Interactions between Non-Hematological and Multiple Myeloma Cells

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Tumors are composed of a plethora of extracellular matrix, tumor and non-tumor cells that form a tumor microenvironment (TME) that nurtures the tumor cells and creates a favorable environment where tumor cells grow and proliferate. In multiple myeloma (MM), the TME is the bone marrow (BM). Non-tumor cells can belong either to the non-hematological compartment that secretes soluble mediators to create a favorable environment for MM cells to grow, or to the immune cell compartment that perform an anti-MM activity in healthy conditions. Indeed, marrow-infiltrating lymphocytes (MILs) are associated with a good prognosis in MM patients and have served as the basis for developing different immunotherapy strategies. However, MM cells and other cells in the BM can polarize their phenotype and activity, creating an immunosuppressive environment where immune cells do not perform their cytotoxic activity properly, promoting tumor progression.

Keywords: multiple myeloma ; bone marrow ; marrow-infiltrating lymphocytes

1. Introduction

Nowadays, it is widely accepted that the tumor microenvironment (TME) is a relevant component in tumors that modulates the response to cancer treatments affecting tumor progression. The TME consists of an extracellular matrix, a plethora of tumor cells, and a variety of non-tumor cells with complex interactions. These interactions, either through cell–cell contact or as soluble mediators, can accelerate tumor progression and the lack of response to cancer therapy [1]. Moreover, the knowledge of these interactions enables the development of non-immunotherapy [2][3][4] and immunotherapy strategies [5][6][7][8][9] in cancer patients.

Non-tumor cells in the TME, including endothelial cells, fibroblasts, and immune cells [7], modulate the responses to chemotherapy cancer treatments. For instance, chemotherapy agents that induce DNA damage, such as doxorubicin, trigger cytokine production by endothelial cells that decrease chemosensitivity of tumor cells to these treatments [10]. DNA-damaging agents also induce a senescence state in cells with the production of a senescence-associated secretory phenotype (SASP), a secretome rich in chemokines and growth factors that promote tumor progression [11]. Indeed, the secretion of SASP by endothelial cells in the TME includes IL6 secretion and chemoresistance development [12]. Tumor-associated macrophages (TAMs) with an M2-like phenotype provide a survival advantage to tumor cells in hypoxic conditions through IL6 receptor-mediated signals [13]; they protect tumor cells against paclitaxel, etoposide, and doxorubicin [14]. Moreover, platinum-based therapy supports monocyte differentiation to M2 macrophages, which associates with tumor progression [15].

Cellular components in the TME also influence the efficacy of radiotherapy treatments. Hence, radiotherapy activates fibroblasts, which become cancer-associated fibroblasts (CAFs). While some studies argue that CAFs promote tumor progression, others claim they are beneficial [16][17]. Thus, CAFs can secrete cytokines, such as IL32 that promote cancer cell invasion and metastasis [18]. However, CAFs in vivo depletion accelerates pancreatic cancer accompanied by epithelial-to-mesenchymal transition and enhanced T-regulatory (regs) cells that is reversed with anti-CTLA4 immunotherapy [19].

Immune cells and their secretome also shape the TME [1], impacting cancer progression and the efficacy of immunotherapy treatments [20]. For instance, tumor-infiltrating cells (TILs) in the TME are the basis for developing immunotherapy strategies based on immune checkpoint inhibition (ICI) that try to reactivate the tumor immune-surveillance activity of TILs [9]. Radiotherapy can promote tumor-specific immunity by activating dendritic cells (DCs) in the TME that support tumor-specific effector CD8 T cells [21]. Moreover, immunotherapy strategies based on the infusion of chimeric antigen receptor (CAR)-modified T cells have significantly improved the treatment of hematological malignancies [22][23][24][25]. However, in solid tumors, the barriers imposed by the TME [26] have delayed the development of efficient

CAR-T cell therapies. Age also seems to play an essential role in the immune cells' activity and, therefore, in immunotherapy. Thus, in hematological malignancies, pediatric patients with acute lymphoblastic leukemia (ALL) have achieved outstanding responses after treatment with CAR-T cells [22]. However, in adult patients with multiple myeloma (MM) [27], a disease where aging is a risk factor and where the TME is more relevant than in ALL, a proportion of patients end-up relapsing. In MM, the progression of the disease is drastically affected by the TME, either by soluble factors or cell–cell interactions in the bone marrow (BM) [28]. Moreover, relapses after administration of CAR-T cells [27], and the lack of efficacy of ICI therapies with significant toxicities in MM [25] might be partly explained by the impact of non-immune and immune cell interactions in the TME.

2. Extracellular Matrix (ECM)

MM is a hematologic malignancy characterized by clonal proliferation of plasma cells in the BM [29]. However, different trafficking events of MM cells allow them to reach distinct niches from the BM, re-circulate to the BM, and finally egress from the BM during the extramedullary stage of the disease [28]. When MM cells re-enter the BM, they use the BM sinusoids, where the interaction CXCR4/CXCL12 is critical to promote both MM cell homing and retention in the BM [30]. In the BM, MM cells will interact first with proteins in the ECM, a complex layer of proteins that serves as a scaffold for many cells. Interactions between MM cells and the ECM are required for cell proliferation, migration, and survival [31]. Specifically, CD138 and VLA-4 on MM cells directly interact with the ECM proteins, such as collagen type 1 and fibronectin. The binding of VLA-4 to fibronectin induces activation of nuclear factor-kB (NFkB), inducing tumor cell survival and cell adhesion-mediated drug resistance [32]. These interactions generate a welcome and growth-supporting environment that stimulates the dissemination of the malignant plasma cells and results in the upregulation of anti-apoptotic proteins and cell cycle dysregulation [33]. Strategies used in the clinic to disrupt these MM–ECM interactions and reduce cell adhesion-mediated drug resistance include the CXCR4 inhibitor AMD3100 or the proteasome inhibitor bortezomib, which downregulates VLA-4 on MM cells [34], leading to the de-adhesion of MM cells from the BM and turning them more sensitive to therapeutic agents [35]. However, although these agents can enhance the efficacy of treatments by disrupting these interactions, they also contribute to the mobilization of MM cells from the BM into the circulation, promoting extramedullary disease [36].

