The immune microenvironment and immunotherapy of multiple myeloma

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Le microenvironnement immun et les immunothérapies dans le myélome multiple

multiple myeloma, tumour immunity, immunoevasion, T lymphocytes, NK lymphocytes, monoclonal antibodies, chimeric antigen receptor T cells, bispecific antibodies
Myélome multiple, immunité antitumorale, immunoévasion, lymphocytes T, lymphocytes NK, anticorps monoclonaux, cellules T à récepteur antigénique chimérique, anticorps bispécifiques

Abstract

Multiple myeloma evolves from a bone marrow clone of plasma cells and is always preceded by a pre-malignant condition. There is a continuum that ranges from monoclonal gammopathy of unknown significance, to smouldering multiple myeloma, to multiple myeloma (MM). It represents a model of clonal evolution and evolution of the microenvironment, with well-defined clinical stages. The bone marrow microenvironment is constituted of stromal cells, haematopoietic stem cells, osteoblasts, osteoclasts, and immune cells such as B, T and NK lymphocytes, dendritic cells, macrophages and myeloid-derived suppressor cells. The dawn of immunotherapies has highlighted the importance of the immune system in MM oncogenesis. Reactivating the immune system is sufficient to induce a clear response in clinical settings. Several immunotherapies are now essential treatments in MM, such as immunomodulators (thalidomide, lenalidomide, pomalidomide, iberdomide), anti-CD38 (daratumumab and isatuximab), CAR-T cells and bispecific antibodies. In this article we describe the immune dysfunction and the main immunotherapies in MM.

Résumé

Le myélome multiple (MM) se développe au niveau de la moelle osseuse et est toujours précédé de stades précurseurs. En effet, il existe un continuum entre la gammapathie monoclonale de signification indéterminée, le MM asymptomatique, et le MM. Il s'agit là d'un modèle d'évolution défini par des entités cliniques bien individualisées permettant l'étude de l'évolution clonale et du microenvironnement médullaire au cours de la progression de la maladie et ainsi d'en comprendre les étapes phylogéniques. Le microenvironnement médullaire est constitué de cellules stromales, de cellules souches hématopoïétiques, d'ostéoblastes, d'ostéoclastes et de cellules immunitaires dont les lymphocytes T et *natural killer*, les cellules dendritiques, les monocytes et les cellules myéloïdes suppressives. L'avènement des immunothérapies ces dernières années a réaffirmé de façon remarquable l'importance des mécanismes d'immunoévasion dans l'oncogenèse du MM. La réactivation du système immunitaire suffit à éradiquer des cellules tumorales. Plusieurs types d'immunothérapies ont maintenant une place centrale dans le MM, dont les immunomodulateurs (thalidomide, lénalidomide, pomalidomide et iberdomide), les anti-CD38 (daratumumab et isatuximab), les CAR-T cells et les anticorps bispécifiques. Dans cet article, nous allons exposer les dysfonctionnements du système immunitaire et les mécanismes d'action des principales immunothérapies dans le MM.

The role of the immune system in the development of multiple myeloma (MM) is increasingly well characterised and understood. In particular, it is illustrated by the advent of numerous immunotherapies which are under development or already in clinical use. In this review, we discuss the different immunosuppressive mechanisms of MM

To cite this article: Gazeau N, Manier S. The immune microenvironment and immunotherapy of multiple myeloma. *Hématologie* 2021; xx(x): 1-8. doi: 10.1684/hma.2021.1708

doi: 10.1684/hma.2021.1708

as well as the modes of action of immunotherapies and the evolution of the medullar environment as the disease progresses in order to elucidate the phylogenetic stages. The bone marrow microenvironment consists of stromal cells, haematopoietic stem cells, osteoblasts, osteoclasts and immune cells including T and NK (natural killer) lymphocytes, dendritic cells, monocytes and myeloid-derived suppressor cells.

The advent of immunotherapies in recent years has remarkably reaffirmed the importance of immunoevasion mechanisms in the oncogenesis of MM. Reactivation of the immune system is sufficient to eradicate tumour cells. Several types of immunotherapies now play a central role in MM, including immunomodulatory drugs (IMiD) (thalidomide, lenalidomide, pomalidomide and iberdomide), anti-CD38 (daratumumab and isatuximab), chimeric antigen receptor T cells (CAR-T cells) and bispecific antibodies.

