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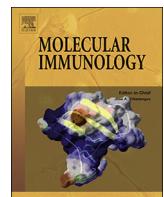
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Checkpoint inhibition in the treatment of multiple myeloma: A way to boost innate-like T cell anti-tumor function?



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ABSTRACT

Multiple myeloma (MM) is a progressive monoclonal B cell malignancy, for which survival and progression largely relies on the crosstalk of tumor cells with the bone marrow (BM) microenvironment, inducing immune escape, angiogenesis, bone destruction and drug resistance. Despite great therapeutic advances, most of the MM patients still relapse and remain incurable. Over the past years, immunotherapy has emerged as a new field in cancer therapy. Here, the immune cells of the patients themselves are activated to target the tumor cells. In MM, several effector cells of the immune system are present in the BM microenvironment; unfortunately, they are mostly all functionally impaired. In this review, we focus on the role of innate-like T cells in MM, particularly CD1d- and MR1-restricted T cells such as respectively invariant natural killer T (iNKT) cells and mucosal associated invariant T (MAIT) cells. These cells have the capacity upon activation to rapidly release copious amounts of cytokines affecting a wide range of innate and adaptive immune responses, and could therefore play a key protective role in anti-tumor immunity. We describe recent observations with regard to functional exhaustion of iNKT and MAIT cells in MM pathology and discuss the potential application of checkpoint inhibition as an attractive target for prolonged activation of these immunomodulatory T cells in the treatment of MM.

1. Multiple myeloma

Multiple Myeloma (MM), also known as Kahler's disease, is an incurable monoclonal B cell malignancy that develops in the bone marrow (BM) (Caers et al., 2008). MM accounts for approximately 1% of all cancers and is the second most prevalent hematologic malignancy after non-Hodgkin lymphoma (Ferlay et al., 2013; Turesson et al., 2010). This uncontrolled accumulation of terminally differentiated plasma cells in the BM is characterized by an overproduction of monoclonal antibodies (mAb) in the serum and/or urine, also known as M-proteins or paraproteins. Immunoglobulins (Ig) G and Ig A are the two most common types of paraproteins detected in MM patients. Moreover, other major clinical manifestations such as bone lesions, kidney failure, anemia, hypercalcemia and a high risk of infections further typically characterize this disease (Caers et al., 2008; Raab et al., 2009). More common in men than in women, MM is twice more present in the African-American populations compared to Caucasians. However, the reason of this uneven race and sex distribution is still

unknown (Landgren and Weiss, 2009). In Europe, the MM incidence is 4.5–6.0 per 100.000 each year with a mortality rate at 4.1 per 100.000. The median age at diagnosis is situated between 60–65 years (Moreau et al., 2013). Also obese people (BMI > 30), people with the Acquired immune-Deficiency Syndrome (AIDS) and persons with a familial history of lympho-hematopoietic cancers appear to be more prone to develop MM (Birmann et al., 2007; Pouli et al., 2001; Lynch et al., 2008).

Almost all of the MM patients evolve from an asymptomatic pre-malignant stage called monoclonal gammopathy of undetermined significance (MGUS), which is present in around 3% of the population above 50. The progress to the MM stage happens at a rate of 1% per year. MGUS is an asymptomatic condition where less than 10% clonal plasma cells are found in the BM, where paraproteins can be detected in the blood and/or the urine (< 3 g/dL) but in absence of any end-organ damage and further clinical signs of MM (Landgren and Weiss, 2009). In certain patients, a more advanced intermediate stage, named smoldering multiple myeloma (sMM) can be observed (Rajkumar et al., 2015). The risk of progression from sMM to symptomatic MM is 10%

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per year and is influenced by the underlying cytogenetic type of the disease. Patients with t(4;14) translocation, 17p deletion, and 1q amplification appear to have a higher risk of progression from sMM to MM (Rajan and Rajkumar, 2015).

2. Immune therapy in multiple myeloma

Treatment of multiple myeloma changed considerably over the last two decades with the introduction of novel therapeutic agents such as proteasome inhibitors, autologous stem cell transplantation (ASCT) and immunomodulatory drugs, as well as combination therapies, significantly improving the survival outcomes for patients (Caers et al., 2008; Ferlay et al., 2013). However few MM patients remain in long-term remission, and the majority still relapses and eventually dies from the disease, usually due to a resistant clone. It is well established today that MM is associated with a progressive immune dysregulation, resulting in a loss of immunosurveillance and subsequently promoting tolerance and tumor progression (Ferlay et al., 2013). Although MM is primarily a disease of the B cell lineage, the T cell compartment is also strongly affected (Tuesson et al., 2010). A significant loss in CD4 and CD8 T cell subsets is reported, while on the other hand a recruitment and a concomitant rise in suppressor cells, including Treg cells, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSC) is observed (Raab et al., 2009).

MM immune dysregulation also disturbs other aspects of the immune system as well, directly affecting antigen presentation and the upregulation of inhibitory antigens (checkpoint molecules, see below) that promote immune escape and growth advantage for malignant clones (Rajan and Rajkumar, 2015; Shapiro-shelef and Calame, 2004). Therefore, there is a crucial need to continue preclinical research and to find new therapeutic strategies to target the immune system in the treatment for MM. Currently, immunotherapy with the goal to boost the patient's own immune system to combat malignancy, is revolutionizing the landscape of cancer treatment as it has the potential to improve patient outcomes significantly. The success is based on progress in both preclinical and clinical science, and this has led to the development of new immunotherapeutic strategies already being approved or under clinical evaluation. Most relevant immune-targeting strategies in MM include Immunomodulatory drugs (IMiDs) such as thalidomide and its less toxic analogues lenalidomide and pomalidomide (Engelhardt et al., 2014), vaccines (peptide-based (Zhou et al., 2010), Idiotype-based (Reihardt et al., 1999; Yi et al., 2010), DC/MM fusion vaccines (Rosenblatt et al., 2013) and ITriMix-DC vaccines (Benteyn et al., 2012; De Keersmaecker et al., 2014)), antibody therapies such as Anti-CS-1 (SLAMF7) antibody (elotuzumab) (Lonial et al., 2015) and Anti-CD38 antibody (Daratumumab) (Lonial et al., 2016) and Chimeric antigen receptor (CAR) T cell based therapy (Atanackovic et al., 2016; Gay et al., 2017).