3. Control of the Stroma by BM Mesenchymal Stromal Cells (BM-MSCs)

In physiological conditions, the primary cell population in the BM stroma, known as bone marrow mesenchymal stromal cells (BM-MSCs), support the maintenance and differentiation of hematopoietic lineages, regulate bone homeostasis and contribute to the spatial delimitation of the endosteal and vascular niches [37]. However, in MM, BM-MSCs, as part of the BM microenvironment, play a crucial role in the pathology of the disease. Despite being at low proportions in the BM (0.01 to 0.001% of mononuclear cells) [38], BM-MSCs are the main population among BM stromal cells that interact with MM cells by direct cell–cell contact or through paracrine secretion of different pro-survival cytokines. Thus, for instance, binding VLA-4 on MM cells to VCAM-1 on BM-MSCs promotes activation of NFkB increasing MM cell survival and proliferation [39]. Moreover, the integrin lymphocyte function-associated antigen 1 (LFA-1) on MM cells and its transmembrane binding partner Mucin 1 (MUC1) bind to ICAM-1 in adjacent BM-MSCs, resulting in the activation of different pathways associated with poor prognosis and disease progression in patients [40]. The strong impact of the interactions with BM-MSCs in the physiology of MM cells and their acquisition of multidrug resistance phenotype justifies their consideration as targets for MM therapy. Indeed, some drugs have been developed to disrupt these interactions and tested in MM patients, such as Natalizumab, a recombinant humanized IgG4 monoclonal antibody (MoAb) which binds α4 integrin impairing the interaction VLA-4/VCAM-1 (NCT00675428). Other promising approaches have been preclinically evaluated, such as the LFA-1 inhibitor LFA878 [41].

Soluble mediators are also required for MM plasma cell survival and proliferation in the BM. Thus, MM cells induce BM-MSCs to secrete cytokines that will be used for their benefit. Specifically, the main secreted cytokine is interleukin-6 (IL6), which is involved in MM growth, survival, migration, and drug resistance [42]. In turn, MM cells use IL6 to enhance the secretion of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Then, both VEGF and bFGF bind to their receptors on BM-MSCs, re-stimulating IL6 production [43]. Whereas inhibition of IL6 has not shown clinical benefit in MM [44], blocking of IL6 receptor with tocilizumab has shown efficacy in MM patients [45]. Furthermore, MM cell interactions with BM-MSCs cells are mediated through Notch pathways and Dickkopf-1 (DKK1), which induce the secretion of IL6, VEGF, and insulin-like growth factor (IGF-1) in BM-MSCs [46][47]. Moreover, MSC-derived exosomes contain the long intergenic noncoding RNA LINC00461, which promotes MM cell proliferation and suppresses the beneficial effect of dexamethasone treatment. Indeed, the knockdown of LINC00461 enhances the beneficial impact of dexamethasone in preclinical studies [48].

B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) are additional mediators with a protective effect on MM cells [49]. BAFF is a member of the tumor necrosis factor (TNF) family expressed on the surface of BM-MSCs and as a soluble form. BAFF stimulates B cell growth, and ligation of BAFF leads to increased proliferation and survival of MM cells [50]. APRIL is a secreted protein by BM-MSC that binds to B-cell maturation antigen (BCMA) and to transmembrane activator and calcium-modulator and cyclophilin ligand (TACI) on MM cells [51]. Therefore, APRIL-based CARs target MM cells expressing either BCMA or TACI with high efficacy at pre-clinical levels [52]. Moreover, BM-MSCs also protect MM cells against the lytic machinery of CAR-T cells [53].

Another member of the TNF family involved in this stromal training is TNFα, which induces the expression of adhesion molecules, such as LFA-1, ICAM-1, VCAM-1, and VLA-4 on MM cells, as well as ICAM-1 on BM-MSCs, resulting in increased binding of MM cells to BM-MSCs and further enhancing IL6 secretion [54]. These paracrine loops are critical for maintaining the constant growth of MM cells through the activation of different signaling pathways. In addition, MM cell interactions with BM-MSCs, added to the senescent status of cells in the BM in MM, further enhance the secretion of cytokines, chemokines, and soluble factors secreted by BM-MSCs to the BM milieu, which induce further MM proliferation and survival. TNFα, crucial in inflammation, is related to bone resorption and enhanced in MM patients. Thus, targeting TNFα could improve MM responses to treatments [55]. However, reports in inflammatory diseases suggest that anti-TNF-α inhibitors enhance the risk of having future hematological malignancies [56].

In summary, these interleukins and growth factors secreted by BM-MSCs cause tumor growth and drug resistance, limiting current MM treatments' impact. Indeed, they are promising targets for developing anti-MM therapies that avoid the negative effect of BM-MSCs on dexamethasone treatment [48], on CAR-T cell therapies [53], or the negative impact of IL6 secretion. Thus, tocilizumab, an anti-IL6R [57], BHQ880, a monoclonal antibody against DKK1 [58], or tabalumab, a potent and selective fully human IgG4 MoAb with neutralizing activity against membrane-bound and soluble BAFF [59] are strategies that could be added to MM treatment.

4. Osteoclast/Osteoblast Imbalance in the Endosteal Niche

As previously mentioned, MM cells not only interact with the stromal compartment they also alter the endosteal and vascular niches in the BM. In the endosteal niche, healthy bone remodeling in the BM is maintained by a balance between bone formation (osteoblastogenesis) versus bone degradation (osteoclastogenesis). However, MM cells alter this dynamic balance, enhancing bone resorption to enable space for MM proliferation, causing the osteolytic lesions characteristic of myeloma bone disease (MBD) in around 80–90% of MM patients [60]. The negative impact of MBD on patient survival, quality of life, and public health costs has led to the development of different approaches to block MM-endosteal niche interactions. Strategies for patients to treat and avoid MBD have recently been reviewed [61].

