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Advances in Immunotherapy with Chimeric Antigen Receptor Invariant Natural Killer T cells (CAR-iNKT cells); Therapeutic Implications in Multiple Myeloma

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Abstract

In Multiple Myeloma (MM), a highly CD1d expressing tumor, defects in endogenous invariant Natural Killer T cells (iNKT cells) along with CD1d downregulation through epigenetic silencing in advanced relapses, promotes myeloma immune escape. Especially CD1d expression is totally lost on plasma cells from extramedullary relapses and in myeloma cell lines, showing that downregulation of CD1d on plasma cells happens when they become independent from the bone marrow microenvironment. Myeloma cells aberrantly overexpressed GM3, a glycolipid that promotes osteoclastogenesis and myeloma cell survival. A-galactosyl-ceramide, a glycolipid extracted from a marine sponge, can expand, and manipulate iNKT cell in-vivo but efforts to reverse iNKT cells defect and restore antimyeloma cytotoxicity with this agent, failed. Advanced relapses of MM patients are difficult to treat and today CAR-T cell based immunotherapeutic approaches are evolving with encouraging results. The development of CAR-iNKT cells technology can overcome restrictions of CAR-T cells and confers the ability to attack myeloma cells by two ways; the CAR receptor as long with the invariant TCR receptor in the context of CD1d.

Invariant Natural Killer T-cells (iNKT-cells) Defects in Multiple Myeloma Permits Myeloma Immune Escape

The CD1d/ invariant Natural Killer T cell (iNKT) / glycolipid immune axis belongs to the innate immunity and consists a system highly conserved among species. iNKT cells are a natural clone expressing a limited repertoire of T-cell Receptors (TCR) (Va24/ Vb11) in humans that can interact with all immune cells[1]. They have the ability upon TCR ligation to secrete rapidly robust amounts of Th1/Th2/Th17 cytokines and through them to regulate a huge variety of immune responses[2]. Considering antitumor immunity, iNKT cells can go both ways and either suppress or promote antitumor immunity in a huge variety of solid and hematologic CD1d-expressing malignancies. Whether antitumor iNKT responses eventuates as cytotoxic or anergic is mostly determined by the mode of iNKT activation and the cytokine microenvironment[3].

In multiple myeloma (MM), an incurable hematologic malignancy, suppression of the immune system of the host is essential in order myeloma to evade antitumor immunity[4,5]. In an intriguing interplay, myeloma cells suppress iNKT-dependent immune responses through IFNγ suppression during the evolution from the premalignant MGUS to active myeloma[6]. Additionally, iNKT cells in MM migrate from the periphery toward the bone marrow and are turned to an abnormal cytokine profile characterized by upregulation of

RANKL, contributing thus to osteoclast (OC) activation and consequently to the pathogenesis of myeloma bone diseases[7,8]. Thus in myeloma iNKT cells migrate towards the bone marrow but their cytotoxic efficacy is reduced. In many cancers different glycolipids aberrantly overexpressed by tumor cells suppress iNKT mediated cytotoxicity. We speculate that GM3, the glycolipind most abundantly expressed on myeloma cells, is binding on plasma cells CD1d and turns iNKT cells to an anergic phenotype. A study used lenalidomide and a-galactosyl-ceramide aiming to restore the antimyeloma cytotoxicity of endogenous iNKT cells showed limited efficacy[9]. In many cancers migration of iNKT cells from the periphery to the tumor bed has been reported and high tumor infiltration with iNKT cells is related with better prognosis of solid tumors[10,11].

On the other hand, malignant plasma cells from newly diagnosed myeloma patients express high levels of CD1d, an expression lost almost totally in advance stages of the disease[10]. CD1d, apart from an antigen-presenting (APC) molecule, represents a death receptor for myeloma cells. A variety of anti-CD1d monoclonal antibodies (MoAbs) can trigger myeloma cell apoptosis in a caspase-independent manner through Bax accumulation, in a process preceded by cell aggregation and disruption of the Mitochondrial Membrane Potential (MMP)[10].

Quantitative and qualitative changes of the cellular glycosphingolipid (GSL) profile have long been recognized as a trait of malignant transformation and acquisition of novel cellular functions that promote tumor survival, growth, metastasis and angiogenesis[12,13]. Glycomic analysis of GSL expression patterns in MM showed that GM3 was the most abundant polar GSL expressed in myeloma cell lines and in CD138+ primary myeloma cells[14]. GM3 has been recognized as a tumor neoantigen regulating metastasis, cell motility and EGFR signaling in solid tumors[15-18]. In MM the overexpressed GM3 is subsequently sledded into tumor microenvironment and enhance the formation and function of mature OC in a process needed permissive quantities of RANKL and promoted by insulin growth factor-1 (IGF-1)[14].