However, one of the most encouraging approaches to activate therapeutic anti-tumor immunity is the blockade of immune checkpoints (Melero et al., 2015). Immune checkpoint proteins, such as programmed death 1 (PD-1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), are inhibitory molecules that are essential for maintaining self-tolerance and for modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to prevent collateral tissue damage (Farkona et al., 2016). So they maintain immune equilibrium in health but unfortunately are also upregulated in the presence of malignancy, fostering a state of immune tolerance towards tumor cells. The upregulation of these negative costimulatory (co-inhibitory) signals induces an anergic state of T cells, blunting T cell activation and expansion, and blocking T cell-mediated killing of cancer cells (Hamanishi et al., 2016). PD-1 ligands, PD-L1 or PD-L2, are frequently expressed on tumor cells and can further minimize the immune response. Remarkable results with different PD-1 (nivolumab, pidilizumab and pembrolizumab) and PD-L1 (atezolizumab and avelumab) inhibitors indicated promising clinical efficacy

and a well-tolerated toxicity profile in various cancer patients, including those with melanoma, non-small cell lung cancer, head and neck cancer or kidney cancers (Cheah et al., 2015; Topalian et al., 2015; Patnaik et al., 2015). Also, in hematological malignancies immune checkpoint blockade looks like a promising strategy to revert anergy of the immune system (Armand, 2015). PD-L1 is expressed in a variety of hematological malignancies such as leukemia and peripheral T-cell lymphoma and has been correlated to poor prognosis (Shi et al., 2013; Kiyasu et al., 2015; Miyoshi et al., 2016). With regard to MM, studies have demonstrated the expression of PD-L1 (which is normally not expressed on plasma cells) on MM cells and immune cells and expression of PD-1 on different T cell subsets and natural killer cells within the MM microenvironment (Yousef et al., 2015; Zelle-Rieser et al., 2016; Benson et al., 2010). Preclinical work showed anti-myeloma activity by inhibiting PD-1/PD-L1 signaling (Hallett et al., 2011). Unfortunately, up to this moment, results from clinical trials remain unsatisfactory (Armand, 2015). This might indicate that monotherapy treatment with PD-1/PD-L1 inhibitors in MM does not show the same efficacy as for some solid tumors or Hodgkin lymphoma, suggesting that a combination approach is needed (Jelinek and Hajek, 2016). Currently, a phase II trial is ongoing to assess efficacy of a DC/tumor vaccine in combination with anti-PD-1 mAb CT-11 (pidilizumab) following ASCT (Karp et al., 2015). Multiple other studies using different anti-PD-1 antibodies have been initialized in advanced MM patients, including pembrolizumab with pomalidomide, following lenalidomide/dexamethasone or ASCT (for more details, see reviews (Gay et al., 2017; Bianchi et al., 2015; Atanackovic et al., 2017) and ClinicalTrials.gov).

Blockade of the CTLA-4 and PD-1/PD-L1 pathways only represent the tip of the iceberg in the realm of potential immune checkpoint targets. Ongoing studies of the regulation of immune responses have identified other checkpoint pathways and molecules such as lymphocyte activation gene 3 protein (LAG-3), T cell immunoglobulin mucin receptor 3 (TIM-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT) and V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA) (Anderson et al., 2012; Wang et al., 2011). It is possible that these molecules are co-expressed on tumor-infiltrating lymphocytes. Therefore, combination therapy targeting different checkpoint molecules might be a promising strategy (Jing et al., 2015).

3. NKT and MAIT cell function is exhausted in multiple myeloma

Invariant Natural Killer T cells (iNKT) and Mucosal associated invariant T (MAIT) cells are two important subsets of unconventional, innate-like T cells (Salio et al., 2014). Both express a semi-invariant TCR and show antigen restriction towards non-polymorphic MHC-like molecules (CD1d and MR1, respectively) by which they can respond to microbial derived products (glycolipid molecules and vitamin B2 metabolites, respectively). Their specificity is mainly conserved between species but numeric differences exist. In mice, MAIT cells are 5 to 10-fold less abundant compared to frequencies in man and the opposite seems true for iNKT cells (Godfrey et al., 2015). For an extended view on their development and phenotypical/functional features we refer to recent reviews related to this topic (Nair and Dhodapkar, 2017; Godfrey et al., 2018; Teyton, 2017).

iNKT cells are capable of maturing DCs and activating B cells, and thus are crucial in enhancing antigen-specific B- and T-cell responses (McEwen-Smith et al., 2015). Their capacity to modify the immune microenvironment influences the capability of the host to control tumor growth, making them an interesting population to be harnessed in the clinic for the development of anti-cancer therapeutics. Indeed, one of the advantages of iNKT cell-targeted anti-tumor immunotherapy is that, compared with current anti-cancer immunotherapies (that only target one arm of the immune system), a variety of anti-tumor immune effector cells, such as NK cells, B cells, neutrophils, DCs and cytotoxic and helper T cells, can be targeted simultaneously. In addition, other benefits of iNKT cell-targeted anti-tumor immunotherapy over a more

traditional T cell-based immunotherapy is the competency of iNKT cells to target both MHC-negative and MHC-positive cancer cells and the expected lack of allogeneic responses against host MHC (Fujii et al., 2013). Remarkably, in patients showing solid malignancies or hematological malignancies (including MM), reduced iNKT cell numbers in circulation have been observed, further underscoring a role of iNKT cells in tumor immunosurveillance (Dhopakar et al., 2003; Crough et al., 2004; Tahir et al., 2001). Next to iNKT cells, we discovered that MAIT cells are numerically and functionally impaired in MM as well (Favreau et al., 2017a), an observation recently confirmed by others (Gherardin et al., 2018). Moreover, MAIT cells were capable of targeting and killing myeloma cell lines pulsed with the MR1 restricted ligand 5-OP-RU, highlighting the anti-tumor potential of these cells in MM (Gherardin et al., 2018).

