targeted therapies with the potential to target stroma-driven therapy resistance in AML are displayed in the diagram. Note that targeting cytokine/chemokine axes may additionally suppress their immunomodulatory effects (e.g. CCL2-mediated recruitment of myeloid-derived suppressor cells [MDSCs] and IL-6-driven polarisation to M2-like macrophages [Mφs]) and restore immune regulation in the bone marrow. AKT, protein kinase B; BCL-2, B-cell lymphoma-2; ERK, extracellular-regulated kinase; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase-3β; IL-1, interleukin-1; IL-1R, interleukin-1 receptor; IL-6R, interleukin-6 receptor; IRAK-4, interleukin-1 receptor-associated kinase 4; JAK2, Janus kinase 2; MCL-1, myeloid cell leukaemia 1; MEK, mitogen-activated protein kinase kinase (MAPKK); mTORC1, mammalian target of rapamycin complex 1; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; OXPHOS, oxidative phosphorylation; PI3K, phosphoinositide 3-kinase; RAF, mitogen-activated protein kinase kinase kinase (MAPKKK); RAS, rat sarcoma virus; sIL-6Rα, soluble IL-6 receptor alpha; STAT3, signal transducer and activator of transcription 3. Created with BioRender.com

8.3 | CCL2

CCL2 is a chemokine involved in the directed chemotaxis and transendothelial migration of **C–C motif chemokine receptor 2 (CCR2)**-expressing monocytes. Preliminary evidence reported a role of CCL2 in acute myeloid leukaemia, as bone marrow-MSCs isolated from acute myeloid leukaemia patients expressed significantly higher levels of *CCL2* (Jacamo et al., 2014). In turn, bone marrow- and peripheral

blood-derived acute myeloid leukaemia blasts displayed high expression of the cognate CCL2 receptor, CCR2, responding to CCL2-mediated chemotactic signals and homing to the bone marrow (Macanas-Pirard, Quezada, et al., 2017). Neutralising monoclonal antibodies against CCR2, as well as small-molecule inhibitors of the CCL2/CCR2 interaction, not only prevented CCL2-mediated migration of human acute myeloid leukaemia cells in culture (Macanas-Pirard, Quezada, et al., 2017) but also reduced infiltration of tumour-promoting M2-like macrophages into the spleen of leukaemia-bearing mice (Jacamo et al., 2015). The immunosuppressive role of fibroblast-derived CCL2 in the tumour stroma has been confirmed in other cancers. For example, FAP^+ CAF-derived CCL2 enhanced tumour growth in a syngeneic murine model of hepatoma, with the authors attributing this effect to recruitment of CCR2-expressing immunosuppressive myeloid-derived suppressor cells, accompanied by reduced infiltration of cytotoxic T-cells (Yang et al., 2016). Although exogenous addition of CCL2 does not protect acute myeloid leukaemia cells against cytarabine-induced apoptosis, it is tempting to speculate that stroma-derived CCL2 may indirectly contribute towards acute myeloid leukaemia therapy resistance by promoting migration and homing of $CCR2^+$ leukaemic blasts and immunosuppressive immune cell subsets into bone marrow niches, where other cell-to-cell interactions may provide protection against the killing effects of therapeutics (e.g. **CXCL12/C–X–C motif chemokine receptor 4 [CXCR4]** and **selectin P ligand/E-selectin (CD62E)** interactions) (Erbani et al., 2020; Ladikou et al., 2020).