In this article, we present the dysfunctions of the immune system and the mechanisms of action of the main immunotherapies in MM.

Dysfunction of the immune system

The immunoevasion necessary for the development of MM is made possible due to dysfunction of the various components of the immune system, rendering the tumour microenvironment immunosuppressive.

T lymphocytes

T lymphocytes (T cells) are cells of adaptive immunity through the presence of their antigen-specific T-cell receptors (TCRs) and are dependent on the major histocompatibility complex (MHC) for their activation (Class I for CD8+ and Class II for CD4 +). Antigen presentation (peptides expressed by MHC molecules) takes place through antigen presenting cells, such as dendritic cells. Costimulation is then essential to protect T cells from anergy or early apoptosis. The co-stimulatory signal occurs by interaction between CD80 or CD86 expressed on the surface of dendritic cells in the lymph node and CD28 expressed on the surface of T cells.

After antigen recognition and activation, CD8+ T cells have cytotoxic activity towards tumour cells. CD4 T cells proliferate. Some of them become effector or helper lymphocytes (T helper, Th), others become T cells with regulatory activity (T regulators). During differentiation, some of the CD4+ and CD8+ T cells become memory T cells with a capacity for prolonged life and rapid expansion upon re-exposure to the given antigen.

Once the immune response is complete, the number of specific T cells decreases, due to the disappearance of the antigen but also due to regulatory mechanisms, involving CTLA-4 (cytotoxic T lymphocyte associated antigen-4), or the programmed cell death-1 inhibiting molecules (PD-1) and T-cell immunoglobulin and immunoreceptor tyrosine based inhibitory motif domains (TIGIT). TIGITs play an important regulatory role in preventing overactivation of the T cell immune response.

Several levels of evidence demonstrate that immune escape from myeloma cells occurs at all stages of T cell activation:

– at the stage of monoclonal gammopathy of undetermined significance (MGUS), the CD4/CD8 ratio is reduced, and this phenomenon increases as the disease progresses to MM,

– *in vitro* studies have shown that the stimulation of tumour-specific T-cell development leads to apoptosis of abnormal plasma cells, thus demonstrating the importance of the cytotoxic effect of CD8+ T-cells in this disease and especially of immunoescape as a means of defence in MM,

– a decrease in the expression of CD80 (a molecule present on tumour plasmacytes) induces the inhibition of CD28 (a costimulatory molecule expressed

by T lymphocytes, and which allows its activation). This reduction in expression leads to T-cell anergy,
– MM cells always express CD86 which interacts with CTLA-4 (in addition to CD28), upon presentation of tumour antigen by the dendritic cell to the naive T cell, thus inhibiting its transformation into an effector T cell,
– similarly, the interaction between PD-1 and its ligand, PD-L1, is also involved in the immunoevasion of MM, representing a negative feedback system of T cells. An increase in PD-L1 levels in tumour plasma cells occurs, leading to T cell inhibition in the effector phase,
– an abnormal increase of the TIGIT receptor on T cells from MM patients was observed. This is another system that inhibits the T cell response. Monoclonal antibodies directed against TIGIT allow a reactivation of T lymphocytes and a parallel decrease in tumour development *in vivo*,
– finally, an increase in the development of regulatory T cells by tumour cells has been identified as a tumour escape phenomenon *in vitro*.

Natural killer lymphocytes

NK cells are part of innate immunity and–unlike T cells–are independent of antigen presentation. Their action is dependent on activation and inhibition signals conditioned by tumour cells, cytokines or viral or bacterial antigens. The main inhibitory receptors are represented by the killer immunoglobulin-like receptors (KIRs) and NKG2A; activation receptors by CD16 and NKG2D, among others.
Disrupting this activation/inhibition balance is a mechanism of tumour escape from MM.
One of the most important inhibitory signals to the NK lymphocyte is the interaction between the histocompatibility molecule HLA-E, which has a primary function in its regulation, and NKG2A, a membrane receptor of the NK lymphocyte. About 40% of the population expresses HLA-E strongly. Studies have shown that in individuals with a high level of HLA-E, progression-free survival is lower than in those with a low level.
Cytokine production (interleukin [IL]-6, IL-10, prostaglandin E2) by myeloma cells, regulatory T cells (Treg) or bone marrow stromal cells induces under-expression of NK cell activating receptors via NKG2D and CD16, and thus their inhibition.