Several iNKT cell-directed therapeutic options have been explored thus far in the clinic, including administration of iNKT cell-activating ligands and administration of antigen presenting cells (APCs) pulsed with α -GalCer or the transfer of ex vivo-expanded and/or activated iNKT cells (McEwen-Smith et al., 2015; Favreau et al., 2016). Of note, clinical studies described to date have used the prototypical iNKT ligand α -galactosylceramide (α -GalCer) for iNKT cell stimulation, mainly because of the strong adjuvant-like activity. Synthetic α -GalCer analogues inducing more skewed NKT1 (IFN- γ) responses have been tested successfully in animal models (e.g. (Aspeslagh et al., 2011, 2013)) but they still await clinical validation.

Although generally well tolerated (with no serious side effects), results with α -GalCer based therapies remain disappointing and far from effective. This is understandable since iNKT cell-based immunotherapies are associated with some challenges (Dhopakar and Richter, 2011). For example, there is quite a large range of peripheral blood circulating iNKT cell numbers between individuals (for which a clear explanation still remains to be found) and this possibly contributes to the heterogeneity in clinical responses (Bendelac et al., 2007; Kronenberg, 2005). Although it is known that low numbers of iNKT cells are associated with poor prognosis in autoimmune or malignant diseases, it is unknown if high or low numbers of iNKT cells are an important biomarker for the outcome of an iNKT cell-based therapy (Dhopakar and Richter, 2011; Najera Chuc et al., 2012; Molling et al., 2007). Studies have shown that the use of α -GalCer results in iNKT cell over-activation and an exhausted functional state (Parekh et al., 2009, 2005; Singh et al., 2014; Sullivan and Kronenberg, 2005). We found that iNKT cells in blood as well as bone marrow samples from MM patients (as compared to healthy subjects) show a marked increase in PD-1 expression (Favreau et al., 2017a). Moreover, the use of an anti-PD-1 checkpoint inhibitor in a MM animal model (5T33MM) restored iNKT anti-tumor properties and subsequently combining PD-1 blockade with α -GalCer stimulation led to a superior anti-MM effect. Interestingly, we also found elevated PD1 levels on MAIT cells from MM patients (Favreau et al., 2017a). Blocking PD-1 together with iNKT cell stimulation partially restored the MAIT cell functionality. The exact nature and role of a MAIT-NKT crosstalk remain unknown and certainly needs further investigation. Indeed, a dysregulation of MAIT cells in cancer and especially in non-mucosa-associated cancer types is gaining interest in the field (Ling et al., 2016; Won et al., 2016). Still, it has to be addressed whether MAIT cells show clear (direct) anti-tumor activity in vivo, potentially with a specific role for certain MAIT cell subsets (Kurioka et al., 2017), or whether they mainly modulate other immune cells to induce anti-tumor responses.

4. Leptin receptor signaling: a new check-point inhibitor to boost iNKT cell function in MM?

The BM is the perfect microenvironment where various cross-talks occur between MM cells and the different BM compartments. Close interactions with the cellular components of the BM, including endothelial cells, osteoblasts, osteoclasts, adipocytes and immune cells,

confer survival and growth to MM cells and induce angiogenesis, bone destruction, drug resistance and immune escape, ultimately leading to relapse (Lemaire et al., 2011). Remarkably, fatty deposits can occupy up to 70% of the BM cavity with aging (Caers et al., 2007), yet surprisingly little attention has been given to the role of BM adipocytes in MM development. Clinical studies showed an association between BM adipocytes and an increased risk of MM, but specific biological mechanisms still have to be elucidated (Vogl et al., 2012; Liu et al., 2015). It has been demonstrated that adipocytes isolated from femoral BM biopsies supported MM cell proliferation and migration. When MM cells invade the BM (showing a diffuse growth pattern), BM adipocytes disappear during disease development, suggesting a role of BM adipocytes at the initial stage of the disease, before full remodeling of the BM microenvironment has occurred (Caers et al., 2007). BM adipocytes may support MM oncogenesis by stimulating migration and proliferation of malignant plasma cells through secretion of myeloma-supportive adipokines such as leptin. Interestingly, leptin is known to play a crucial role in energy homeostasis but it also displays immunomodulatory properties (Matarese et al., 2018). This led us to investigate whether there was a crosstalk between BM adipose tissue and iNKT cells mediated by leptin, and its potential role in the onset of MM. Our results showed a marked and progressive increase in leptin levels and upregulated leptin receptor (LR) expression levels on iNKT cells during multiple myeloma progression, both in human disease (as compared to BMI and age-matched healthy donors and MGUS patients) and the 5T33 myeloma animal model (Favreau et al., 2017b). MM cells and leptin synergistically counteracted the anti-tumor function of both murine and human iNKT cells in vitro, and in vivo blockade of LR signalling in combination with iNKT stimulation resulted in superior tumor protection. The effects of LR blockade on the α GalCer mediated protection in MM development were lacking in the CD1d-/- myeloma mice, underscoring a major role for leptin in modulating iNKT cell dependent anti-myeloma effects. Additionally, in the presence of leptin receptor antagonism repeated α -GalCer administration in myeloma mice did not lead to a profound iNKT cell anergy, using persistent IFNy secretion as a hallmark of NKT reactivity. A partial rescue of iNKT cell functionality could also be observed in non-diseased mice. Moreover, by means of intravital dual-photon microscopy in Cxcr6^{GFP/+} mice we could demonstrate a partial restoration of liver iNKT cell dynamics in vivo upon leptin antagonism, in contrast to the unresponsiveness observed after repeated α -GalCer injections.