8.4 | CXCL12

The chemokine CXCL12, also known as stromal cell-derived factor-1 (SDF-1), is mainly secreted by perivascular bone marrow-MSCs in response to signals from $CD169^+$ stromal macrophages and is crucial in the homing/retention of $CXCR4^+$ haematopoietic stem and progenitor cells within bone marrow niches (Chow et al., 2011) (Figure 2). During acute myeloid leukaemia, leukaemic cells exploit the CXCL12/CXCR4 chemotactic axis to adhere to the protective niche of the bone marrow and withstand the effects of anti-cancer therapies (Ladikou et al., 2020). Importantly, acute myeloid leukaemia cells often up-regulate CXCR4 expression in response to chemotherapy, displaying enhanced response to CXCL12-mediated chemotaxis and promoting **AKT** and ERK pro-survival signalling, collectively reducing therapeutic efficacy (Sison et al., 2013). Studies have demonstrated that CXCR4 receptor inhibition via **plerixafor/AMD3100** or disruption of CXCL12/CXCR4 interaction (e.g. peptide E5), results in reduced acute myeloid leukaemia burden and improved survival in murine acute myeloid leukaemia models, in combination with chemotherapeutic agents (Han et al., 2015; Li, Guo, et al., 2015). Paradoxically, genetic ablation of CXCL12 in endothelial cells and mesenchymal progenitor cells of MLL-AF9 mice did not prevent leukaemia establishment, suggesting that CXCL12 function in acute myeloid leukaemia may, in fact, be dispensable (Ramakrishnan et al., 2020).

9 | ALTERATIONS IN THE BONE MARROW STROMA AS PROGNOSTIC INDICATORS AND TARGETS FOR ACUTE MYELOID LEUKAEMIA-DIRECTED THERAPIES

Due to our current understanding of the bone marrow microenvironment and its protective role against conventional and targeted therapies, we will discuss the potential prognostic impact and therapeutic potential of cancer-associated fibroblasts (CAFs)/ cancer-associated fibroblasts (MSCs)/extracellular matrix and their signalling pathways in acute myeloid leukaemia (Table 1). This knowledge has been acquired in recent years, following a temporary cessation in the preliminary CAF/MSC acute myeloid leukaemia research (Garrido et al., 2001; Konopleva et al., 2002).

Analyses of CAFs by scRNA-seq have demonstrated the heterogeneity of CAF populations, with identification of a single target population for therapeutic intervention hindered by the expression of many CAF markers on other cell types (Kieffer et al., 2020). For example, FAP^+ and/or $FSP\text{-}1^+$ tumour-associated macrophage populations have been described in the stroma of various malignancies, suggesting that dual targeting of these specific CAF and macrophage populations may enhance therapeutic benefit (Arnold et al., 2014; Tchou et al., 2013; Zhang et al., 2013). Acute myeloid leukaemia patients with higher bone marrow reticulin fibre density display worse overall prognosis, as a result of an increased number of bone marrow CAFs, highlighting the prognostic value of CAFs and their extracellular matrix modelling/remodelling capacity (Zhai et al., 2016). Specifically, fibre density was higher in refractory acute myeloid leukaemia patients, as compared to patients in complete remission, whereas prognosis of patients in complete remission could also be determined based on the percentage of reticulin fibres (Zhai et al., 2016).

Data from reverse-phase protein array of acute myeloid leukaemia patient samples have linked increased FAK expression to unfavourable cytogenetics in acute myeloid leukaemia (Carter et al., 2017). The authors speculated that overexpression of FAK may be mediated by acute myeloid leukaemia-expressed **integrin β3** ligation to the stroma, leading to proto-oncogene tyrosine-protein kinase (SRC)-dependent signalling in leukaemic cells (Carter et al., 2017; Despeaux et al., 2011) (Figure 3). These data, in combination with evidence of higher expression of integrin β3 in acute myeloid leukaemia with unfavourable cytogenetics/mutational status, highlight the potential of therapeutically targeting integrin β3/downstream signalling proteins to overcome contact-dependent, stroma-mediated therapy resistance (Yi et al., 2016). Moreover, activation of FAK downstream of VLA-4/fibronectin adhesion has been linked to minimal residual disease and ultimately disease recurrence, despite indications of high VLA-4 expression associated with improved relapse-free survival (RFS) and overall survival (OS) (Becker et al., 2009; Matsunaga et al., 2003; Walter et al., 2010) (Figure 3). Therapeutic intervention at the level of VLA-4 may simultaneously block cell-to-extracellular matrix (PI3K/AKT/BCL-2) and cell-to-cell (VLA-4/VCAM-1) interactions,