Chimeric Antigen Receptor T-cells Against Multiple Myeloma

In Relapsed Refractory MM, the median overall survival (OS) of patients who progressed after exposure to ≥3 prior therapies are approximately 13 months consisting a population characterized as an unmet medical need. Chimeric Antigen Receptors (CAR) are artificial fusion proteins that incorporate a tumor antigen recognition domain and a T signalling domain. CARs are transduced into homologous T cells by lenti or retro-viruses and generate CAR-T cells specific to recognize a tumor antigen in an HLA unrestricted manner[19].

Selection of the target antigen is essential for an effective and safe CAR-T cell-based immunotherapy. B Cell Maturation Antigen (BCMA) or CD269 is a member of the tumor necrosis factor superfamily, expressed on some B cells, normal plasma cells, and on MM cells. BCMA was uniformly expressed in most cases of MM by immunohistochemistry. The preferential expression of BCMA on plasma cells together with its limited expression on normal cells makes BCMA attractive as target for immunotherapy. CAR-T therapy targeting BCMA has provided promising results for management of relapsed and refractory MM. However, a significant portion of patients still relapse with progressive disease after monospecific anti-BCMA CAR-T treatment[20]. Toxicity issues (Cytokine Release Syndrome CRS, neurotoxicity) issues of limited effectiveness due to antigen escape, issues of CAR-T cells viability, issues of co-stimulatoty molecules binding to CAR receptor are limiting the effectiveness of CAR-T immunotherapy[21]. Next generation CARs are being tested in clinical trials in order to overcome all this issues by using many different approaches.

LCAR-B38M is a structurally differentiated CAR-T cell therapy containing a 4-1BB co-stimulatory domain and 2 BCMA-targeting single-domain antibodies designed to confer avidity. In a first-in-human phase 1 study included 74 pts with RRMM, LCAR-B38M displayed a manageable safety profile consistent with its known mechanism of action and with a median follow-up of 19 months, demonstrated deep and durable responses in patients with RRMM[21].

JNJ-68284528 (JNJ-4528) is a CAR-T cell therapy, identical to LCAR-B38M, containing two BCMA-targeting single-domain antibodies designed also to confer better avidity. A Phase 1b results from the ongoing CARTITUDE-1 with JNJ-4528 showed an overall response rate of 91% (19/21 pts), with 4 stringent complete responses (sCRs), 2 CRs, 7 very good partial responses (VGPR), and 6 partial responses (PR), also with a manageable safety profile[22] .

CAR-T design as bb2121 and the product carrying the same construct but adds the phosphoinositide 3-kinase inhibitor bb007 during ex vivo culture to enrich the drug product for memory-like T cells (named bb21217) shows encouraging preliminary results in heavily pretreated MM patients. In a phase I trial 22 patients (median age 63) received bb2121 (12 at 150, 6 at 300 and 4 at 450) and in the extended phase including the initial patients and at least 15 additional patients treated with up to 450 x 10^6 CAR+ T cells and patients outcome was encouraging. All patients had BCMA levels above 50% on their malignant cells and had received ≥ 3 lines of treatment. CRS affected 84% of patients that received the dose of 450 x 10^6 CAR+ T cells but severe CRS was diagnosed in 8% of patients while neurotoxicity reported at the rate of 24%. Fast responses with 18 of 41 patients achieving VGPR or CR that are

ongoing was reported in ASH 2019 meeting[23]. The bb21217 CART cell in a phase 1 trial in 7 evaluable for response patients showed 86% response rate (1 CR, 3 VGPR, 2 PR).

To further improve the efficacy and to reduce relapse, a bispecific CAR-T targeting both BCMA and CD19 has been designed. Pre-clinical data demonstrated BCMA-CD19 CAR-T cells are effective in eliminating MM tumor cells both *in vitro* and *in vivo*. The first-in-human clinical trial also showed extraordinary safety profile and efficacy of BCMA-CD19 bispecific CAR-T in 7 R/R MM aged less than 60 years of age[24]. Another clinical trial (ChiCTR1800018143) using a bispecific CAR-T (BM38 CAR), incorporating the anti-CD38 and anti-BCMA is under conduction, showing improved efficacy with a high ORR and manageable toxicities[25]. Preliminary results from a phase 1 trial including 16 RRMM patients reported ORR 87,5% (14/16) with 8 pts achieving sCR, 2 VGPR and 4 PR, while CRS syndrome occurred in 10.16 pts treated.