Dendritic cells

Dendritic cells are antigen-presenting cells that form a link between innate and adaptive immunity. They are derived from a haematopoietic progenitor which, during differentiation in the bone marrow, shifts to either the monocytic or the dendritic lineage.
High levels of IL-6 in MM prevent the genesis of dendritic cells and their normal function. This phenomenon also stimulates CD34+ cells and influences their differentiation into monocytes–which, unlike dendritic cells, do not have the function of presenting tumour antigens to T cells.
It has been shown that excess IL-6 prevents dendritic cells from presenting the tumour epitope for lymphocyte activation. A vaccine comprising dendritic cells engineered not to express the IL-6 receptor induced increased production of cytotoxic CD8 T cells and improved progression-free survival in a mouse model.

Macrophages

Macrophages are phagocytic cells capable of capturing elements of various sizes (antigens, microbial agents, cells or cellular debris) before destroying them and presenting them to the cells of adaptive immunity. They also produce many important cytokines at all stages of the immune response.

They activate as M1 or M2 macrophages. M1 macrophages have a pro-inflammatory activity with the production of tumour necrosis factor (TNF-α) and IL-12 which, via an antiangiogenic mechanism, prevents the tumour from growing. The macrophages active in neoplasia correspond to M2, which have immunosuppressive activity, notably by increasing the level of PD-L1 or by activating angiogenesis, which favours tumour progression.
Tumour plasma cells in MM produce cytokines (including PGE2) that promote macrophage polarisation into the M2 phenotype.
The predominance of M2 macrophages is directly related to resistance to daratumumab and IMiDs. Strategies are therefore being developed to restore the polarisation of M1 macrophages and restore a response to treatment.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of cells of immature myeloid origin with the property of inhibiting the T cell response. MDSCs develop rapidly during infection or inflammation in order to avoid hyperactivation of the T response and uncontrolled cytokine release. The level of MDSC is about five times higher in MM at diagnosis than in healthy control subjects.

Immunotherapies in multiple myeloma (*table 1*)

Many MM treatments, including new generations of therapies, have an immunomodulatory effect, including IMiDs, monoclonal antibodies, conjugated antibodies, bispecific antibodies and CAR-T cells.

Table 1

Immunotherapies used in the management of multiple myeloma.

Therapeutic class	Drug	Phase of development
IMiD	Lenalidomide	Marketing authorisation from first line
	Pomalidomide	Marketing authorisation as second line
	Iberdomide	Phase 2, CC-220-MM001
Anti-CD38	Daratumumab	Marketing authorisation from first line
	Isatuximab	ATI Cohort
	TAK-079	Phase 1
ADC	Belantamab	Phase III
	CC-93269	Phase 2
Bispecific BCMA-CD3	JNJ-64007957	Phase 1
	AMG-701	Phase 1
CAR-T cells BCMA	bb2121	Phase 3, KarMMa-3
	JNJ4528	Phase 3, Cartitude-4

IMiD: immunomodulatory drugs; ADC: antibody drug conjugate.

The choice of target for these immunotherapies is crucial, since it must be sufficiently specific to tumour cells to avoid side effects on healthy cells, and also sufficiently expressed in tumour cells to allow maximum efficacy. The main targets in MM are represented by CD38, BCMA, CS1, and GPRC5D, among others.
The side effects are both dependent on the target cell–e.g. hypogammaglobulinaemia when destroying plasma cells–and related to immune system activation, including cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) related to hypersecretion of lL-6 and lL-1, respectively.

