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Cytokine induced killer cells as adoptive immunotherapy strategy to augment graft versus tumor after hematopoietic cell transplantation

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Donor lymphocyte infusion (DLI) is used to increase the graft versus tumor (GVT) effect after allogeneic hematopoietic cell transplant (HCT). The limited spectrum of activity and high risk of graft versus host disease (GVHD) remain major limitations of this approach. The finding of new cell populations for adoptive immunotherapy, with the ability to separate GVT from GVHD, would be useful. Here we review the main basic, preclinical and clinical research on cytokine-induced killer (CIK) cells, highlighting the aspects of their antitumor and alloreactive potentials that might favourably affect the balance between GVT and GVHD. CIK cells are *ex vivo*-expanded T lymphocytes sharing NK markers and endowed with a potent MHC-unrestricted antitumor activity against haematological and solid malignancies. Studies in preclinical animal models have demonstrated their low GVHD potential when infused across MHC-barriers, and recent clinical studies seem to confirm these findings in patients with hematological malignances relapsing after HCT. If consolidated with larger clinical trials, adoptive immunotherapy with CIK cells might represent an effective alternative to classic DLI, helping HCT to succesfully meet current challenges like the extension across major HLA-barriers and application to solid tumors.

Keywords: cytokine-induced killer, donor lymphocyte infusion, graft versus host disease, graft versus tumor

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1. Introduction

Hematopoietic cell transplantation (HCT) is an established treatment for various hematological malignancies and it has recently been extended, as an experimental treatment, to metastatic solid tumors [1-3]. The efficacy of HCT relies on the effects of both high-dose chemotherapy and graft versus tumor (GVT) activity operated by the donor immune system. Potentiating the GVT effect is a crucial issue to ameliorate and consolidate the efficacy of HCT. In the setting of hematological malignancies, refractory or relapsing diseases are in fact still a major clinical problem.

The infusion of donor lymphocytes (DLI) after HCT is a promising approach to increase the GVT effect and treat relapsing or residual disease [4]. The major drawback associated with DLI is the risk of developing graft versus host disease (GVHD) that can lead to potentially severe and even fatal clinical consequences [5,6]. The finding of new strategies to decrease the risk of GVHD and increase the GVT power of DLI would have a great effect on treatment outcome. A

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refinement of current DLI strategies would be crucial also in new experimental settings, like the recent HCT applications to solid tumors. In this context, even if proofs of activity have been provided, no durable responses have currently been obtained and further immunotherapeutic interventions are needed [1-3].

One possible way to improve DLI would be to identify and selectively infuse lymphocyte subsets capable of a GVT activity but with a null or reduced GVHD potential. In this scenario cytokine induced killer (CIK) cells might represent an appealing new therapeutic option.

In the following paragraphs we review the principal biological characteristics of CIK cells, pointing out how they might produce results helpful for a post-HCT adoptive immunotherapy strategy. We especially focus on the biological properties of antitumor activity and alloreactivity and the possible implications for the delicate balance between GVT and GVHD. We ideally start from *in vitro* studies and, passing through preclinical animal models, arrive at the recent early clinical applications. Finally, we try to envision the future challenges and potentialities of CIK-cell-based immunotherapy.

2. Expansion and phenotype of CIK cells

CIK cells are a heterogeneous population of polyclonal T lymphocytes sharing NK phenotype and functional properties. Initially described by Schmidt-Wolf *et al.* in 1991 [7], CIK cells are endowed with a potent MHC-unrestricted antitumor activity and can be efficiently expanded *in vitro* from bone marrow or PBMC by the timed addition of IFN-γ, Ab anti-CD3 and IL-2 [7-11]. After 2 – 3 weeks of *ex vivo* culture the expansion of CIK cells is described to range from few to more than 1000-fold [7,8,10]. Within the bulk culture of expanded CIK cells two main subpopulations can be distinguished, one co-expressing the CD3 and CD56 molecules ($CD3^+CD56^+$) while the other presenting a $CD3^+CD56^-$ phenotype. The antitumor activity of CIK cells has been reported to be associated with the $CD56^+$ fraction [10,12]. The majority of expanded CIK cells are $CD8^+$ but $CD4^+CD8^-$ cells can be found as well. Double positive ($CD4^+CD8^+$) or double negative ($CD4^-CD8^-$) phenotypes are present to a lesser extent within bulk CIK cultures. The $CD3^+CD56^+$ subset of CIK cells presents with a more terminally differentiated late effector phenotype ($CD45RO^+$; $CD27^{low}$; $CD28^{low}$; $CD62L^-$; $CCR7^-$) than their $CD3^+CD56^-$ counterparts that exhibit earlier memory characteristics [13].