Taken together, these findings suggest that the leptin-leptin receptor axis acts like an immune checkpoint barrier (specifically with regard to iNKT cell function), enhancing immune suppression and evasion in pathologic conditions with elevated leptin/ leptin receptor levels, such as detected in MM (Fig. 1). Of note, it is currently unknown whether this is also true for other innate-like T cells such as MAIT cells. Leptin could affect MM progression in two ways. On the one hand, it might directly promote cancer cell growth, migration and invasion (Moreira et al., 2015; Garofalo and Surmacz, 2006). Patients with weak leptin receptor expression tend to have a longer progression-free survival compared to patients with strong leptin expression (Alexandrakis et al., 2004). And Yu et al. recently demonstrated that upregulated leptin could stimulate proliferation of MM and reduce the anti-tumor effect of chemotherapy possibly via the activation of AKT and STAT3 pathways (Yu et al., 2016). On the other hand, leptin can counteract anti-tumor immunity by contributing to the iNKT cell anergy, as we have shown (Favreau et al., 2017b).

Our work unmasks a previously unknown link between leptin and iNKT cells in the context of MM immunosuppression. It appears that the large accumulation of adipocytes in the aging BM might play a key role in suppressing anti-tumor immunity. These data suggest that leptin receptor antagonism might work as a new checkpoint inhibitor, an interesting strategy for iNKT based immunotherapy in MM. Moreover, this could also be relevant in the treatment of a large variety of other cancers such as breast, pancreas, lung, thyroid, endometrial and

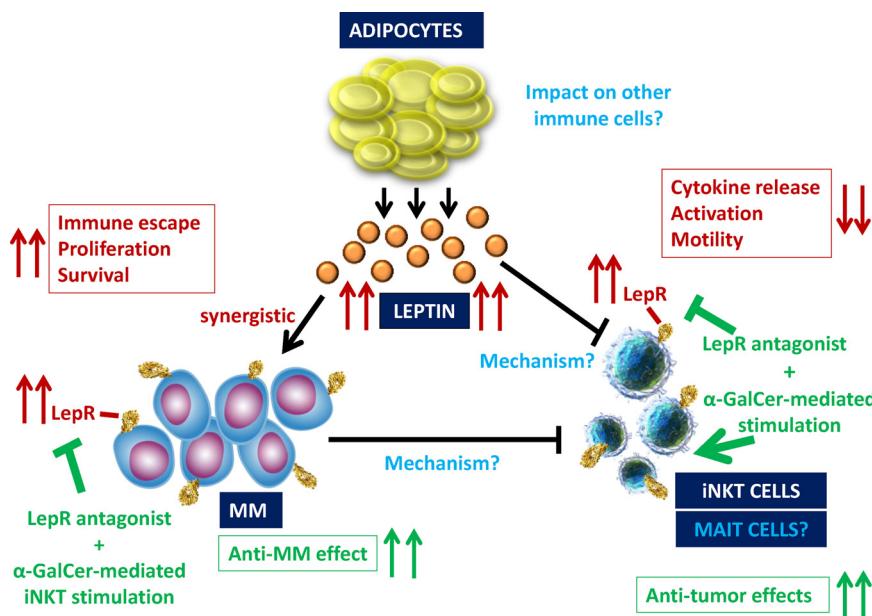


Fig. 1. Leptin receptor antagonism leads to a boost in iNKT cell mediated anti-MM responses. Concept figure illustrating the progressive increase of leptin secreted by adipocytes and Leptin receptor (LepR) expression levels on iNKT cells during MM progression. Leptin profoundly inhibited iNKT cell function and stimulated MM cells (red arrows). iNKT stimulation and LepR antagonism (with specific 2.17 nanobodies) induced enhanced activation (alleviated anergy), proliferation, motility response (shown by dual photon imaging) and cytokine release of iNKT cells in MM, with cross-activation of NK cells observed (green arrows). Complete tumor protection was observed with dual treatment with α -GalCer and LepR antagonism in a MM animal model (for details we refer to (Favreau et al., 2017b)). Several questions still remain including the mechanism(s) behind the increase in leptin and the leptin induced silencing of iNKT cells, the impact of leptin on other immune cells (e.g. MAIT) and the role of other adipokines (light blue) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

gastrointestinal cancer, where the leptin-leptin receptor axis also clearly plays a role in pathology (Garofalo and Surnacz, 2006). Many questions such as the specificity of the effect and its inner mechanisms remain unanswered. Studies in leptin and leptin receptor conditional knockouts should provide some answers on this matter.

5. Concluding remarks

Multiple myeloma is a cancer of the plasma cell where, despite the major therapeutic advances, the majority of patients still relapses and develops into a non-responsive disease, highlighting the unmet medical need. New treatment options for MM patients are needed, with a special focus on immune therapy. The scientific turning point for the emergence of checkpoint inhibitors in cancer immunotherapy came with the understanding that T cell immune responses are controlled through on and off switches, so called 'immune checkpoints' that protect the body from possibly damaging immune responses. Checkpoint inhibition in cancer treatment is assumed to work primarily via CD8 + T cell-and NK cells mediated responses. However, our work shows that semi-invariant innate-like T cells such as iNKT and MAIT cells are additional important immunomodulatory target cells of checkpoint inhibition. Both cell types appear to present a state of functional anergy that might be supported by activation and signaling via checkpoint molecules such as PD-1 and potentially also leptin. This pathway might be exploited by cancer cells to avoid immune surveillance and could for example also be an explanation for the lack of therapeutic success with α -GalCer based interventions. Our work contributes to the understanding of iNKT and MAIT cell biology in MM and has provided new insights in the concept of anergy impeding innate-like T cell-based immunotherapy, with a particular role for the PD-1/PDL and the leptin-leptin receptor signaling pathways. Finally, this could not only be beneficial for the treatment of MM patients but potentially shines a light on future therapeutic avenues for aggressive cancers with currently limited treatment options as well. Further work will help us validate the leptin-leptin receptor molecules are potential useful targets of immunotherapy in MM and other cancers.