TABLE 1 Single-agent and combination therapies currently in clinical trials with the potential to overcome stroma-mediated therapy resistance in acute myeloid leukaemia

Treatment regimen	Mechanism of action/target	Purpose/eligibility	Clinical trial details[a]
LY3214996 (temuterkib)	ERK inhibitor	Assessing the safety and DLT of the investigational drug LY3214996 in R/R adult AML (≥18 years)	NCT04081259 Phase I: Recruiting Estimated completion: 30 April 2023
PRT1419	Selective MCL-1 inhibitor	Assessing DLT and RP2D of PRT1419 in R/R haematological malignancies (≥18 years)	NCT05107856 Phase I: Recruiting Estimated completion: November 2023
IACS-010759	Selective complex I inhibitor (metabolic targeting)	Assessing the MTD, DLT, PK and efficacy of IACS-010759 in adult R/R AML (≥18 years)	NCT02882321 Phase I: Active, not recruiting Estimated completion: 1 September 2022
Tocilizumab, idarubicin, cytarabine	Tocilizumab: Anti-IL-6Rα mAb Idarubicin: Anti-neoplastic agent Cytarabine: Anti-neoplastic agent	Assessing the addition of tocilizumab to standard induction chemotherapy (idarubicin + cytarabine) in high-risk adult AML (≥18 years)	NCT04547062 Phase I: Recruiting Estimated completion: 15 June 2023
Ruxolitinib, venetoclax	Ruxolitinib: JAK1/JAK2 inhibitor Venetoclax: Selective BCL-2 inhibitor	Assessing the safety, efficacy and MTD of combination therapy in adult R/R AML (≥18 years)	NCT03874052 Phase I: Recruiting Estimated completion: 31 December 2023
CYC065, venetoclax	CYC065 (fadraciclib): Dual MCL-1 and CDK2/5/9 inhibitor Venetoclax: Selective BCL-2 inhibitor	Assessing the safety and efficacy of combination therapy in R/R adult AML or MDS (≥18 years)	NCT04017546 Phase I: Unknown status Estimated completion: 31 December 2020—No updates as of January 2022
AZD5991, venetoclax	AZD5991: Selective MCL-1 inhibitor Venetoclax: Selective BCL-2 inhibitor	Assessing the MTD, DLT, PK and preliminary anti-tumour activity of AZD5991 (± venetoclax) in R/R haematological malignancies (≥18 and ≤85 years)	NCT03218683 Phase I/Ib/IIa: Temporarily suspended Estimated completion: 24 June 2024
S64315, venetoclax	S64315: Selective MCL-1 inhibitor Venetoclax: Selective BCL-2 inhibitor	Assessing the DLT, PK, anti-tumour activity and RP2D of combination therapy in adult de novo AML, s-AML or t-AML (≥18 years)	NCT03672695 Phase Ib: Recruiting Estimated completion: 31 January 2024
S64315, azacitidine	S64315: Selective MCL-1 inhibitor Azacitidine: HMA	Assessing the DLT, PK and anti-leukaemic activity of S64315 in combination with azacitidine in adult de novo AML, s-AML or t-AML (≥18 years)	NCT04629443 Phase I/II: Recruiting Estimated completion: March 2024
VOB560, MIK665	VOB560: Selective BCL-2 inhibitor MIK665: Selective MCL-1 inhibitor	Assessing the DLT, PK and anti-leukaemic activity of combination therapy in adult R/R non-Hodgkin lymphoma, R/R AML or R/R MM (≥18 years)	NCT04702425 Phase Ib: Recruiting Estimated completion: 2 January 2025
BP1002, decitabine	BP1002: Liposomal BCL-2 antisense oligodeoxynucleotide Decitabine: HMA	Assessing the DLT, PK, PD and RP2D of BP1002 ± decitabine in adult R/R AML (≥18 years)	NCT05190471 Phase I/Ib: Not yet recruiting Estimated completion: September 2024
RAD001, PKC412	RAD001 (everolimus): mTORC1 inhibitor PKC412 (midostaurin): Multi-TKI (e.g. SYK and c-Src)	Assessing the DLT, PK, PD and MTD of combination therapy in adult R/R or high-risk AML or MDS (≥18 years)	NCT00819546 Phase I: Active, not recruiting Estimated completion: December 2021—No updates as of January 2022
Trametinib, azacitidine, venetoclax	Trametinib: MEK1/2 inhibitor Azacitidine: HMA Venetoclax: Selective BCL-2 inhibitor	Assessing the efficacy of combination treatment in haematological malignancies (≥18 years)	NCT04487106 Phase II: Recruiting Estimated completion: 1 June 2024