Other phenotypic characteristics of CIK cells are the expression of HLA-DR [11,14], CD57, CD11b and CD5 molecules while they are missing the Fcy receptor CD16, mediator of antibody-dependent cellular cytotoxicity (ADCC) mechanisms. CIK cells, unlike classic NKT cells, are independent from CD1 molecules for their expansion and present with a polyclonal TCR repertoire. The *ex vivo* expansion of CIK cells can be obtained by simply culturing the starting PBMC population with IFN-γ, Ab anti-CD3 and IL-2 [7]. Antibody to CD3 acts as a mitogenic stimulus on T cells that are then expanded in IL-2-containing medium [15-18].

The addition of IFN-γ on day 0 seems to increase the cytotoxicity and expansion of CIK cells, likely stimulating the monocytes in the culture. Activated monocytes provide both a contact-dependent factor [CD58/ lymphocyte function-associated antigen-3 (LFA-3)] and a soluble factor (IL-12) crucial for the expansion and acquisition of a T_H1 phenotype of CIK cells [19-21]. Some works suggest that IL-7 can also be used to generate CIK cells with high cytotoxicity activity [22,23].

Lu & Negrin demonstrated that T lymphocytes rather than NK cells are the precursors of CIK cells. T cells and NK cells were isolated from peripheral blood and separately cultured in 'CIK conditions'. It was observed that only the first could generate CIK cells while NK cells maintained their initial phenotype throughout the culture period. It seems that $CD3^+CD56^+$ CIK cells are preferentially generated from $CD3^+CD8^-CD4^-$ precursors and, to a lesser extent, from $CD4^+CD8^+$ and $CD4^-CD8^+$ T cells [10].

Besides the classical expansion from circulating PBMC, it has been demonstrated that a successful expansion of CIK cells can also be obtained from G-CSF-mobilized bone marrow and from cord blood cells [24,25]. Ex vivo culture conditions can be effectively reproduced in Good Manufacturering Practices conditions [25]. Recently it has been shown that, after allogeneic HCT, CIK cells could be efficiently expanded from engrafted patients even during immune-suppression treatment, their expansion degree and functional activity was similar to that observed in their correspondent healthy donors [12].

3. Antitumor activity and GVT potential of CIK cells

3.1 Mechanism of action and *in vitro* data

CIK cells are endowed of a potent MHC-unrestricted cytotoxicity against both hematological and solid malignancies.

The antitumor activity of CIK cells is mainly restricted to the $CD3^+CD56^+$ fraction [10,12] and it is not due to the higher percentage of $CD8^+$ cells compared with the $CD56^-$ fraction. The mechanisms underlying the cytotoxicity of CIK cells have not been completely clarified, however some key molecules and pathways have recently been identified. Tests with blocking antibodies against CD2, CD3, CD8, CD28, CD56, very late antigen 4 (VLA-4), TCR-α-β, and MHC class I and II molecules failed to inhibit the cytotoxic activity while a significant inhibition was obtained blocking LFA-1 and intracellular cell adhesion molceule 1 (ICAM-1) [26]. Treament of CIK cells with dibutyryl (db)-cAMP, which prevents the conversion of LFA-1 into a high affinity receptor for ICAM-1, inhibited perforin and granzyme degranulation of CIK cells triggered by both CD3Ab and tumor targets [27].