MM cells, through different mechanisms, upregulate osteoclast activity and differentiation resulting in imbalanced bone resorption, causing the osteolytic lesions of the MBD [62]. Specifically, MM cells secrete macrophage inflammatory protein-1α (MIP1α) and MIP1β that directly activate osteoclast formation and activity [63][64]. In turn, osteoclasts secrete IL6 to stimulate their self-proliferation and the proliferation of MM cells [65]. This interaction upregulates Chondroitin synthase 1 (CHSY1), which induces Notch signaling promoting MM cell survival and stimulating the recruitment of osteoclast precursors to increase bone resorption [66]. Macrophage-colony stimulating factor (M-CSF) and receptor activator of NFkB (RANK) ligand (RANKL) are additional factors required for osteoclast differentiation. Osteocytes produce RANKL, which promotes osteoclast activity through binding to RANK on osteoclastic lineage cells [67]. Nevertheless, MM cells' interaction with BM-MSCs leads to the secretion of RANKL by BM-MSCs, further stimulating osteoclast activation and differentiation and enhancing bone lysis. This interaction also leads to the production of cytokines by BM-MSCs, such as IL6, which further promotes osteoclast growth [68]. In this way, amino-bisphosphonates have been administered in MM patients as first-line therapy for MBD due to their capacity to inhibit osteoclast activity [69]. Moreover, Denosumab, a fully human monoclonal antibody against RANKL, has also shown clinical benefit in MM patients [70]. Denosumab inhibits the development and activity of osteoclasts, decreases bone resorption, and increases bone density [71].

On the other hand, MM cells prevent osteoblast progenitor cell maturation and inhibit osteoblast activation, to continue impairing bone formation. Direct cell–cell interactions of MM cells through binding to VCAM-1 on osteoblast progenitors downregulate RUNX2 activity, essential for osteoblast differentiation [72]. Moreover, osteoblasts and BM-MSCs produce osteoprotegerin (OPG), which prevents the development of bone alterations caused by osteoclast/osteoblast imbalance. However, the binding of VLA-4 on MM cells to VCAM-1 on BM-MSCs decreases OPG secretion, forcing the balance in favor of osteoclasts and bone degradation [73]. Disrupting this VLA-4/VCAM-1 interaction with monoclonal antibodies, such as Natalizumab, could prevent bone lysis in MM patients, as described in preclinical models [74]. On the other hand,

BHQ880, the DKK1-neutralizing antibody, can increase osteoblast differentiation, blocking the negative effect of MM cells on osteoblastogenesis and reducing IL6 secretion in MM patients [75].

5. Angiogenesis Promotion in the Vascular Niche

During the development of MM, an alteration in the neovascularization process occurs that affects the vascular niche. Neovascularization is the formation of new vessels from existing ones through endothelial cells (angiogenesis) or from endothelial precursors (vasculogenesis). Interactions between plasma cells and the BM microenvironment can modify this biological process [76][77][78].

Angiogenesis in cancer involves an early balanced avascular phase that gives rise to an uncontrolled and unlimited in-time vascular phase [79]. In the context of MM, Rajkumar et al. demonstrated that the BM microvascular density is increased in MM patients [80]. In this environment, the accumulation of MM cells in the BM generates hypoxic tumors highly expressing hypoxia-inducible factor-1 alpha (HIF-1α). HIF-1α will upregulate angiogenesis to deliver oxygen and nutrients and remove catabolites [81]. Different cytokines control angiogenesis, such as VEGF, fibroblast growth factor-2 (FGF-2), and hepatocyte growth factor (HGF). In MM, MM plasma cells become CD45-negative and produce VEGF [82]. Moreover, endothelial cells in the BM of MM modify their phenotype, expressing surface receptors related to angiogenesis, such as VEGFR-2 and Tie2/Tek, and increased expression of the β3-integrin and endoglin [83]. This differentiated phenotype in endothelial cells of the BM enhances MM cell interaction with the new-formed blood vessels and favors the entry and dissemination of MM cells into the circulation. This angiogenic phenotype in MM cells can also be induced by oncogenes, such as C-MYC, C-FOS, C-JUN, and ETS-1, which become active as a consequence of the genetic instability and immunoglobulin translocations in MM [84].

On the other hand, the differentiation of endothelial progenitors termed angioblasts during embryogenesis causes the development of the vascular system, known as vasculogenesis [85]. Studies suggest that vasculogenesis is responsible for the neovascularization in the BM in MM [86][87]. Indeed, endothelial markers such as VIII-related antigen (FVIII-RA), vascular endothelial-cadherin (VE-cadherin), VEGFR-2, TIE/Tek, and CD133 are expressed in endothelial cells of the neovessel wall [88]. Moreover, interactions of MM cells with BM-MSCs in the BM also impact vasculogenesis. Thus, MM cells stimulate BM-MSCs in the vascular niche to secrete HGF, VEGF, and IL8, further inducing neovascularization [89]. In turn, endothelial cells in MM will produce IGF1 and IL6 to promote MM cell growth, causing an autocrine loop in endothelial cells, which will enhance their production of VEGF, platelet-derived growth factor (PDGF), Ang-1, HGF, and IL1 to promote angiogenesis constantly [90].

The relevance of angiogenesis in the development of MM has led to the development of different treatments targeting this process. For instance, amino-bisphosphonates that inhibit osteoclasts also present anti-angiogenic activities and are administered in MM patients as supportive therapy for bone disease [69]. Ria et al. reviewed different strategies in MM mainly based on VEGF inhibition, such as monoclonal antibodies anti-VEGF (Bevacizumab) [91]. However, the addition of bevacizumab to anti-MM therapies did not result in a significant improvement in the outcome of patients [92][93]. Derivatives of quinolone and quinazoline, which inhibit a variety of tyrosine kinases, including VEGFRs, EGFR, and PDGFR have also been tested in MM patients. Despite their in vitro activity and reduced plasma levels of VEGF in treated MM patients, no responses or clinical benefits were achieved [94][95]. These disappointing results inhibiting a single proangiogenic cytokine could be related to the role played by hypoxia and other active pro-angiogenic pathways in the BM microenvironment, and greater efficacy could be feasible with drugs that simultaneously block multiple cytokines. Moreover, immunomodulators (IMIDs), such as thalidomide or lenalidomide, have revealed anti-angiogenic activity and inhibition of the secretion of angiogenic cytokines in MM patients [96][97].