Declaration of competing interests

There are no competing interests to declare.

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References

- Alexandrakis, M.G., Passam, F.H., Sfiridakis, A., Pappa, C.A., Moschandrea, J.A., Kandidakis, E., Tsirakis, G., Kyriakou, D.S., 2004. Serum levels of leptin in multiple myeloma patients and its relation to angiogenic and inflammatory cytokines. *Int. J. Biol. Markers* 19, 52–57.
- Anderson, A.C., Joller, N., Kuchroo, V.K., 2012. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* 44, 989–1004.
- Armand, P., 2015. Immune checkpoint blockade in hematologic malignancies. *Blood* 125, 3393–3400.
- Aspeslagh, S., Li, Y., Yu, E.D., Pauwels, N., Trappeniers, M., Girardi, E., Decruy, T., Van Beneden, K., Venken, K., Drennan, M., Leybaert, L., Wang, J., Franck, R.W., Van Calenbergh, S., Zajonc, D.M., Elewaut, D., 2011. Galactose-modified iNKT cell agonists stabilized by an induced fit of CD1d prevent tumour metastasis. *EMBO J.* 30, 2294–2305.
- Aspeslagh, S., Nemcovic, M., Pauwels, N., Venken, K., Wang, J., Van Calenbergh, S., Zajonc, D.M., Elewaut, D., 2013. Enhanced TCR footprint by a novel glycolipid increases NKT dependent tumor protection. *J. Immunol.* 191.
- Atanackovic, D., Radhakrishnan, S.V., Bhardwaj, N., Luetkens, T., 2016. Chimeric Antigen Receptor (CAR) therapy for multiple myeloma. *Br. J. Haematol.* 172, 685–698.
- Atanackovic, D., Luetkens, T., Radhakrishnan, S., Kroger, N., 2017. Coinhibitory molecule PD-1 as a therapeutic target in the microenvironment of multiple myeloma. *Curr. Cancer Drug Targets* 17, 839–845.
- Bendelac, A., Savage, P.B., Teyton, L., 2007. The biology of NKT cells. *Annu. Rev. Immunol.* 25, 297–336.
- Benson Jr, D.M., Bakan, C.E., Mishra, A., Hofmeister, C.C., Efebera, Y., Becknell, B., Baiocchi, Ra., Zhang, J., Yu, J., Smith, M.K., Greenfield, C.N., Porcu, P., Devine, S.M., Rotem-yehudar, R., Lozanski, G., Byrd, J.C., Caliguri, Ma., 2010. The PD-1 / PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti – PD-1 antibody. *Blood* 116, 2286–2294.
- Bentyn, D., Van Nuffel, A.M.T., Wilgenhof, S., Corthals, J., Heirman, C., Neyns, B., Thielemans, K., Bonehill, A., 2012. Characterization of CD 8 + T-Cell responses in the peripheral blood and skin injection sites of melanoma patients treated with mRNA electroporated autologous dendritic cells (TriMixDC-MEL). *Biomed Res. Int.* 2013, 1–8.
- Bianchi, G., Richardson, P.G., Anderson, K.C., 2015. Promising therapies in multiple myeloma. *Blood* 126, 300–310.
- Birmann, B.M., Giovannucci, E., Rosner, B., Anderson, K.C., Colditz, G.A., 2007. Body mass index, physical activity, and risk of multiple myeloma. *Cancer Epidemiol. Biomarkers Prev.* 16, 1474–1478.
- Caers, J., Deleu, S., Belaid, Z., De Raeve, H., Van Valckenborgh, E., De Bruyne, E.,