Immunomodulators

IMiDs such as thalidomide, lenalidomide, pomalidomide and iberdomide have both direct antitumour and immunomodulatory actions. Their recently discovered mechanism of action involves the degradation of Ikaros zinc finger protein 1 and 3 (IKZFl and IKZF3) by hijacking the ubiquitin-proteasome system. Indeed, these compounds bind to both cereblon (CRBN), which is a component of a ubiquitin ligase (CRL4crbn), and to IKZF1 and IKZF3. This leads to their ubiquitination and degradation. IKZF1 and IKZF3 are transcription factors involved in the cell differentiation of B lymphopoiesis. They regulate genes such as interferon regulatory factor 4 (*IRF4*) and *MYC*, which allow the differentiation of plasma cells. Their degradation induces the apoptosis of tumour cells.
Furthermore, IKZF1 and IKZF3 are also expressed in T cells, where they repress 1L-2 expression. Degradation of IKZFl and 1KZF3 by IMiD at the T cell level induces increased secretion of 1L-2 and activation of CD8+ T lymphocytes.
IMiDs are now part of the treatment of MM at all stages of the disease, in combination with proteasome inhibitors and synergistically with monoclonal antibodies, and, perhaps in the future, bispecific antibodies or CAR-T cells.

Monoclonal antibodies

Monoclonal antibodies recognise specific antigens on tumour plasma cells, thus generating an immune response against the tumour.

Anti-CD38

Daratumumab and isatuximab are the main monoclonal antibodies targeting CD38. Daratumumab is a human antibody and isatuximab is a chimeric murine-human antibody. Their mechanism of action involves antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). However, daratumumab has also been shown to induce depletion of regulatory T cells (Treg), which is responsible for the removal of effector cell inhibition. This would probably explain the synergy between anti-CD38 and IMiDs seen in clinical practice. Phase 3 studies of daratumumab in combination with lenalidomide and dexamethasone (POLLUX) or bortezomib and dexamethasone (CASTOR) have led to its approval for patients in relapse. The first-line trials in patients ineligible for autograft, ALYCONE (daratumumab, bortezomib, melphalan and prednisone) and MAIA (daratumumab, lenalidomide and dexamethasone) have resulted in the approval of daratumumab as first-line therapy. In young subjects, the CASS1OPEA trial showed efficacy of D-VTD in induction and consolidation of autograft.

Elotuzumab

Elotuzumab is a humanised anti-CS1 monoclonal antibody (also called SLAMF7). Its use as a single agent has not shown efficacy, but its combination with lenalidomide has led to its approval in Europe (ELOQUENT-2 trials). However, this drug has not been granted marketing authorisation in France.

Conjugated antibodies

Belantamab mafodotin is a monoclonal antibody targeting BCMA and conjugated to a tubulin inhibitor, monomethyl auristatin F (mafodotin). The Phase 2 DREAMM 2 study reported an overall response rate of 31% at 2.5 mg/kg in patients refractory to an IMiD, a proteasome inhibitor and an anti-CD38. Ocular toxicity, with corneal deposits, limits its use to higher doses. This drug is currently available under a temporary authorisation for use (*autorisation temporaire d'utilisation,* ATU) in France.

Bispecific antibodies

Bispecific antibodies combine the specificity of two antibodies: one targets a T-cell epitope, CD3, the other an epitope of the tumour cell. Through this double specificity, bispecific antibodies recreate the immunological synapse allowing the activation of T lymphocytes against tumour cells. There are two types of bispecific mAbs: the so-called immunoglobulin G (IgG)-like, consisting of a complete immunoglobulin (variable part and fragment crystallizable region [Fc]) and the non-IgG-like, consisting of a fragment of immunoglobulin. The former have a longer half-life and show ADCC activity, through their Fc component (except in case of FcRg inactivation); the latter have a very short half-life, requiring their continuous administration, however, they may offer better tissue penetration. The preferential target of bispecific antibodies in MM is B-cell maturation antigen (BCMA), a member of the family of TNF receptors, expressed in almost 100% of cases of MM. BCMA is essential for the differentiation of B-cells into plasma cells and their proliferation. The first product in this class was AMG420, a bispecific non-1gG-like antibody, targeting BCMA and CD3. A Phase I dose escalation study demonstrated remarkable efficacy in 10 patients treated at the effective, non-toxic dose of 400 mg/24 h, with an overall response rate of 70%, including 50% of patients with negative minimal residual disease. However, the company decided to stop its development because of the requirement for continuous administration of the drug. A second bispecific antibiotic targeting BCMA and CD3 was introduced: CC93269. This is a bispecific IgG-like antibiotic with FcRg modification, bivalent for BCMA and monovalent for CD3 (called 2 + 1). The Phase I dose escalation study reported remarkable efficacy, with nearly 90% overall response with 6 mg and 10 mg, including 80% very good partial response or better. Tolerance was marked by the occurrence of CRS in all patients who received the effective doses of 6 mg or 10 mg, which may require the use of a ramp-up strategy at treatment initiation.