The use of immunosuppressive drugs like Cyclosporine and FK506 prevented degranulation of CIK cells induced by CD3–TCR stimulation but could not block the cytotoxicity triggered by the interaction with tumor targets [27]. These findings suggest a crucial role for the LFA-1–ICAM-1 interaction in the TCR triggering of CIK cells antitumor activity. The molecule that seems to play the most important role in tumor recognition of CIK cells is probably the NKG2D receptor. It is located within the NK gene complex and is a member of the c-type lectin activating receptor family [28]. NKG2D appears to be highly expressed in expanded CIK cells and it is not restricted to the $CD3^+$ $CD56^+$ subpopulation. The most known ligands for NKG2D are relatively restricted to malignant cells and include MHC class I polypeptide-related sequence (MIC)-A, MIC-B and members of the unique long 16-binding protein (ULBP) family (ULBP1, 2 and 3) [29-31]. Studies with antibodies blocking the NKG2D molecules, siRNA experiments and redirected cytolysis indicated that the majority of the MHC-unrestricted cytotoxicity of CIK cells is exerted trough the NKG2D interaction rather than TCR engagement [12,32]. The action of NKG2D is probably associated with the upregulation of the adaptor protein DAP10, induced by the high dose of IL-2 present in the culture medium of CIK cells [32].

While NKG2D mediates the interaction between CIK cells and tumor targets, the final cytolytic effect is perforin- and granzyme-mediated [8].

3.2 Murine models demonstrating CIK anti-tumor activities

Animal models with SCID mice were initially used to test the *in vivo* antitumor activity of CIK cells. The first reported data demonstrated the antitumor activity against lymphoma cells, envisioning the potential future clinical role. It described how CIK cells were able to effectively purge tumor-contaminated bone marrow, resulting in the increased survival of recipient animals with the majority of them showing no signs of tumor growth for more than 100 days after the injection [7]. Following studies demonstrated how the adoptive infusion of CIK cells significantly prolonged the survival of SCID mice that received grafts of human B-cell lymphoma cells (SU-DHL-4) compared with both untreated controls or mice infused with equal amounts of lymphokine-activated killer (LAK) cells [7,10]. CIK activity was found to be independent of exogenous IL-2 administration but seemed to require the active proliferation of CIK cells since their activity was abrogated by irradiation (15 cGy). The beneficial effect of CIK cells was maintained regardless if the infusion was performed intravenously or intraperitoneally. Also in a syngeneic bone marrow transplantation (BMT) model, the infusion of CIK cells significantly prolonged the survival of lymphoma-bearing mice [8].

To rule out a possible role of allogeneic stimulation in the reported tumor killing activity, further experiments demonstrated that CIK cells were fully capable of killing autologous chronic myeloid leukemia (CML) blasts both *in vitro* and after engraftment in SCID mice [24,33]. CIK cells were demonstrated to be safe against autologous bone marrow cells, a certain degree of cytolysis occurred instead against allogeneic bone marrow but to a much lesser extent compared with tumor targets [34]. As previously described, NKG2D is the molecule that mostly accounts for the interaction between CIK and tumor cells while the cytotoxic effect seems to be perforin-mediated. CIK cells generated from mice strains deficient in FAS ligand (FASL) were fully capable of exerting *in vitro* cytotoxicity while CIK cells expanded from perforin-knockout mice had lost their tumor-killing ability [34]. Similarly the adoptive infusion, after allogeneic HCT, of CIK cells generated from FASL-deficient or wild-type mice protected the animals from a lethal dose of lymphoma cells while no tumor protection was observed following the infusion of perforin-deficient CIK cells [34]. Using a HCT model with luciferase expressing (luc^+) CIK cells it was recently possible to visualize the *in vivo* trafficking of CIK cells and confirm their direct infiltration of tumor sites. Lymphoma-bearing mice received infusions of luc^+ CIK cells, and a strong signal indicative of tumor infiltration was detected on day 21 resulting in the inhibition of tumor growth [35]. CIK cells preferentially localized to tumor sites expressing NKG2D ligands an their killing activity was inhibited by the *in vivo* administration of NKG2D blocking antibodies [35]. In the same model it was demonstrated that there was a low expression of NKG2D ligands at GVHD target organ sites, further evidence of the low GVHD risk associated with CIK cells [35].