References

1. Etxebeste-Mitxeltorena, M.; Del Rincón-Loza, I.; Martín-Antonio, B. Tumor Secretome to Adoptive Cellular Immunotherapy: Reduce Me Before I Make You My Partner. Front. Immunol. 2021, 12, 717850.
2. Li, W.; Ng, J.M.-K.; Wong, C.C.; Ng, E.K.W.; Yu, J. Molecular Alterations of Cancer Cell and Tumour Microenvironment in Metastatic Gastric Cancer. Oncogene 2018, 37, 4903–4920.
3. Wu, S.-M.; Lin, W.-Y.; Shen, C.-C.; Pan, H.-C.; Keh-Bin, W.; Chen, Y.-C.; Jan, Y.-J.; Lai, D.-W.; Tang, S.-C.; Tien, H.-R.; et al. Melatonin Set out to ER Stress Signaling Thwarts Epithelial Mesenchymal Transition and Peritoneal Dissemination via Calpain-Mediated C/EBPβ and NFκB Cleavage. J. Pineal Res. 2016, 60, 142–154.

4. Huang, J.; Xiao, D.; Li, G.; Ma, J.; Chen, P.; Yuan, W.; Hou, F.; Ge, J.; Zhong, M.; Tang, Y.; et al. EphA2 Promotes Epithelial-Mesenchymal Transition through the Wnt/β-Catenin Pathway in Gastric Cancer Cells. Oncogene 2014, 33, 2737–2747.
5. Baghban, R.; Roshangar, L.; Jahanban-Esfahlan, R.; Seidi, K.; Ebrahimi-Kalan, A.; Jaymand, M.; Kolahian, S.; Javaheri, T.; Zare, P. Tumor Microenvironment Complexity and Therapeutic Implications at a Glance. Cell Commun. Signal. 2020, 18, 59.
6. Oliver, A.J.; Lau, P.K.H.; Unsworth, A.S.; Loi, S.; Darcy, P.K.; Kershaw, M.H.; Slaney, C.Y. Tissue-Dependent Tumor Microenvironments and Their Impact on Immunotherapy Responses. Front. Immunol. 2018, 9, 70.
7. Hirata, E.; Sahai, E. Tumor Microenvironment and Differential Responses to Therapy. Cold Spring Harb. Perspect Med. 2017, 7, a026781.
8. Balkwill, F.R.; Capasso, M.; Hagemann, T. The Tumor Microenvironment at a Glance. J. Cell Sci. 2012, 125, 5591–5596.
9. Bagchi, S.; Yuan, R.; Engleman, E.G. Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annu. Rev. Pathol. 2021, 16, 223–249.
10. Tavora, B.; Reynolds, L.E.; Batista, S.; Demircioglu, F.; Fernandez, I.; Lechertier, T.; Lees, D.M.; Wong, P.-P.; Alexopoulou, A.; Elia, G.; et al. Endothelial-Cell FAK Targeting Sensitizes Tumours to DNA-Damaging Therapy. Nature 2014, 514, 112–116.
11. Battram, A.M.; Bachiller, M.; Martín-Antonio, B. Senescence in the Development and Response to Cancer with Immunotherapy: A Double-Edged Sword. Int. J. Mol. Sci. 2020, 21, 4346.
12. Bent, E.H.; Gilbert, L.A.; Hemann, M.T. A Senescence Secretory Switch Mediated by PI3K/AKT/MTOR Activation Controls Chemoprotective Endothelial Secretory Responses. Genes Dev. 2016, 30, 1811–1821.
13. Jeong, S.K.; Kim, J.S.; Lee, C.G.; Park, Y.-S.; Kim, S.D.; Yoon, S.O.; Han, D.H.; Lee, K.Y.; Jeong, M.H.; Jo, W.S. Tumor Associated Macrophages Provide the Survival Resistance of Tumor Cells to Hypoxic Microenvironmental Condition through IL-6 Receptor-Mediated Signals. Immunobiology 2017, 222, 55–65.
14. Shree, T.; Olson, O.C.; Elie, B.T.; Kester, J.C.; Garfall, A.L.; Simpson, K.; Bell-McGuinn, K.M.; Zabor, E.C.; Brogi, E.; Joyce, J.A. Macrophages and Cathepsin Proteases Blunt Chemotherapeutic Response in Breast Cancer. Genes Dev. 2011, 25, 2465–2479.
15. Dijkgraaf, E.M.; Heusinkveld, M.; Tummers, B.; Vogelpoel, L.T.C.; Goedemans, R.; Jha, V.; Nortier, J.W.R.; Welters, M.J.P.; Kroep, J.R.; van der Burg, S.H. Chemotherapy Alters Monocyte Differentiation to Favor Generation of Cancer-Supporting M2 Macrophages in the Tumor Microenvironment. Cancer Res. 2013, 73, 2480–2492.
16. Wang, Z.; Tang, Y.; Tan, Y.; Wei, Q.; Yu, W. Cancer-Associated Fibroblasts in Radiotherapy: Challenges and New Opportunities. Cell Commun. Signal 2019, 17, 47.
17. Tommelein, J.; De Vlieghere, E.; Verset, L.; Melsens, E.; Leenders, J.; Descamps, B.; Debucquoy, A.; Vanhove, C.; Pauwels, P.; Gespach, C.P.; et al. Radiotherapy-Activated Cancer-Associated Fibroblasts Promote Tumor Progression through Paracrine IGF1R Activation. Cancer Res. 2018, 78, 659–670.
18. Wen, S.; Hou, Y.; Fu, L.; Xi, L.; Yang, D.; Zhao, M.; Qin, Y.; Sun, K.; Teng, Y.; Liu, M. Cancer-Associated Fibroblast (CAF)-Derived IL32 Promotes Breast Cancer Cell Invasion and Metastasis via Integrin B3-P38 MAPK Signalling. Cancer Lett. 2019, 442, 320–332.
19. Özdemir, B.C.; Pentcheva-Hoang, T.; Carstens, J.L.; Zheng, X.; Wu, C.-C.; Simpson, T.R.; Laklai, H.; Sugimoto, H.; Kahlert, C.; Novitskiy, S.V.; et al. Depletion of Carcinoma-Associated Fibroblasts and Fibrosis Induces Immunosuppression and Accelerates Pancreas Cancer with Reduced Survival. Cancer Cell 2014, 25, 719–734.
20. Bui, J.D.; Schreiber, R.D. Cancer Immunosurveillance, Immunoediting and Inflammation: Independent or Interdependent Processes? Curr. Opin. Immunol. 2007, 19, 203–208.
21. Gupta, A.; Probst, H.C.; Vuong, V.; Landshammer, A.; Muth, S.; Yagita, H.; Schwendener, R.; Pruschy, M.; Knuth, A.; van den Broek, M. Radiotherapy Promotes Tumor-Specific Effector CD8+ T Cells via Dendritic Cell Activation. J. Immunol. 2012, 189, 558–566.
22. Maude, S.L.; Laetsch, T.W.; Buechner, J.; Rives, S.; Boyer, M.; Bittencourt, H.; Bader, P.; Verneris, M.R.; Stefanski, H.E.; Myers, G.D.; et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N. Engl. J. Med. 2018, 378, 439–448.
23. Ortíz-Maldonado, V.; Rives, S.; Castellà, M.; Alonso-Saladrigues, A.; Benítez-Ribas, D.; Caballero-Baños, M.; Baumann, T.; Cid, J.; Garcia-Rey, E.; Llanos, C.; et al. CART19-BE-01: A Multicenter Trial of ARI-0001 Cell Therapy in Patients with CD19+ Relapsed/Refractory Malignancies. Mol. Ther. 2021, 29, 636–644.