(Continues)

TABLE 1 (Continued)

Treatment regimen	Mechanism of action/target	Purpose/eligibility	Clinical trial details[a]
Ruxolitinib, **fedratinib**, decitabine	Ruxolitinib: JAK1/JAK2 inhibitor Fedratinib: Selective JAK2 inhibitor Decitabine: HMA	Assessing the efficacy of decitabine in combination with ruxolitinib or fedratinib before HSCT in AML originating from MPN (accelerated/blast phase MPN) (≥18 years)	NCT04282187 Phase II: Recruiting Estimated completion: 11 November 2024
CA-4948, azacitidine, venetoclax	CA-4948 (emavusertib): Small-molecule inhibitor of IRAK-4 Azacitidine: HMA Venetoclax: Selective BCL-2 inhibitor	Dose escalation and expansion study to determine MTD, RP2D and efficacy of CA-4948 (± azacitidine or venetoclax) in high-risk AML or MDS (≥18 years)	NCT04278768 Phase I/IIa: Recruiting Estimated completion: 1 February 2022

Note: Information correct as of 31 January 2022.

Abbreviations: AML, acute myeloid leukaemia; BCL-2, B-cell lymphoma-2; CDK, cyclin-dependent kinase; c-Src, proto-oncogene tyrosine-protein kinase Src; DLT, dose-limiting toxicity; ERK, extracellular-regulated kinase; HMA, hypomethylating agent; HSCT, haematopoietic stem cell transplantation; IL-6Rα, interleukin-6 receptor alpha; IRAK-4, interleukin-1 receptor-associated kinase 4; JAK, Janus kinase; mAb, monoclonal antibody; MCL-1, myeloid cell leukaemia-1; MDS, myelodysplastic syndrome; MEK, mitogen-activated protein kinase kinase; MM, multiple myeloma; MPN, myeloproliferative neoplasm; MTD, maximum tolerated dose; mTORC1, mammalian target of rapamycin complex 1; PD, pharmacodynamics; PK, pharmacokinetics; R/R, relapsed/refractory; RP2D, recommended Phase 2 dose; s-AML, secondary acute myeloid leukaemia; SYK, spleen tyrosine kinase; t-AML, therapy-related acute myeloid leukaemia; TKI, tyrosine kinase inhibitor.

[a]All clinical trials are registered at clinicaltrials.gov and can be accessed by the study ID provided in the table.

providing enhanced therapeutic potential (Matsunaga et al., 2008; Ruan et al., 2022). Examples of VLA-4 targeting therapies include the humanised anti-α4 integrin monoclonal antibody natalizumab (RRID:AB_2910857), approved by the Food and Drug Administration (FDA) for the treatment of relapsed multiple sclerosis, and the novel, small-molecule VCAM-1 mimetic AVA4746 that effectively displaces VCAM-1 from VLA-4 and re-sensitises B-acute lymphoblastic leukaemia cells to chemotherapy in murine models (Ruan et al., 2022) (Figure 3).