3.3 Clinical studies demonstrating CIK anti-tumor activities

The first clinical application of CIK cells after allogeneic HCT in a Phase I trial was recently reported [36]. Eleven patients with refractory hematological malignancies received the adoptive infusion of donor CIK cells and successfully demonstrated the feasibility and the low toxicity profile of this approach. It was also possible however to describe the contribution given to clinical responses, indicative of GVT effect of CIK cells *in vivo*. Disease progression and death occurred in six patients. One patient had stable disease, one had hematologic improvement and three achieved complete responses. In a similar setting, preliminary data reported by the Stanford University group confirmed the feasibility of adoptive CIK cell infusions and showed evidence of GVT effect with clinical activity. They reported an event-free and overall survival of 20% and 76% respectively [37].

More data about the clinical anti-tumor activity of CIK cells are available from the autologous setting. Even if the allogeneic setting is the main subject of this review, a brief analysis of the *in vivo* killing ability of autologous CIK cells can provide important information on the feasibility, safety and potential targets of this strategy. The first clinical experience was attempted infusing CIK cells in 10 patients affected by

metastatic renal carcinoma, colorectal cancer and lymphoma. Circulating CIK cells persisted for up to 2 weeks after the infusion, an increase in serum IFN-γ, GM-CSF and TGF-β was observed along with an increased cytotoxic activity of total peripheral blood lymphocytes. One CR was reported in a lymphoma patient while six patients had progressive diseases and three did not experience any change. A mild fever in three patients was the only reported side effect. It is worthy of note that, in this first study, CIK cells were able to auto-generate IL-2 after being preemptively transfected with a plasmid containing the IL-2 gene [38]. Other clinical trials subsequently demonstrated the potential clinical benefit of CIK cells against both hematologic and solid tumors, all confirming the low toxicity profile of this strategy. In a Phase I trial, nine patients with relapsed Hodgkin's and non-Hodgkin's lymphomas received autologous CIK cells reporting a minimal toxicity along with two partial responses and two instances of disease stabilization [39]. A very recent Phase I study reported on 12 patients with advanced non-Hodgkin's lymphoma (NHL), metastatic renal cancer or hepatocellular carcinoma (HCC). Along with a very favorable toxicity profile, the adoptive infusion of autologous CIK cells resulted in three complete responses and two stabilizations of disease Two of the complete responses were observed in metastatic renal cancer and HCC, these patients received the simultaneous subcuteneous injection of low dose IL-2 and IFN-α respectively [40]. A randomized trial of adjuvant autologous CIK cells after resection of hepatocellular carcinoma showed a significant increase in disease-free survival while no statistically significant differences were observed in overall survival [41]. A prospective study, on 59 patients, of chemotherapy (Docetaxel + Cisplatin) in combination with CIK cells for advanced NSCLC reported prolonged progression-free and overall survival compared with control patients treated with only chemotherapy [42]. CIK cells' activity has also been reported in patients with advanced gastric cancer [43].

The main clinical studies with CIK cells, including both allogeneic and autologous settings, are summarized in **Table 1**.

These early results appear very promising and will hopefully lead to more large and controlled clinical trials in these settings.

4. Alloreactivity and GVHD potential of CIK cells

GVHD is the most frequent and severe complication associated with the adoptive infusion of allogeneic lymphocytes after HCT. Since from early studies, CIK cells appeared to be endowed with a reduced alloreactive potential, compared with conventional T cells, making an appealing and promising alternative to classic DLI. Initial observations came from *in vitro* studies and were subsequently confirmed by preclinical animal models that helped to highlight crucial mechanisms responsible for the reduced GVHD potential. Recently new data have been made available about initial clinical trials that seem to confirm the reduced propensity of CIK cells to induce GVHD.

4.1 *In vitro* data

The first studies to assess the alloreactivity of CIK cells were based on *in vitro* mixed lymphocyte reactions (MLR) across major MHC-barriers. Expanded murine CIK cells, in contrast to fresh naïve splenocytes, did not exhibit increased proliferation when cultured with irradiated MHC-mismatched stimulators [34]. In these experiments however, it would have been difficult to detect the real contribution to proliferation given by the allogeneic stimulation since CIK cells were already actively proliferating under the influence of IL-2. Recently similar experiments have been reported with human CIK cells tested in HLA-mismatched MLR but with low dose of IL-2. It has been shown how CIK cells, when tested as a bulk population, maintained an alloreactive proliferation similar to that observed with fresh lymphocytes. If tested separately, it was clear how the majority of the observed prolifetation was due to the $CD3^+CD56^-$ subset while $CD3^+CD56^+$ cells showed only minimal alloreactive capacity [12].