- DeFresne, M.P., Van Riet, I., Van Camp, B., Vanderkerken, K., 2007. Neighboring adipocytes participate in the bone marrow microenvironment of multiple myeloma cells. *Leukemia* 21, 1580–1584.
- Caers, J., Vande, I., Raeve, H.D., Michaux, L., Trullemans, F., Schots, R., Camp, B.V., Vanderkerken, K., 2008. Multiple myeloma – an update on diagnosis and treatment. *Eur. J. Haematol.* 81, 329–343.
- Cheah, C.Y., Fowler, N.H., Neelapu, S.S., 2015. Targeting the programmed death-1/programmed death-ligand 1 axis in lymphoma. *Curr. Opin. Oncol.* 27, 384–391.
- Crough, T., Purdie, D.M., Okai, M., Maksoud, A., Nieda, M., Nicol, a.J., 2004. Modulation of human Valpha24(+)/Beta11(+) NKT cells by age, malignancy and conventional anticancer therapies. *Br. J. Cancer* 91, 1880–1886.
- De Keersmaecker, B., Fostier, K., Corthals, J., Wilgenhof, S., Heirman, C., Aerts, J.L., Thielemans, K., Schots, R., 2014. Immunomodulatory drugs improve the immune environment for dendritic cell-based immunotherapy in multiple myeloma patients after autologous stem cell transplantation. *Cancer Immunol. Immunother.* 63, 1023–1036.
- Dhodapkar, M.V., Richter, J., 2011. Harnessing natural killer T (NKT) cells in human myeloma: progress and challenges. *Clin. Immunol.* 140, 160–166.
- Dhodapkar, M.V., Geller, M.D., Chang, D.H., Shimizu, K., Fujii, S.-I., Dhodapkar, K.M., Krasovsky, J., 2003. A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma. *J. Exp. Med.* 197, 1667–1676.
- Engelhardt, M., Terpos, E., Kleber, M., Gay, F., Wäsch, R., Morgan, G., Cavo, M., Van de Donck, N., Beilhack, A., Bruno, B., Johnsen, H.E., Hajek, R., Driessens, C., Ludwig, H., Beksa, M., Boccadoro, M., Straka, C., Brighen, S., Gramatzki, M., Larocca, A., Lokhorst, H., Magarotto, V., Morabito, F., Dimopoulos, M.A., Einsele, H., Sonneveld, P., Palumbo, A., 2014. European Myeloma Network recommendations on the evaluation and treatment of newly diagnosed patients with multiple myeloma. *Haematologica* 99, 232–242.
- Farkona, S., Diamandis, E.P., Blasutig, I.M., 2016. Cancer immunotherapy: the beginning of the end of cancer? *BMC Med.* 14, 73.
- Favreau, M., Vanderkerken, K., Elewaut, D., Venken, K., Menu, E., 2016. Does an NKT-cell-based immunotherapeutic approach have a future in multiple myeloma? *Oncotarget* 7 (Apr. (17)). <https://doi.org/10.18632/oncotarget.17440>. 23128–40.
- Favreau, M., Venken, K., Faict, S., Maes, K., De Veirman, K., De Bruyne, E., Leleu, X., Boon, L., Elewaut, D., Vanderkerken, K., Menu, E., 2017a. Both mucosal-associated invariant and Bendelacnatural killer T-cell deficiency in multiple myeloma can be countered by PD-1 inhibition. *Haematologica* 102, e266–e270.
- Favreau, M., Menu, E., Gaublomme, D., Vanderkerken, K., Faict, S., Maes, K., De Bruyne, E., Govindarajan, S., Drennan, M., Van Calenbergh, S., Leleu, X., Zabeau, L., Tavernier, J., Venken, K., Elewaut, D., 2017b. Leptin receptor antagonism of iNKT cell function: a novel strategy to combat multiple myeloma. *Leukemia* 31, 2678–2685.
- Perlay, J., Stiliarova-foucher, E., Lorret-tieulent, J., Rosso, S., 2013. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur. J. Cancer* 49, 1374–1403.
- Fujii, S.-I., Shimizu, K., Okamoto, Y., Kunii, N., Nakayama, T., Motohashi, S., Taniguchi, M., 2013. NKT cells as an ideal anti-tumor immunotherapeutic. *Front. Immunol.* 4.
- Garofalo, C., Surmacz, E.V.A., 2006. Leptin and Cancer. *J. Cell. Physiol.* 207, 12–22.
- Gay, F., D'Agostino, M., Giaccone, L., Genuardi, M., Festuccia, M., Boccadoro, M., Bruno, B., 2017. Immuno-oncologic approaches: CAR-T cells and checkpoint inhibitors. *Clin. Lymphoma Myeloma Leuk.* 17, 471–478.
- Gherardin, N.A., Loh, L., Admojo, L., Davenport, A.J., Richardson, K., Rogers, A., Darcy, P.K., Jenkins, M.R., Prince, H.M., Harrison, S.J., Quach, H., Fairlie, D.P., Kedzierska, K., McCluskey, J., Uldrich, A.P., Neeson, P.J., Ritchie, D.S., Godfrey, D.I., 2018. Enumeration, functional responses and cytotoxic capacity of MAIT cells in newly diagnosed and relapsed multiple myeloma. *Sci. Rep.* 8, 4159.
- Godfrey, D.I., Uldrich, A.P., McCluskey, J., Rossjohn, J., Moody, D.B., 2015. The burgeoning family of unconventional T cells. *Nat. Immunol.* 16, 1114–1123.
- Godfrey, D.I., Le Nours, J., Andrews, D.M., Uldrich, A.P., Rossjohn, J., 2018. Unconventional t cell targets for cancer immunotherapy. *Immunity* 48, 453–473.
- Hallett, W.H., Jing, W., Drobyski, W.R., Johnson, B.D., 2011. Immunosuppressive effects of multiple myeloma are overcome by PD-L1 blockade. *Biol. Blood Marrow Transplant.* 17, 1133–1145.
- Hamaniishi, J., Mandai, M., Matsumura, N., Abiko, K., Baba, T., Konishi, I., 2016. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. *Int. J. Clin. Oncol.* 21, 1–12.
- Jelinek, T., Hajek, R., 2016. PD-1/PD-L1 inhibitors in multiple myeloma: the present and the future. *Oncoimmunology* 5, e1254856.
- Jing, W., Gershon, J.A., Weber, J., Tlomak, D., McOlash, L., Sabatos-Peyton, C., Johnson, B.D., 2015. Combined immune checkpoint protein blockade and low dose whole body irradiation as immunotherapy for myeloma. *J. Immunother. Cancer* 3, 2–16.
- Karp, L.R., Cho, H.J., Avigan, D., 2015. Immunotherapy for multiple myeloma, past, present, and future: monoclonal antibodies, vaccines, and cellular therapies. *Curr. Hematol. Malig. Rep.* 10, 395–404.
- Kiyasu, J., Miyoshi, H., Hirata, A., Arakawa, F., Ichikawa, A., Niino, D., Sugita, Y., Yufu, Y., Choi, I., Abe, Y., Uike, N., Nagafuji, K., Okamura, T., Akashi, K., Takayanagi, R., Shiratsuchi, M., Ohshima, K., 2015. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. *Blood* 126, 2139–2201.
- Kronenberg, M., 2005. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu. Rev. Immunol.* 23, 877–900.
- Kurioka, A., Jahun, A.S., Hannaway, R.F., Walker, L.J., Fergusson, J.R., Sverremark-Ekstrom, E., Corbett, A.J., Ussher, J.E., Willberg, C.B., Klenerman, P., 2017. Shared and distinct phenotypes and functions of human CD161++ Valpha7.2+ t cell subsets. *Front. Immunol.* 8, 1031.
- Landgren, O., Weiss, B.M., 2009. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/racial groups: support for genetic factors in pathogenesis. *Leukemia* 23, 1691–1697.