Elevated levels of certain cytokines, chemokines and their cognate receptors are also implicated in acute myeloid leukaemia patient prognosis and provide attractive therapeutic targets for eradication of contact-independent therapy resistance. Up-regulation of CXCR4 on acute myeloid leukaemia blasts was detected in patients and correlates with worse event-free survival, relapse-free survival and overall survival (Konoplev et al., 2007; Spoo et al., 2007). The CXCL12/CXCR4 axis has long been the target of therapeutic strategies (e.g. plerixafor), with a Phase IIa clinical trial (NCT01838395) assessing the safety/efficacy of **motixafortide** (CXCR4 inhibitor) in combination with high-dose cytarabine in relapsed/refractory acute myeloid leukaemia completed recently (Borthakur et al., 2021). A CXCL12-neutralising RNA oligonucleotide (**olaptesed pegol**) has also been developed and assessed in the context of chronic lymphocytic leukaemia (NCT01486797) (Hoellenriegel et al., 2014; Steurer et al., 2019) (Figure 3). In patients resistant to etoposide, plasma IL-8 levels are significantly higher than patients responsive to etoposide (Chen et al., 2019) and higher expression of CXCR2 correlates with worse clinical manifestations, including low haemoglobin levels, high dependence on blood transfusions and poor overall survival (Schinke et al., 2015). Although therapeutic interventions at the level of IL-8/CXCR2 and CCL2/CCR2 have yet to be described for acute myeloid leukaemia, the experimental compounds BMS-986253 (anti-IL-8 monoclonal antibody) and BMS-813160 (dual CCR2/CCR5 antagonist) are currently in clinical trials for myelodysplastic syndrome and advanced pancreatic or colorectal cancer, respectively, and could potentially have clinical utility in acute myeloid leukaemia (Bilusic et al., 2019; Le et al., 2018). Similarly, the anti-**IL-1 receptor** antagonist **anakinra**, approved for the treatment of rheumatoid arthritis (RA), is currently being investigated in treatment-naive chronic lymphocytic leukaemia patients (NCT04691765) and may also be applicable in acute myeloid leukaemia treatment, based on preliminary efficacy/safety in viral-induced pneumonia in acute myeloid leukaemia (Day et al., 2020). Moreover, increased concentrations of IL-6 have also been linked to poor prognosis in both adult acute myeloid leukaemia (Sanchez-Correa et al., 2013) and paediatric acute myeloid leukaemia (Stevens et al., 2017). In the context of targeting IL-6, the humanised anti-IL-6Rα monoclonal antibody, **tocilizumab** (RRID:AB_2911441), is currently in clinical trials for acute myeloid leukaemia (NCT04547062), following its initial FDA approval for RA (Oldfield et al., 2009) (Figure 3). This targeting method may be effective in acute myeloid leukaemia patients that display enhanced autocrine secretion of IL-6 but may also prevent stroma-mediated therapy resistance. Another novel therapeutic compound is the selective STAT3 proteolysis-targeting chimera (PROTAC) SD-36, which targets STAT3 protein for proteasomal-mediated degradation (Bai et al., 2019). Both tocilizumab and SD-36 could also be beneficial in combination with the indirect MCL-1 inhibitor **fadraciclib** in acute myeloid leukaemia, with high expression of MCL-1 correlating with poor response to therapy, disease recurrence and reduced overall survival (Li et al., 2019) (Figure 3). Combination regimens of fadraciclib with venetoclax, cytarabine and azacitidine have previously demonstrated synergistic activity in acute myeloid leukaemia and fadraciclib is currently being tested in combination with venetoclax in the treatment of relapsed/refractory acute myeloid leukaemia

(NCT04017546) (Borthakur et al., 2019; Chantkran et al., 2021). Lastly, intervention at the level of NF-κB, for example, via the NF-κB inhibitor parthenolide,or the **interleukin-1 receptor-associated kinase 4 (IRAK-4)** inhibitor **emavusertib (CA-4948)**, may simultaneously prevent NF-κB target gene expression in both acute myeloid leukaemia and stromal cells (Flores-Lopez et al., 2018; Gummadi et al., 2020).