24. Perez-Amill, L.; Suñe, G.; Antoñana-Vildosola, A.; Castella, M.; Najjar, A.; Bonet, J.; Fernández-Fuentes, N.; Inogés, S.; López, A.; Bueno, C.; et al. Preclinical Development of a Humanized Chimeric Antigen Receptor against B Cell Maturation Antigen for Multiple Myeloma. Haematologica 2021, 106, 173–184.
25. Castella, M.; Fernández de Larrea, C.; Martín-Antonio, B. Immunotherapy: A Novel Era of Promising Treatments for Multiple Myeloma. Int. J. Mol. Sci. 2018, 19, 3613.
26. Martinez, M.; Moon, E.K. CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front. Immunol. 2019, 10, 128.
27. Raje, N.; Berdeja, J.; Lin, Y.; Siegel, D.; Jagannath, S.; Madduri, D.; Liedtke, M.; Rosenblatt, J.; Maus, M.V.; Turka, A.; et al. Anti-BCMA CAR T-Cell Therapy Bb2121 in Relapsed or Refractory Multiple Myeloma. N. Engl. J. Med. 2019, 380, 1726–1737.
28. García-Ortiz, A.; Rodríguez-García, Y.; Encinas, J.; Maroto-Martín, E.; Castellano, E.; Teixidó, J.; Martínez-López, J. The Role of Tumor Microenvironment in Multiple Myeloma Development and Progression. Cancers 2021, 13, 217.
29. Kumar, S.K.; Rajkumar, V.; Kyle, R.A.; van Duin, M.; Sonneveld, P.; Mateos, M.-V.; Gay, F.; Anderson, K.C. Multiple Myeloma. Nat. Rev. Dis. Primers. 2017, 3, 17046.
30. Bone Marrow Niches in Haematological Malignancies | Nature Reviews Cancer. Available online: https://www.nature.com/articles/s41568-020-0245-2 (accessed on 20 June 2022).
31. Glavey, S.V.; Naba, A.; Manier, S.; Clauser, K.; Tahri, S.; Park, J.; Reagan, M.R.; Moschetta, M.; Mishima, Y.; Gambella, M.; et al. Proteomic Characterization of Human Multiple Myeloma Bone Marrow Extracellular Matrix. Leukemia 2017, 31, 2426–2434.
32. Landowski, T.H.; Olashaw, N.E.; Agrawal, D.; Dalton, W.S. Cell Adhesion-Mediated Drug Resistance (CAM-DR) Is Associated with Activation of NF-KB (RelB/P50) in Myeloma Cells. Oncogene 2003, 22, 2417–2421.
33. Targeting the Bone Marrow Microenvironment in Multiple Myeloma-Kawano-2015-Immunological Review-Wiley Online Library. Available online: https://onlinelibrary.wiley.com/doi/10.1111/imr.12233 (accessed on 23 May 2022).
34. Kouroukis, T.C.; Baldassarre, F.G.; Haynes, A.E.; Imrie, K.; Reece, D.E.; Cheung, M.C. Bortezomib in Multiple Myeloma: Systematic Review and Clinical Considerations. Curr. Oncol. 2014, 21, 573–603.
35. Ghobrial, I.M.; Liu, C.-J.; Zavidij, O.; Azab, A.K.; Baz, R.; Laubach, J.P.; Mishima, Y.; Armand, P.; Munshi, N.C.; Basile, F.; et al. Phase I/II Trial of the CXCR4 Inhibitor Plerixafor in Combination with Bortezomib as a Chemosensitization Strategy in Relapsed/Refractory Multiple Myeloma. Am. J. Hematol. 2019, 94, 1244–1253.
36. Ghobrial, I.M. Myeloma as a Model for the Process of Metastasis: Implications for Therapy. Blood 2012, 120, 20–30.
37. Méndez-Ferrer, S.; Michurina, T.V.; Ferraro, F.; Mazloom, A.R.; MacArthur, B.D.; Lira, S.A.; Scadden, D.T.; Ma'ayan, A.; Enikolopov, G.N.; Frenette, P.S. Mesenchymal and Haematopoietic Stem Cells Form a Unique Bone Marrow Niche. Nature 2010, 466, 829–834.
38. Dazzi, F.; Ramasamy, R.; Glennie, S.; Jones, S.P.; Roberts, I. The Role of Mesenchymal Stem Cells in Haemopoiesis. Blood Rev. 2006, 20, 161–171.
39. Hideshima, T.; Mitsiades, C.; Tonon, G.; Richardson, P.G.; Anderson, K.C. Understanding Multiple Myeloma Pathogenesis in the Bone Marrow to Identify New Therapeutic Targets. Nat. Rev. Cancer 2007, 7, 585–598.
40. Asosingh, K.; Vankerkhove, V.; Riet, I.V.; Camp, B.V.; Vanderkerken, K. Selective in Vivo Growth of Lymphocyte Function- Associated Antigen-1–Positive Murine Myeloma Cells: Involvement of Function-Associated Antigen-1–Mediated Homotypic Cell-Cell Adhesion. Exp. Hematol. 2003, 31, 48–55.
41. Schmidmaier, R.; Mandl-Weber, S.; Gaul, L.; Baumann, P.; Bumeder, I.; Straka, C.; Emmerich, B. Inhibition of Lymphocyte Function Associated Antigen 1 by LFA878 Induces Apoptosis in Multiple Myeloma Cells and Is Associated with Downregulation of the Focal Adhesion Kinase/Phosphatidylinositol 3 Kinase/Akt Pathway. Int. J. Oncol. 2007, 31, 969–976.
42. Harmer, D.; Falank, C.; Reagan, M.R. Interleukin-6 Interweaves the Bone Marrow Microenvironment, Bone Loss, and Multiple Myeloma. Front. Endocrinol. 2019, 9, 788.
43. Hideshima, T.; Bergsagel, P.L.; Kuehl, W.M.; Anderson, K.C. Advances in Biology of Multiple Myeloma: Clinical Applications. Blood 2004, 104, 607–618.
44. Orlowski, R.Z.; Gercheva, L.; Williams, C.; Sutherland, H.; Robak, T.; Masszi, T.; Goranova-Marinova, V.; Dimopoulos, M.A.; Cavenagh, J.D.; Špička, I.; et al. A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study of Siltuximab (Anti-IL-6 MAb) and Bortezomib versus Bortezomib Alone in Patients with Relapsed or Refractory Multiple Myeloma. Am. J. Hematol. 2015, 90, 42–49.