- Lemaire, M., Deleu, S., De Bruyne, E., Van Valckenborgh, E., Menu, E., Vanderkerken, K., 2011. The microenvironment and molecular biology of the multiple myeloma tumor. *Adv. Cancer Res.* 110, 19–42.
- Ling, L., Lin, Y., Zheng, W., Hong, S., Tang, X., Zhao, P., Li, M., Ni, J., Li, C., Wang, L., Jiang, Y., 2016. Circulating and tumor-infiltrating mucosal associated invariant T (MAIT) cells in colorectal cancer patients. *Sci. Rep.* 6, 20358.
- Liu, Z., Xu, J., He, J., Liu, H., Lin, P., Wan, X., Nora, M., Tong, Q., Kwak, L.W., Orlowski, R.Z., Yang, J., 2015. Mature adipocytes in bone marrow protect myeloma cells against chemotherapy through autophagy activation. *Oncotarget* 6, 34329–34341.
- Lonial, S., Dimopoulos, M., Palumbo, A., White, D., Grosicki, S., Spicka, I., Walter-Croneck, A., Moreau, P., Mateos, M.-V., Magen, H., Belch, A., Reece, D., Beksa, M., Spencer, A., Oakervee, H., Orlowski, R.Z., Taniwaki, M., Röllig, C., Einsele, H., Wu, K.L., Singhal, A., San-Miguel, J., Matsumoto, M., Katz, J., Bleickardt, E., Poulat, V., Anderson, K.C., Richardson, P., 2015. Elotuzumab therapy for relapsed or refractory multiple myeloma. *N. Engl. J. Med.* 373, 621–631.
- Lonial, S., Durie, B., Palumbo, A., 2016. Monoclonal antibodies in the treatment of multiple myeloma: current status and future perspectives. *Blood Rev.* 30, 101–110.
- Lynch, H.T., Ferrara, K., Barlogie, B., Coleman, E.A., Lynch, J.F., Weisenburger, D., Sanger, W., Watson, P., Nipper, H., Witt, V., Thomé, S., 2008. Familial myeloma. *N. Engl. J. Med.* 359, 152–157.
- Matarese, G., Moschos, S., Christos, S., 2018. Leptin Immunol. <https://doi.org/10.4049/jimmunol.174.6.3137>(2016).
- McEwen-Smith, R.M., Salio, M., Cerundolo, V., 2015. The regulatory role of invariant NKT cells in tumor immunity. *Cancer Immunol. Res.* 3, 425–435.
- Melero, I., Berman, D.M., Aznar, M.A., Korman, A.J., Perez Gracia, J.L., Haanen, J., 2015. Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nat. Rev. Cancer* 15, 457–472.
- Miyoshi, H., Kiyasu, J., Kato, T., Yoshida, N., Shimono, J., Yokoyama, S., Taniguchi, H., Sasaki, Y., Kurita, D., Kawamoto, K., Kato, K., Imaizumi, Y., Seto, M., Ohshima, K., 2016. PD-L1 expression on neoplastic or stromal cell is respectively poor or good prognostic factor for adult T-cell leukemia/lymphoma. *Blood* 128, 1374–1381.
- Molling, J.W., Langius, J.A., Langendijk, J.A., Leemans, C.R., Bontkes, H.J., Van der Vliet, H.J., Von Blomberg, B.M., Schepers, R.J., Van den Eertwegh, A.J., 2007. Low levels of circulating invariant natural killer T cells predict poor clinical outcome in patients with head and neck squamous cell carcinoma. *J. Clin. Oncol.* 25, 862–868.
- Moreau, P., San Miguel, J., Ludwig, H., Schouten, H., Mohty, M., Dimopoulos, M., Dreyling, M., 2013. Clinical practice guidelines Multiple myeloma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up † clinical practice guidelines. *Ann. Oncol.* 24, vi133–137.
- Moreira, Á., Pereira, S.S., Costa, M., Moraes, T., Pinto, A., 2015. Adipocyte secreted factors enhance aggressiveness of prostate carcinoma cells. *PLoS One* 10, e0123217.
- Nair, S., Dhodapkar, M.V., 2017. Natural killer t cells in Cancer immunotherapy. *Front. Immunol.* 8, 1178.
- Najera Chuc, A.E., Cervantes, L.A.M., Retiguen, F.P., Ojeda, J.V., Maldonado, E.R., 2012. Low number of invariant NKT cells is associated with poor survival in acute myeloid leukemia. *J. Cancer Res. Clin. Oncol.* 138, 1427–1432.
- Parekh, V.V., Wilson, M.T., Olivares-villagómez, D., Singh, A.K., Wu, L., Wang, C.-r., Joyce, S., Van Kaer, L., 2005. Glycolipid antigen induces long-term natural killer T cell anergy in mice. *J. Clin. Invest.* 115, 2572–2583.
- Parekh, V.V., Lalani, S., Kim, S., Halder, R., Azuma, M., Yagita, H., Kumar, V., Wu, L., Kaer, L.V., 2009. PD-1/PD-L1 blockade prevents anergy induction and enhances the anti-tumor activities of glycolipid-activated invariant NKT cells. *J. Immunol.* 182, 2816–2826.
- Patnaik, A., Kang, S.P., Rasco, D., Papadopoulos, K.P., Elassaiss-Schaap, J., Beeram, M., Drengler, R., Chen, C., Smith, L., Espino, G., Gergich, K., Delgado, L., Daud, A., Lindia, J.A., Nicole Li, X., Pierce, R.H., Yearley, J.H., Wu, D., Laterza, O., Lehnert, M., Iannone, R., Tolcher, A.W., 2015. Phase I study of pembrolizumab (MK-3475; Anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin. Cancer Res.* 21, 4286–4293.
- Pouli, A., Lemessiou, H., Rontogianni, D., Papanastassiou, C., Tsakanikas, S., Gerassimou, A., Stamatiou, M., 2001. Multiple myeloma as the first manifestation of acquired immunodeficiency syndrome: a case report and review of the literature. *Ann. Hematol.* 80, 557–560.
- Raab, M.S., Podar, K., Breitkreutz, I., Richardson, P.G., Anderson, K.C., Anderson, K., 2009. Multiple myeloma. *The Lancet* 374, 324–339.
- Rajan, A.M., Rajkumar, S.V., 2015. Interpretation of Cytogenetic Results in Multiple Myeloma for Clinical Practice 5. Nature Publishing Group, pp. e365.
- Rajkumar, S.V., Landgren, O., Mateos, M.V., 2015. Smoldering multiple myeloma. *Blood* 125, 3069–3076.
- Reihardt, V.L., Okada, C.Y., Liso, A., Stockerl-Goldstein, K.E., Auffermann-Gretzinger, S., Benike, C.J., Reichardt, V., van Beckhoven, A., Rajapaksa, R., Engleman, E.G., Blume, K.G., Levy, R., 1999. Idiotypic vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Blood* 7, 2411–2419.
- Rosenblatt, J., Avivi, I., Vasir, B., Uhl, L., Munshi, N.C., Katz, T., Dey, B.R., Somaiya, P., Mills, H., Campigotto, F., Weller, E., Joyce, R., Levine, J.D., Tzachanis, D., Richardson, P., Laubach, J., Raje, N., Boussiotis, V., Yuan, Y.E., Bisharat, L., Held, V., Rowe, J., Anderson, K., Kufe, D., Avigan, D., 2013. Vaccination with dendritic cell / tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. *Clin. Cancer Res.* 19, 3640–3648.
- Salio, M., Silk, J.D., Jones, E.Y., Cerundolo, V., 2014. Biology of CD1- and MR1-restricted t cells. *Annu. Rev. Immunol.* 32, 323–366.
- Shapiro-shelef, M., Calame, K., 2004. Plasma cell differentiation and multiple myeloma.