4.2 Murine studies

To assess if the reduced alloreactivity observed in the MLR essays could result in a reduced GVHD risk, the adoptive infusion of CIK cells was first tested in murine experimental models of HCT.

Bone marrow from C57BL/6 mice was used to reconstitute letally irradiated BALB/c mice; subsequently increasing numbers of either C57BL/6 derived CIK cells or naïve splenocytes were adoptively infused. Compared with naïve splenocytes, CIK cells infused across MHC-barriers caused minimal GVHD, persisting into the peripheral circulation up to 3 weeks after the infusion and mediating effective GVT [34]. The reduced incidence of GVHD could be explained if the infused CIK cells have a very limited lifespan after the prolonged *ex vivo* expansion. It is true that the majority of $CD3^+CD56^+$ cells present with a terminally differentiated late effector phenotype [13] and are endowed with a limited proliferative potential. The $CD3^+CD56^-$ subpopulation however represents a considerable percentage of the final bulk population of CIK cells and exhibits an earlier effector phenotype with a greater proliferative capacity [12,13]. The phenotype of infused cells could thus not be the only explanation for the low GVHD incidence and a crucial role seemed to be played by certain cytokines produced by CIK cells. Attention has been focused in particular on the abundant production of IFN-γ, spontaneously occuring in expanded CIK cells, and known to be protective against GVHD [44]. Further evidence in support of this hypothesis has been found in an elegant HCT preclinical experiment, in which CIK cells, generated from IFN-γ knockout mice, rapidly induced lethal GVHD when infused across MHC-barriers, in contrast to the wild-type counterpart that confirmed a minimal GVHD potential [8]. Recently, HCT murine models

Table 1. Clinical studies with the adoptive infusion of CIK cells.

Disease	Patients	Type of CIKs	Toxicity	GVHD	Clinical responses	Ref.
Relapsed AML; HD; CML ALL; MDS	11	Allogeneic	None relevant	Acute: 4 (Grade I–II) Chronic: 2	CR (3); SD (1)	[36]
Relapsed AML; NHL; MM; HD	10	Allogeneic	*Ventricular arrhythmias: 2 (Grade 3 – 4)	Acute: 1 (Grade I) Chronic: 2	1 year EFS 20% 1 year OS 76% TTP 90 days (9 – 577)	[37]
Colorectal and renal carcinoma; NHL	10	Autologous	Fever: 3	NA	CR (1) SD (3)	[38]
Relapsed HD; NHL	9	Autologous	Fever: 1 Mild hypotension: 1	NA	PR (2); SD (2)	[39]
NHL; renal carcinoma; HCC	12	Autologous	Fever: 2	NA	CR(3); SD (2)	[40]
Advanced NSCLC	59 (randomized)	Autologous (+ Chemotherapy)	None relevant	NA	Increased PFS and OS compared with control group (chemotherapy alone)	[42]
Resected HCC (adjuvant setting)	127 (randomized)	Autologous	Fever: 5	NA	Increased DFS compared with control group (no adjuvant treatment)	[41]
HCC (adjuvant setting)	85 (randomized)	Autologous	None relevant	NA	Decreased recurrence rate compared with controls (no adjuvant treatment)	[47]
Gastric cancer (Stage IV)	57 (randomized)	Autologous (+ chemotherapy)	None relevant	NA	Decreased tumor markers Improved QOL Increased 2 year life-span (compared with chemotherapy alone)	[43]
HCC	13	Autologous	Transient fever (most patients)	NA	Reduced tumor volume (3) Improved symptoms Decreased HBV-DNA load	[48]