45. Matsuyama, Y.; Nagashima, T.; Honne, K.; Kamata, Y.; Iwamoto, M.; Okazaki, H.; Sato, K.; Ozawa, K.; Minota, S. Successful Treatment of a Patient with Rheumatoid Arthritis and IgA-κ Multiple Myeloma with Tocilizumab. Intern. Med. 2011, 50, 639–642.

46. Corre, J.; Mahtouk, K.; Attal, M.; Gadelorge, M.; Huynh, A.; Fleury-Cappellesso, S.; Danho, C.; Laharrague, P.; Klein, B.; Rème, T.; et al. Bone Marrow Mesenchymal Stem Cells Are Abnormal in Multiple Myeloma. Leukemia 2007, 21, 1079–1088.

47. Zdzisińska, B.; Bojarska-Junak, A.; Dmoszyńska, A.; Kandefer-Szerszeń, M. Abnormal Cytokine Production by Bone Marrow Stromal Cells of Multiple Myeloma Patients in Response to RPMI8226 Myeloma Cells. Arch. Immunol. Ther. Exp. 2008, 56, 207.

48. Deng, M.; Yuan, H.; Peng, H.; Liu, S.; Xiao, X.; Wang, Z.; Zhang, G.; Xiao, H. Mesenchymal Stem Cells Inhibit the Effects of Dexamethasone in Multiple Myeloma Cells. Stem Cells Int. 2022, 2022, e4855517.

49. BAFF and APRIL Protect Myeloma Cells from Apoptosis Induced by Interleukin 6 Deprivation and Dexamethasone | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/103/8/3148/18054/BAFF-and-APRIL-protect-myeloma-cells-from (accessed on 23 May 2022).

50. Expression of BCMA, TACI, and BAFF-R in Multiple Myeloma: A Mechanism for Growth and Survival | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/103/2/689/17829/Expression-of-BCMA-TACI-and-BAFF-R-in-multiple (accessed on 23 May 2022).

51. APRIL and BCMA Promote Human Multiple Myeloma Growth and Immunosuppression in the Bone Marrow Microenvironment | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/127/25/3225/35206/APRIL-and-BCMA-promote-human-multiple-myeloma (accessed on 23 May 2022).

52. Lee, L.; Draper, B.; Chaplin, N.; Philip, B.; Chin, M.; Galas-Filipowicz, D.; Onuoha, S.; Thomas, S.; Baldan, V.; Bughda, R.; et al. An APRIL-Based Chimeric Antigen Receptor for Dual Targeting of BCMA and TACI in Multiple Myeloma. Blood 2018, 131, 746–758.

53. Holthof, L.C.; van der Schans, J.J.; Katsarou, A.; Poels, R.; Gelderloos, A.T.; Drent, E.; van Hal-van Veen, S.E.; Li, F.; Zweegman, S.; van de Donk, N.W.C.J.; et al. Bone Marrow Mesenchymal Stromal Cells Can Render Multiple Myeloma Cells Resistant to Cytotoxic Machinery of CAR T Cells through Inhibition of Apoptosis. Clin. Cancer Res. 2021, 27, 3793–3803.

54. The Role of Tumor Necrosis Factor α in the Pathophysiology of Human Multiple Myeloma: Therapeutic Applications | Oncogene. Available online: https://www.nature.com/articles/1204623 (accessed on 23 May 2022).

55. Tsubaki, M.; Komai, M.; Itoh, T.; Imano, M.; Sakamoto, K.; Shimaoka, H.; Ogawa, N.; Mashimo, K.; Fujiwara, D.; Takeda, T.; et al. Inhibition of the Tumour Necrosis Factor-Alpha Autocrine Loop Enhances the Sensitivity of Multiple Myeloma Cells to Anticancer Drugs. Eur. J. Cancer 2013, 49, 3708–3717.

56. Calip, G.S.; Lee, W.-J.; Lee, T.A.; Schumock, G.T.; Chiu, B.C.-H. Tumor Necrosis Factor-Alpha Inhibitor Medications for Inflammatory Conditions and Incidence of Multiple Myeloma. Blood 2015, 126, 2954.

57. Sebba, A. Tocilizumab: The First Interleukin-6-Receptor Inhibitor. Am. J. Health Syst. Pharm. 2008, 65, 1413–1418.

58. Iyer, S.P.; Beck, J.T.; Stewart, A.K.; Shah, J.; Kelly, K.R.; Isaacs, R.; Bilic, S.; Sen, S.; Munshi, N.C. A Phase IB Multicentre Dose-Determination Study of BHQ880 in Combination with Anti-Myeloma Therapy and Zoledronic Acid in Patients with Relapsed or Refractory Multiple Myeloma and Prior Skeletal-Related Events. Br. J. Haematol. 2014, 167, 366–375.