Contrary to the apparent role of stroma in promoting tumour growth, some studies have also indicated anti-tumourigenic properties of certain CAF/MSC populations. For example, selective depletion of α-SMA$^+$ CAFs in pancreatic ductal adenocarcinoma resulted, paradoxically, in cancer progression (Özdemir et al., 2014). Another population of FSP1$^+$ fibroblasts prevented establishment of aggressive epithelial tumours in murine models of chemically induced fibrosarcoma by collagen encapsulation of the carcinogen (Zhang et al., 2013). These findings are consistent with previous observations where FSP1 can be a marker of both resting and activated (CAF-like) fibroblasts (Frassanito et al., 2014). Furthermore, MSCs restore expression of the thrombopoietin receptor in undifferentiated chronic myelogenous leukaemia cells, promoting their differentiation in response to the receptor agonist **eltrombopag** and reducing leukaemic burden in chronic myelogenous leukaemia mouse models (Zuo et al., 2021). It is therefore suggested that the presence of cancer cells, and the plethora of cancer secreted factors, results in the establishment of distinct, highly heterogeneous stromal populations, the functions and cellular phenotypes of which will be largely context dependent (Gieniec et al., 2019; Mao et al., 2021).

Evidence also suggests that extracellular matrix remodelling in the leukaemic bone marrow may not be directly driven by stromal elements. A study by Izzi et al. (2017) identified two distinct populations of leukaemic precursor cells based on the expression of extracellular matrix-related genes. One population overlapped with normal haematopoietic stem cells (termed early leukaemic cells), whereas the other population had a distinct expression pattern of the tested genes (termed definitive leukaemic cells). Indeed, B-acute lymphoblastic leukaemia cells induce the expression of the extracellular matrix remodelling enzyme MMP9 in bone marrow-MSCs, which ultimately enhances leukaemic cell survival and mobilisation to sites of extramedullary haematopoiesis (Verma et al., 2020). These findings support the notion that leukaemic cells can directly influence their surrounding extracellular matrix microenvironment to optimally support their survival/expansion, thus providing a rationale to target the extracellular matrix in acute myeloid leukaemia.

Thus, optimal clinical benefits from therapeutic intervention at the level of the tumour stroma in acute myeloid leukaemia will most likely be dependent upon identification of specific signalling pathways, adhesive interactions to stromal cells/extracellular matrix, alongside alterations in extracellular matrix organisation and dual targeting of the stroma and the acute myeloid leukaemia cells. Novel therapeutic development will ultimately be dependent upon successful characterisation and distinction of pro-tumourigenic versus anti-tumourigenic stromal cell populations, efforts that will be facilitated by the establishment of novel cell sorting techniques, such as the newly described method of flow cytometry and high-speed image-enabled cell sorting (ICS) (Schraivogel et al., 2022).

10 | IMPROVING MODELLING OF THE ACUTE MYELOID LEUKAEMIA BONE MARROW MICROENVIRONMENT: ESTABLISHMENT OF NOVEL CELL LINES AND THREE-DIMENSIONAL (3D) MODELS

Although many examples of bone marrow microenvironment modelling have been discussed, each method has its limitations. The HS-5 and HS-27/HS-27A cell lines improve our understanding of the role of bone marrow stromal cells in acute myeloid leukaemia. However, they are not derived from a leukaemic bone marrow and therefore fail to recapitulate the complexity of acute myeloid leukaemia–stroma interaction. To our knowledge, there have been no reports on isolation of pure acute myeloid leukaemia bone marrow-derived fibroblasts, whereas the isolation and long-term culture of primary bone marrow-MSCs is challenging (Brown et al., 2019; DiGirolamo et al., 1999). Recently, a CAF-derived murine cell line, HXWMF-1 (RRID:CVCL_A8QE), was established in a model of acute lymphoblastic leukaemia, displaying expression of the CAF markers α-SMA, FAP and FSP-1 among others (Li & Gu, 2021). This is the first report of the development of a CAF-like cell line in blood cancer. The authors also isolated two additional CAF-like cell lines from separate xenograft models, HXWMF-2 and HXWMF-3, with similar fibroblast activation markers, indicating the reproducibility of this process and further strengthening the potential of this method for future application in more accurate reflection of the malignant bone marrow microenvironment *in vitro*.