*One of the two patients experienced also transient elevation of transaminases. One more patient experienced ventricular arrhythmia and transient hypotension during the infusion of CIK cells.
ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; CR: Complete response; DFS: Disease-free survival; EFS: Event-free survival; HCC: Hepatocellular carcinoma; HD: Hodgkin's disease; MDS: Myelodisplastic syndrome; MM: Multiple myeloma; NA: Not applicable; NHL: Non-Hodgkin's lymphoma; OS: Overall survival; PFS: Progression-free-survival; PR: Partial response; QOL: Quality of life; SD: Stable disease; TTP: Time to progression

with the infusion of luciferase-expressing CIK cells allowed to visualize the traffic and fate of these cells in allogeneic HCT recipients and operate a direct comparison with conventional T cells responsible for GVHD. Infused CIK cells displayed an early homing to spleen and lymph nodes with an expansion peak within the first week. This pattern was similar to that observed with fresh lymphocytes that, however, had a more rapid signal increase and resulted in the death of all recipient mice due to acute GVHD. Allogeneic CIK cells trafficked trough the same GVHD target organs but more transiently and with much less infiltration compared with conventional T cells [35]. Furthermore CIK cells, compared with conventional T cells, demonstrated a significantly lower acquisition of homing molecules, required for the entry of inflamed and GVHD target organs ($\alpha4\beta7$, CCR9, E-selectin, CXCR3 and CCR5) and a higher susceptibility to apoptosis. The same experiments confirmed a high and stable production of IFN-γ from CIK cells [35]. Interestingly with a similar murine model, but in minor-mismatched setting, it was demonstrated for the first time that proliferation and spreading of CIK cells may be driven also by differences in minor histocompatibility antigens. The observed pattern was similar to that observed in major-mismatched settings but with a lower speed of tissue propagation and a reduced intensity in the peak of photon emission. These findings are particularly interesting because they could be representative of the clinical settings, where the majority of HCT is performed between HLA-identical siblings, that display differences only at the minor antigen level.

4.3 Clinical studies

Early clinical applications of CIK-cells-based adoptive immunotherapy after allogeneic HCT confirmed the feasibility of this approach and the reduced propensity of these cells to cause GVHD. Following repeated infusions of donor derived CIK cells, in patients with hematological malignancies who relapsed after HCT, Introna *et al*. reported 4/11 (36%) cases of acute GVHD (≤ grade II). Three episodes occurred after HCT from HLA-identical siblings and one after HCT from a HLA-matched unrelated donor; two cases progressed to extensive chronic GVHD [36]. A similar low incidence of GVHD was described in a preliminary report from the Stanford group (one grade I acute and two limited chronic GVHD events) of ten patients, with relapsed hematological malignancies after allogeneic HCT, treated with infusions of donor CIK cells. The infusions were generally well tolerated, it is worthy of note is that two patients reported from the Stanford group experienced ventricular arrhythmias [37].

5. Conclusions

The infusion of donor lymphocytes after HCT has the power to increase the GVT effect in selected diseases but is associated with a high risk of inducing severe GVHD. The adoptive infusion of *ex vivo* expanded CIK cells hold great promises to overcome the main limitations of classic DLI. Their high *ex vivo* expansion rate, wide MHC-unrestricted antitumor activity and low GVHD potential represent the crucial properties for realizing an effective and safer alternative immunotherapy approach. Preclinical models clearly demonstrated how CIK cells, compared with conventional T lymphocytes, can operate a functional segregation between GVT and GVHD. Initial clinical trials successfully reported the feasibility and low GVHD incidence of adoptive infusion of CIK cells after HLA-identical HCT. New and larger clinical studies are intended to confirm these results and extend this approach across major HLA-barriers (e.g. HLA-aploidentical HCT), classically associated with a higher risk of GVHD.