59. Raje, N.S.; Moreau, P.; Terpos, E.; Benboubker, L.; Grząśko, N.; Holstein, S.A.; Oriol, A.; Huang, S.-Y.; Beksac, M.; Kuliczkowski, K.; et al. Phase 2 Study of Tabalumab, a Human Anti-B-Cell Activating Factor Antibody, with Bortezomib and Dexamethasone in Patients with Previously Treated Multiple Myeloma. Br. J. Haematol. 2017, 176, 783–795.

60. Roodman, G.D. Osteoblast Function in Myeloma. Bone 2011, 48, 135–140.

61. Gau, Y.-C.; Yeh, T.-J.; Hsu, C.-M.; Hsiao, S.Y.; Hsiao, H.-H. Pathogenesis and Treatment of Myeloma-Related Bone Disease. Int. J. Mol. Sci. 2022, 23, 3112.

62. Valentin-Opran, A.; Charhon, S.A.; Meunier, P.J.; Edouard, C.M.; Arlot, M.E. Quantitative Histology of Myeloma-Induced Bone Changes. Br. J. Haematol. 1982, 52, 601–610.

63. Choi, S.J.; Oba, Y.; Gazitt, Y.; Alsina, M.; Cruz, J.; Anderson, J.; Roodman, G.D. Antisense Inhibition of Macrophage Inflammatory Protein 1-α Blocks Bone Destruction in a Model of Myeloma Bone Disease. J. Clin. Investig. 2001, 108, 1833–1841.

64. Abe, M.; Hiura, K.; Wilde, J.; Moriyama, K.; Hashimoto, T.; Ozaki, S.; Wakatsuki, S.; Kosaka, M.; Kido, S.; Inoue, D.; et al. Role for Macrophage Inflammatory Protein (MIP)-1alpha and MIP-1beta in the Development of Osteolytic Lesions in Multiple Myeloma. Blood 2002, 100, 2195–2202.

65. Macrophage Inflammatory Protein-1α Is an Osteoclastogenic Factor in Myeloma That Is Independent of Receptor Activator of Nuclear Factor KB Ligand | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/97/11/3349/107426/Macrophage-inflammatory-protein-1-is-an (accessed on 23 May 2022).

66. Notch-Directed Microenvironment Reprogramming in Myeloma: A Single Path to Multiple Outcomes | Leukemia. Available online: https://www.nature.com/articles/leu20136 (accessed on 23 May 2022).

67. Boyce, B.F.; Xing, L. Functions of RANKL/RANK/OPG in Bone Modeling and Remodeling. Arch. Biochem. Biophys. 2008, 473, 139–146.

68. Michigami, T.; Shimizu, N.; Williams, P.J.; Niewolna, M.; Dallas, S.L.; Mundy, G.R.; Yoneda, T. Cell-Cell Contact between Marrow Stromal Cells and Myeloma Cells via VCAM-1 and Alpha (4) Beta (1)-Integrin Enhances Production of Osteoclast-Stimulating Activity. Blood 2000, 96, 1953–1960.

69. Terpos, E.; Raje, N.; Croucher, P.; Garcia-Sanz, R.; Leleu, X.; Pasteiner, W.; Wang, Y.; Glennane, A.; Canon, J.; Pawlyn, C. Denosumab Compared with Zoledronic Acid on PFS in Multiple Myeloma: Exploratory Results of an International Phase 3 Study. Blood Adv. 2021, 5, 725–736.

70. Huang, S.-Y.; Yoon, S.-S.; Shimizu, K.; Chng, W.J.; Chang, C.-S.; Wong, R.S.-M.; Gao, S.; Wang, Y.; Gordon, S.W.; Glennane, A.; et al. Denosumab Versus Zoledronic Acid in Bone Disease Treatment of Newly Diagnosed Multiple Myeloma: An International, Double-Blind, Randomized Controlled Phase 3 Study—Asian Subgroup Analysis. Adv. Ther. 2020, 37, 3404–3416.

71. Hanley, D.A.; Adachi, J.D.; Bell, A.; Brown, V. Denosumab: Mechanism of Action and Clinical Outcomes. Int. J. Clin. Pract. 2012, 66, 1139–1146.

72. Myeloma Cells Block RUNX2/CBFA1 Activity in Human Bone Marrow Osteoblast Progenitors and Inhibit Osteoblast Formation and Differentiation | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/106/7/2472/21687/Myeloma-cells-block-RUNX2-CBFA1-activity-in-human (accessed on 23 May 2022).

73. Osteoprotegerin Is Bound, Internalized, and Degraded by Multiple Myeloma Cells | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/100/8/3002/106454/Osteoprotegerin-is-bound-internalized-and-degraded (accessed on 23 May 2022).

74. Mori, Y.; Shimizu, N.; Dallas, M.; Niewolna, M.; Story, B.; Williams, P.J.; Mundy, G.R.; Yoneda, T. Anti-A4 Integrin Antibody Suppresses the Development of Multiple Myeloma and Associated Osteoclastic Osteolysis. Blood 2004, 104, 2149–2154.

75. Padmanabhan, S.; Beck, J.T.; Kelly, K.R.; Munshi, N.C.; Dzik-Jurasz, A.; Gangolli, E.; Ettenberg, S.; Miner, K.; Bilic, S.; Whyte, W.; et al. A Phase I/II Study of BHQ880, a Novel Osteoblat Activating, Anti-DKK1 Human Monoclonal Antibody, in Relapsed and Refractory Multiple Myeloma (MM) Patients Treated with Zoledronic Acid (Zol) and Anti-Myeloma Therapy (MM Tx). Blood 2009, 114, 750.

76. Vacca, A.; Ria, R.; Reale, A.; Ribatti, D. Angiogenesis in Multiple Myeloma. Angiogenes. Lymphangiogenes. Clin. Implic. 2014, 99, 180–196.

77. Ribatti, D.; Nico, B.; Vacca, A. Multiple Myeloma as a Model for the Role of Bone Marrow Niches in the Control of Angiogenesis. Int. Rev. Cell Mol. Biol. 2015, 314, 259–282.

78. Mondello, P.; Cuzzocrea, S.; Navarra, M.; Mian, M. Bone Marrow Micro-Environment Is a Crucial Player for Myelomagenesis and Disease Progression. Oncotarget 2017, 8, 20394–20409.

79. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The next Generation. Cell 2011, 144, 646–674.

80. Rajkumar, S.V.; Mesa, R.A.; Fonseca, R.; Schroeder, G.; Plevak, M.F.; Dispenzieri, A.; Lacy, M.Q.; Lust, J.A.; Witzig, T.E.; Gertz, M.A.; et al. Bone Marrow Angiogenesis in 400 Patients with Monoclonal Gammopathy of Undetermined Significance, Multiple Myeloma, and Primary Amyloidosis. Clin. Cancer Res. 2002, 8, 2210–2216.

81. Bhaskar, A.; Tiwary, B.N. Hypoxia Inducible Factor-1 Alpha and Multiple Myeloma. Int. J. Adv. Res. 2016, 4, 706–715.

82. Angiogenic Switch during 5T2MM Murine Myeloma Tumorigenesis: Role of CD45 Heterogeneity | Blood | American Society of Hematology. Available online: https://ashpublications.org/blood/article/103/8/3131/18020/Angiogenic-switch-during-5T2MM-murine-myeloma (accessed on 24 May 2022).

83. Ria, R.; Todoerti, K.; Berardi, S.; Coluccia, A.M.L.; De Luisi, A.; Mattioli, M.; Ronchetti, D.; Morabito, F.; Guarini, A.; Petrucci, M.T.; et al. Gene Expression Profiling of Bone Marrow Endothelial Cells in Patients with Multiple Myeloma.

Clin. Cancer Res. 2009, 15, 5369–5378.

84. Solimando, A.G.; Summa, S.D.; Vacca, A.; Ribatti, D. Cancer-Associated Angiogenesis: The Endothelial Cell as a Checkpoint for Immunological Patrolling. Cancers 2020, 12, 3380.
85. Ribatti, D.; Vacca, A.; Nico, B.; Roncali, L.; Dammacco, F. Postnatal Vasculogenesis. Mech. Dev. 2001, 100, 157–163.
86. Moschetta, M.; Mishima, Y.; Kawano, Y.; Manier, S.; Paiva, B.; Palomera, L.; Aljawai, Y.; Calcinotto, A.; Unitt, C.; Sahin, I.; et al. Targeting Vasculogenesis to Prevent Progression in Multiple Myeloma. Leukemia 2016, 30, 1103–1115.
87. Tenreiro, M.M.; Correia, M.L.; Brito, M.A. Endothelial Progenitor Cells in Multiple Myeloma Neovascularization: A Brick to the Wall. Angiogenesis 2017, 20, 443–462.
88. Reale, A.; Melaccio, A.; Lamanuzzi, A.; Saltarella, I.; Dammacco, F.; Vacca, A.; Ria, R. Functional and Biological Role of Endothelial Precursor Cells in Tumour Progression: A New Potential Therapeutic Target in Haematological Malignancies. Stem. Cells Int. 2015, 2016, e7954580.
89. Sweeney, M.; Foldes, G. It Takes Two: Endothelial-Perivascular Cell Cross-Talk in Vascular Development and Disease. Front. Cardiovasc. Med. 2018, 5, 154.
90. Dankbar, B.; Padró, T.; Leo, R.; Feldmann, B.; Kropff, M.; Mesters, R.M.; Serve, H.; Berdel, W.E.; Kienast, J. Vascular Endothelial Growth Factor and Interleukin-6 in Paracrine Tumor-Stromal Cell Interactions in Multiple Myeloma. Blood 2000, 95, 2630–2636.
91. Ria, R.; Melaccio, A.; Racanelli, V.; Vacca, A. Anti-VEGF Drugs in the Treatment of Multiple Myeloma Patients. J. Clin. Med. 2020, 9, 1765.
92. Somlo, G.; Lashkari, A.; Bellamy, W.; Zimmerman, T.M.; Tuscano, J.M.; O'Donnell, M.R.; Mohrbacher, A.F.; Forman, S.J.; Frankel, P.; Chen, H.X.; et al. Phase II Randomized Trial of Bevacizumab versus Bevacizumab and Thalidomide for Relapsed/Refractory Multiple Myeloma: A California Cancer Consortium Trial. Br. J. Haematol. 2011, 154, 533–535.
93. White, D.; Kassim, A.; Bhaskar, B.; Yi, J.; Wamstad, K.; Paton, V.E. Results from AMBER, a Randomized Phase 2 Study of Bevacizumab and Bortezomib versus Bortezomib in Relapsed or Refractory Multiple Myeloma. Cancer 2013, 119, 339–347.
94. Yordanova, A.; Hose, D.; Neben, K.; Witzens-Harig, M.; Gütgemann, I.; Raab, M.-S.; Moehler, T.; Goldschmidt, H.; Schmidt-Wolf, I.G. Sorafenib in Patients with Refractory or Recurrent Multiple Myeloma. Hematol. Oncol. 2013, 31, 197–200.
95. Srkalovic, G.; Hussein, M.A.; Hoering, A.; Zonder, J.A.; Popplewell, L.L.; Trivedi, H.; Mazzoni, S.; Sexton, R.; Orlowski, R.Z.; Barlogie, B. A Phase II Trial of BAY 43-9006 (Sorafenib) (NSC-724772) in Patients with Relapsing and Resistant Multiple Myeloma: SWOG S0434. Cancer Med. 2014, 3, 1275–1283.
96. Kumar, S.; Witzig, T.E.; Dispenzieri, A.; Lacy, M.Q.; Wellik, L.E.; Fonseca, R.; Lust, J.A.; Gertz, M.A.; Kyle, R.A.; Greipp, P.R.; et al. Effect of Thalidomide Therapy on Bone Marrow Angiogenesis in Multiple Myeloma. Leukemia 2004, 18, 624–627.
97. Terpos, E.; Katodritou, E.; Symeonidis, A.; Zagouri, F.; Gerofotis, A.; Christopoulou, G.; Gavriatopoulou, M.; Christoulas, D.; Ntanasis-Stathopoulos, I.; Kourakli, A.; et al. Effect of Induction Therapy with Lenalidomide, Doxorubicin and Dexamethasone on Bone Remodeling and Angiogenesis in Newly Diagnosed Multiple Myeloma. Int. J. Cancer 2019, 145, 559–568.