Further improving bone marrow microenvironment modelling is the emergence of 3D cell culture systems, including magnetic levitation systems (Lewis et al., 2017) and 3D tissue-engineered bone marrow (3DTEBM) (de la Puente et al., 2015). The use of 3D systems arises from the understanding that the bone marrow microenvironment contains oxygen and nutrient gradients that optimally support haematopoietic stem cells/haematopoietic stem and progenitor cells (Deynoux et al., 2016; Lu et al., 2019). These conditions are also present in a leukaemic bone marrow, reducing the sensitivity of acute myeloid leukaemia cells to therapy, maintaining leukaemic stem cells, preventing complete disease eradication and leading to acute myeloid leukaemia relapse (Aljitawi et al., 2014; Benito et al., 2015; Drolle et al., 2015). Although conventional two-dimensional (2D) cell culture systems reproduce, to some extent, cell-to-cell interactions, they do not fully reflect important bone marrow microenvironment interactions (Law et al., 2021). Importantly, culture of bone marrow-MSCs in 3D mesenspheres with CD45$^+$ or CD34$^+$ cells reduced chemotherapeutic efficacy and promoted acute myeloid leukaemia cell survival (Forte et al., 2020), confirming that 2D culture systems overestimate

drug efficacy and exhibit enhanced sensitivity to therapies when compared to 3D cell culture systems (Balandrán et al., 2017). More complex 3D hydrogels, consisting of acute myeloid leukaemia cells, MSCs, endothelial cells, extracellular matrix molecules and MMP-responsive sequences that allow extracellular matrix remodelling and leukaemic migration through the hydrogel, have also been developed, allowing for assessment of more intricate bone marrow microenvironment interactions (Bray et al., 2017). In addition to ensuring optimal modelling of the bone marrow microenvironment, the correct mechanical characteristics must also be achieved. For example, adapting the stiffness of an alginate-based hydrogel and altering the amount of integrin-binding sequences for acute myeloid leukaemia attachment can significantly impact the proliferation of leukaemic cells and their response to therapeutic agents (Bray et al., 2017; Shin & Mooney, 2016). Further supporting these findings, bone marrow-MSCs cultured in different topographies adopted different cell morphologies that subsequently altered their cytokine secretion profile (Leuning et al., 2018). Collectively, these studies demonstrate the importance of accurate *in vitro* bone marrow microenvironment modelling for the identification of novel therapeutic targets in acute myeloid leukaemia.

11 | CONCLUSIONS AND FUTURE DIRECTIONS

In summary, our increasing understanding of bone marrow stromal fibroblasts and MSCs, and their role in disease pathogenesis, has the potential to provide novel strategies to overcome bone marrow microenvironment-mediated therapy resistance. Direct cell-to-cell contact between leukaemic cells and stromal elements, mediated via bidirectional communication and/or stromal secreted factors. These collectively shield the acute myeloid leukaemia cells against the killing effects of therapies. In-depth analysis of the phenotypic/functional alterations observed in fibroblasts (cancer-associated fibroblasts) and MSCs in the leukaemic bone marrow is further required to improve our understanding of these highly diverse cell populations. These advancements will be supported by the continuous improvement in current modelling systems (e.g. 3D cell culture), as well as the optimisation of new techniques, such as the newly developed image-enabled cell sorting. Therefore, future studies will enable the identification of specific pro-tumourigenic cell populations, which could be targeted with novel therapeutic compounds, in an effort to improve clinical outcomes of acute myeloid leukaemia patients.

11.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Kelly et al., 2021; Alexander, Christopoulos, et al., 2021; Alexander, Fabbro, et al., 2021a,b).

ACKNOWLEDGEMENTS

We are grateful to Glasgow Caledonian University and all collaborating institutions. We would also like to extend our gratitude to the societies/funding bodies that support our research.

CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

Katerina E. Miari and Mark T. S. Williams contributed equally to this work.

DATA AVAILABILITY STATEMENT

Data availability statement is not applicable to this work.

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How to cite this article: Miari, K. E., & Williams, M. T. S. (2024). Stromal bone marrow fibroblasts and mesenchymal stem cells support acute myeloid leukaemia cells and promote therapy resistance. *British Journal of Pharmacology*, *181*(2), 216–237. https://doi.org/10.1111/bph.16028