6. Expert opinion

Increasing the GVT effect after HTC is a major goal in order to treat disease relapses and to extend this approach beyond the boundaries of hematological malignancies. The adoptive infusion of donor lymphocytes, even if effective in specific settings, is in need of improvements and refinements. The efficacy of DLI can not be considered universal, the associated incidence of severe GVHD is a major limitation and the efficacy against solid tumors remains anecdotal. Current efforts are directed towards manipulation of classic DLI in order to widen the spectrum of antitumor activity and realize a functional segregation between GVT and GVHD. From both preclinical studies and initial clinical trials, CIK cells seem an appealing alternative to conventional T lymphocytes. CIK cells present some crucial properties that compare favorably with other strategies of adoptive immunotherapy, like the infusion of tumor-specific cytotoxic T lymphocytes (CTLs) or even NK cells. The first property is the possibility of CIK cells to be *ex vivo* expanded to adequate numbers for effective and multiple *in vivo* infusions. The main limitations to the clinical application of CTLs specific for tumor-associated antigens (TAA), is in fact the difficulty of their *ex vivo* expansion. Circulating TAA-specific lymphocyte precursors are extremely rare and, currently, the optimal culture conditions for their *ex vivo* expansion are still not clarified. Similar problems are also true for NK cells with the additional pitfall of requiring high doses of IL-2 co-administration, associated with considerable toxicity [45,46]. The second characteristic that favors CIK cells is their wide MHC-unrestricted antitumor activity. This is similar to that exerted by NK cells but overcomes the restriction to a precise HLA-haplotype proper of TAA-specific T cells, consequently expanding the potential number of patients that could benefit from such approach. A third aspect that makes the infusion of CIK cells appealing in HCT settings is their demonstrated low propensity to induce GVHD. This is however not an advantage over TAA-specific T cells that would have an even higher grade of GVHD safety, being TAA not or minimally expressed on normal tissues. The initial clinical trials confirmed only a minimal GVHD incidence after the

infusion of donor-derived CIK cells [36]. It is however to be considered that, even if mild, the reported GVHD episodes occurred across minor-HLA barriers, indicating a residual alloreactive potential that might raise some concerns for their infusion across major-HLA barriers (e.g. haploidentical HCT) [12]. In this perspective, the report of two functionally distinct CIK cell subsets [12] could allow the depletion of the CIK cell subpopulation responsible for the residual alloreactivity, without affecting the tumor killing capacity, and could help in extending this approach across major HLA barriers.

A possible drawback of CIK cells might be their limited lifespan after expansion, lacking the preservation of a memory compartment that can provide an antitumor activity over an extended period of time. In this perspective it is likely that repeated multiple infusions will be required to obtain the maximum beneficial effect for the patients.

To successfully meet the new therapeutic challenges, the field of HCT should move toward a second-generation approach that exalts its immunotherapeutic value. We believe that in the next years HCT might bee envisioned as a functional tool to crate an immunological platform for further immunotherapeutic interventions that might integrate multiple strategies. It might be intended to exploit a MHC-unrestricted tumor attack with CIK cells and combine it with a TAA-specific approach, either infusing TAA-specific CTLs or with TAA vaccinations. Lastly, great interest has recently emerged about the role of T regulatory (Tregs) cells in tumor growth and their possible interaction with anti-tumor immunotherapies. It is expected that future strategies will include interventions intended to deplete or modulate the function of Tregs in patients' favor.

An intriguing future perspective, that might benefit from the adoptive infusion of CIK cells, is the possible extension of HCT beyond hematological boundaries. In the past years HCT as been attempted as an experimental immunotherapeutic treatment for metastatic solid tumors [1,2]. The initial great hopes however have been so far partially disappointed since, even if a GVT effect has been clearly demonstrated, clinical durable responses have remained anecdotal [3]. Additional immunotherapeutic interventions are needed to boost and maintain the demonstrated GVT effect and CIK cells could nicely fit into this picture. Their MHC-unrestricted tumor recognition might help in overcoming important immunological escape mechanisms like the downregulation of MHC molecules and the improper presentation of TAAs. The already discussed low GVHD potential might then induce the clinicians to attempt multiple adoptive infusions of CIK cells that could be started at earlier time points after HCT, hopefully encountering more favorable situations of limited tumor burden. We hope that future clinical trials of allogeneic HCT for patients with solid tumors will consider the adoptive infusion of CIK cells to obtain and boost a durable GVT effect. In conclusion CIK cells might be an extremely valuable tool to potentiate the immunological power of HCT and help with meeting current and new therapeutic challenges.

Declaration of interest

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