- Curr. Opin. Immunol. 16, 226–234.
- Shi, L., Chen, S., Yang, L., Li, Y., 2013. The role of PD-1 and PD-L1 in T-cell immune suppression in patients with hematological malignancies. *J. Hematol. Oncol.* 6.
- Singh, A.K., Gaur, P., Das, S.N., 2014. Natural killer T cell anergy, co-stimulatory molecules and immunotherapeutic interventions. *Hum. Immunol.* 75, 250–260.
- Sullivan, B.A., Kronenberg, M., 2005. Activation or anergy: NKT cells are stunned by alpha-galactosylceramide. *J. Clin. Invest.* 115, 2328–2329.
- Tahir, S.M.A., Cheng, O., Shaulov, A., Koezuka, Y., Bubley, G.J., Wilson, S.B., Balk, S.P., Exley, M.A., 2001. Loss of IFN- γ production by invariant NK t cells in advanced Cancer. *J. Immunol.* 167.
- Teyton, L., 2017. New directions for natural killer t cells in the immunotherapy of Cancer. *Front. Immunol.* 8, 1480.
- Topalian, S.L., Drake, C.G., Pardoll, D.M., 2015. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 27, 451–461.
- Turesson, I., Velez, R., Kristinsson, S.Y., Landgren, O., 2010. Patterns of multiple myeloma during the past 5 decades: stable incidence rates for all age groups in the population but rapidly changing age distribution in the clinic. *Mayo Clin. Proc.* 85, 225–230.
- Vogl, D.T., Wang, T., Pérez, W.S., Edward, A., Heitjan, D.F., Lazarus, H.M., Kyle, R.A., Kamble, R., Weisdorf, D., Roy, V., Gibson, J., 2012. Effect of obesity on outcomes after autologous hematopoietic stem cell transplantation for multiple myeloma. *Biol. Blood Marrow Transplant.* 17, 1765–1774.
- Wang, L., Rubinstein, R., Lines, J.L., Wasieuk, A., Ahonen, C., Guo, Y., Lu, L.-F., Gondek, D., Wang, Y., Fava, R.A., Fiser, A., Almo, S., Noelle, R.J., 2011. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. *J. Exp. Med.* 208, 577–592.
- Won, E.J., Ju, J.K., Cho, Y.-N., Jin, H.-M., Park, K.-J., Kim, T.-J., Kwon, Y.-S., Kee, H.J., Kim, J.-C., Kee, S.-J., Park, Y.-W., 2016. Clinical relevance of circulating mucosal-associated invariant T cell levels and their anti-cancer activity in patients with mucosal-associated cancer. *Oncotarget* 7.
- Yi, Q., Szmania, S., Freeman, J., Qian, J., Rosen, N.A., Viswamitra, S., Cottler-Fox, M., Barlogie, B., Tricot, G., Van Rhee, F., 2010. Optimizing dendritic cell-based immunotherapy in multiple myeloma: intranodal injections of idiotype-pulsed CD40 ligand-matured vaccines led to induction of type-1 and cytotoxic T-cell immune responses in patients. *Br. J. Haematol.* 150, 554–564.
- Yousef, S., Marvin, J., Steinbach, M., Langemo, A., Kovacsics, T., Binder, M., Kröger, N., Luetkens, T., Atanackovic, D., 2015. Immunomodulatory molecule PD-L1 is expressed on malignant plasma cells and myeloma-propagating pre-plasma cells in the bone marrow of multiple myeloma patients. *Blood Cancer J.* 5, e285.
- Yu, W., Cao, D.-D., Li, Q.-B., Mei, H.-L., Hu, Y., Guo, T., 2016. Adipocytes secreted leptin is a pro-tumor factor for survival of multiple myeloma under chemotherapy. *Oncotarget* 7, 86075–86086.
- Zelle-Rieser, C., Thangavadiel, S., Biedermann, R., Brunner, A., Stoitzner, P., Willenbacher, E., Greil, R., Jörhrer, K., 2016. T cells in multiple myeloma display features of exhaustion and senescence at the tumor site. *J. Hematol. Oncol.* 9.
- Zhou, F.-l., Meng, S., Zhang, W.-g., Wei, Y.-c., Cao, X.-m., 2010. Peptide-based immunotherapy for multiple myeloma: current approaches. *Vaccine* 28, 5939–5946.