T cells with chimeric antigen receptor

CAR-T cells are T cells which are modified ex vivo to express a chimeric receptor with a single-chain variable fragment (scfv) of immunoglobulin combined with CD3 and possibly a costimulatory domain, namely CD28 and/or 4-1BB. Their production is complex with a first step of leukapheresis, followed by activation of the patient's T lymphocytes and their transduction by a lentivirus or a retrovirus containing the plasmid of interest, before freezing. Lymphodepletion is then performed, usually with fludarabine and cyclophosphamide, before reinjection into the cells.

The side effects of CAR-T cells are mainly marked by:

– cytokine release syndrome (CRS), resulting from inappropriate IL-6 secretion and often requiring the administration of tocilizumab (anti-IL-6R Ac) or even corticoids,

– neurological toxicity or immune effector cell-associated neurotoxicity syndrome (ICANS), mainly linked to IL-1 over-expression and requiring the administration of corticoids or even anakinra (anti-IL-1R),

– macrophage activation syndrome related to a hyperinflammatory state, similar to CRS,
– cytopenias as a consequence of both the cytokine storm and the targeting of haematopoietic progenitors by CAR-T cells.

As with bispecific antibodies, the target of interest in MM is BCMA, although the efficacy of CAR-T cells targeting CS1 or GPRC5D is being investigated in some studies. bb2121 CAR-T cell therapy is the most advanced and targets BCMA and contains 4-1BB as a costimulatory domain. The Phase 1 dose escalation trial retained the effective, non-toxic dose of 450 million cells. For patients who received at least 150 million cells, the overall response rate was 95% and the median progression-free survival was 11.8 months with a median of 17.7 months for patients achieving undetectable residual disease. The Phase 2 trial, called KarMMa, performed in patients with refractory relapse and at least three prior lines of treatment, achieved an overall response rate of 81% with a progression-free survival of 11.3 months. JNJ-4528 is another CAR-T cell therapy, targeting two epitopes of BCMA, with 4-1BB as a costimulatory domain. It was initially developed by our Chinese colleagues in the Legend-2 trial, a Phase 1 study of relapsed patients who had received at least three lines of treatment; these patients were, however, somewhat less heavily treated than in the previous trials. The overall response rate was 88%, with 68% negative minimal residual disease, associated with a progression-free survival of 19.9 months for the total cohort and 28.2 months for patients with a complete response. The Cartitude-1 Phase 1b/2 trial, using the same CAR-T JNJ-4528 therapy, showed an overall response rate of 100%, with no survival data yet.
The mechanisms of resistance to CAR-T cells in patients with MM are still poorly understood. Loss of BCMA expression does not appear to be common. In the bb2121 trials, peak CAR expansion correlated with response, but this was not confirmed in Cartitude-1, nor was there confirmation of CAR-T cell persistence. There are several levels of evidence that the quality of the T cells collected at the time of leukapheresis affects the response, including their memory character as opposed to a depleted phenotype. This suggests earlier use, before patients have received multiple lines of treatment. Other strategies are evaluating the value of using drugs to enrich the sample with memory T cells at the time of CAR-T manufacture, such as the phosphoinositide 3-kinase (PI3K) inhibitors used in the bb21217 trial.

Conclusion

Immunotherapies have changed the management of MM in recent years. However, a paradox remains, namely very high response rates can be achieved with notably undetectable residual disease, and yet, nonetheless, patients relapse. The hypotheses of the emergence of resistance clones or the appearance of an immunosuppressive microenvironment remain to be explored. Understanding the mechanisms of resistance to immunotherapies will be an important step towards their improvement.

Conflicts of interest: the authors report no conflicts of interest in relation to this article.]

For more information

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Question à l'auteur

Q1 Merci de nous transmettre le titre de la rubrique.