It is known that gamma secretase inhibitors (GSI) increase BCMA surface density, decrease soluble BCMA levels and augment anti-tumor efficacy of BCMA CAR-T cells in preclinical models. In a phase I first-in-human trial (NCT03502577), CAR-T cells expressing a fully human BCMA scFv with an orally administered gamma secretase inhibitor (JSMD194) were combined. This combination led to rapid responses in 6 pts (5 VGPR, 1 PR), achieved with low CAR-T cell doses, including patients that have failed prior BCMA targeted therapy[26].

The type of T cell used in generating CAR-T cells is an important choice. Marrow-infiltrating Lymphocytes (MILsTM) is a novel form of adoptive T cell therapy composed of patient-autologous, polyclonal CD4 and CD8 T cells that are activated and expanded from the bone marrow. Using a CD38-specific, 4-1BB/CD3z-signaling CAR as an initial model, Lutz et al demonstrated the feasibility of producing CAR-modified MILsTM (CAR-MILsTM) and showed that CAR-MILsTM demonstrate superior killing in vitro compared to CAR-T cells generated from patient-matched PBLs (CAR-PBLs). CAR-MILsTM have several advantages over CAR-PBLs, including increased cytolytic potential, enhanced polyfunctionality, increased stemness and less exhaustion, making them more potent and effective than currently approved CAR-T products[27].

Chimeric Antigen Receptor iNKT-cells Against Hematologic Malignancies

The generation of CAR-iNKT cells is a promising development in order to overcome issues raised from the clinical use of CAR-T cells[22]. iNKT cells are rare cells with invariant receptor (Va24/Vb11) in humans recognizing the non-polymorphic CD1d instead of the polymorphic MCH. They need to be expanded before use and can use in out of shelf setting. CD4- iNKT cells can equally be expanded and this subset of iNKT can repress acute graft versus host disease[23,24]. The CD62L+ iNKT cells (central memory) are enriched in the expanded pool of iNKT cells in the presence of IL-21 and therefore expanded iNKT is a cell compartment enriched in memory cells protects against a GVHD and can used in the allogeneic setting[25]. A-galactosyl ceramide (aGalCer) has proved safe when administered in patients and can expand CAR-iNKT cells or modify their function after their infusion in patients[9]. CAR-iNKT GD2 cells are in development for treatment of neuroblastoma and a clinical trial is on the way[26]. Recently, Karadimitris et al, optimized a protocol that offers a robust expansion of CAR 19 engineered iNKT cells from all sources which can be used in out of shelf setting against CD1d+/CD19+ expressing lymphoid tumors. Upfront expansion of iNKT cells and lentiviral transduction of CD3/CD28 activated iNKT cells in the presence of autologous APC and IL-15 generates a pool of active CAR19-iNKT cells[23]. CAR19-iNKTs retain their ability to react against CD1d+ tumors loaded with aGalCer through their invariant TCR receptor and thus they can attack the malignant cell in two ways. The CD1d molecule that is downregulated in relapsed myeloma cells can be restored after ATRA treatment[27]. Therefore, compared to CAR19-T cells, CAR19-iNKT cells regress tumors in mice models more effectively, have more prolonged survival and antitumor action and they penetrate more effectively into the CNS[23]. Based on the above, a clinical trial is recruiting patients with relapsed non-Hodgkin lymphoma and lymphoblastic leukemia offering therapy with CAR19-iNKT cells (NCT03774654). For myeloma the development of CARBCMA iNKT cells is a more rational approach since CD19 is barely expressed on myeloma cells.

Conclusions

CAR- iNKT cell technology evolves as a novel opportunity to overcome issues raised by CAR-T cell-based immunotherapies. CAR- iNKT cells can be modulated in vivo and ex-vivo by aGalCer, are enriched in central memory-like lymphocytes, protects against aGVHD. They can used in out of shelf immunotherapy approach, attacks tumor by two ways CAR and natural TCR and they penetrate more efficiently to body compartments like CNS. In myeloma surface downregulation of CD1d can be restored with ATRA treatment and we envisioned that CAR-iNKT cells expressing anti-BCMA CAR can effectively attack and eliminate myeloma cells.

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