

Short Report

# Cytokine Induced Killer Cells as Promising Immunotherapy for Solid Tumors

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## Abstract

Cytokine-induced killer (CIK) cells are a heterogeneous subset of ex-vivo expanded T lymphocytes which present a mixed T-NK phenotype and are endowed with a MHC-unrestricted antitumor activity. The main functional properties of CIK cells may address some of the main limitations that are currently preventing the successful clinical translation of adoptive immunotherapy strategies. Clinically adequate quantities of immune effectors, sufficient for multiple adoptive infusions, may be obtained based on their relatively easy and inexpensive ex-vivo expansion starting from peripheral blood mononuclear cells. The MHC-unrestricted tumor-killing is mainly based on the interaction between NKG2D molecules on CIK cells and MIC A/B or ULBPs molecules on tumor cells; it has been proved effective against several solid and hematological malignancies and does not require any HLA-restriction increasing the number of patients that might potentially benefit from such approach. Finally, CIK cells present a reduced allo-reactivity across HLA-barriers with important clinical implications for their potential use as alternative to conventional Donor Lymphocyte Infusions after allogeneic hemopoietic cell transplant with a reduced risk of GVHD. In the present report we review the main functional characteristics of CIK cells discussing recent findings and future perspectives to improve their antitumor activity and potential clinical applications.

Key words: CIK cells; adoptive immunotherapy; solid tumors.

## Introduction

Adoptive immunotherapy holds great promises in the scenario of potential new approaches for the treatment of solid tumors refractory to conventional therapies. Crucial issues for all adoptive immunotherapy strategies include the obtainment of sufficient numbers of immune effectors, recognition of tumor targets and possible restriction to specific HLA-haplotypes. Cytokine-Induced Killer (CIK) cells are a heterogeneous subset of ex-vivo expanded T lymphocytes whose biological features make them appealing for adoptive immunotherapy, addressing some limitations associated with other strategies tar-

geting specific Tumor-associated antigens (TAA). The main functional properties that favorably characterize CIK cells are: 1) Ex-vivo expansion 2) Reduced allo-reactivity; 3) MHC-unrestricted tumor-killing.

## Ex-vivo expansion and phenotype of CIK cells

An important limitation preventing the successful clinical translation of several adoptive immunotherapy strategies is the obtainment of sufficient numbers of anti-tumor immune effectors and their in-vivo persistence after infusion into the patients. An

important positive characteristic of CIK cells is their easy, and relatively inexpensive, ex-vivo expansion [1, 2]. CIK cell precursors are CD3+ T lymphocytes, mainly with a naïve, CD4CD8 double negative (CD4-CD8-) phenotype [3]. They can be classically expanded starting from peripheral blood mononuclear cells (PBMC) but may also be generated from bone marrow or umbilical cord blood precursors [3, 4]. The standard culture conditions require three to four weeks with the timed addition of IFN- $\gamma$ , Ab-anti CD3 and IL2 [3]. IFN- $\gamma$  is only added on day 0, its main activity is to activate the monocytes, present in the initial bulk culture, which provide both contact-dependent (CD58/LFA-3) and soluble (IL12) crucial signals that favor the acquisition of a final Th1 phenotype and cytotoxic power of CIK cells [5-7]. The Ab anti-CD3 provides mitogenic signals to T lymphocytes subsequently sustained by IL2 that drives the expansion [8, 9]. Some groups have reported the addition of IL7 as beneficial to increase the cytotoxic potential of CIK cells [10]. After 3-4 weeks of culture the expansion rate is reported to be variable from few to more than 1000 fold [2, 11-13]. Such levels of expansion, also considering the availability of CD3+ starting precursors, are extremely favorable for subsequent clinical applications. The reason of the reported variability in the range of expansion rates is not clear, additional experimental strategies are currently under investigation to further ameliorate such numbers, especially for those patients who may be in the lower range ("poor expanders") and may less benefit from such approach. Alternative strategies are based on additional soluble factors added to culture conditions (Thymoglobulin, IL1, IL7) [14] [10], addition of transient allogeneic stimulation or depletion of T regulatory cells during the expansion culture [15]. Our group recently reported that a transient allogeneic stimulation with allogeneic irradiated PBMC may provide an important additional expansion boost to CIK cells without affecting their antitumor activity of safety profile [16]. Standard CIKs' culture conditions have been successfully validated under Good Manufacturers Practice (GMP) conditions [4] and have allowed recent applications into clinical trials. At the end of the expansion there is a heterogeneous population of CD3+ T lymphocytes, with two main subsets, respectively positive (CD3+CD56+) and negative (CD3+CD56-) for the simultaneous expression of CD56 membrane molecule. The CD3+CD56+ fraction is considered the main responsible for the MHC-unrestricted antitumor activity [3, 13]. Mature CIK cells are mainly CD8+ but CD4+CD8- cells can also be present in the bulk culture, less frequent are subsets with a double positive (CD4+CD8+) or double

negative (CD4-CD8-) phenotype. The terminally differentiated late effector phenotype (CD45RO+; CD27low; CD28low; CD62L-; CCR7-) is the more represented among CD3+CD56+ CIK cells while the CD3+CD56- counterpart exhibits more earlier memory characteristics [17].

### Alloreactivity of CIK Cells

A second clinically relevant property of CIK cells is their reduced alloreactive potential across MHC barriers that may result in a reduced risk for GVHD if adoptively infused after allogeneic Hemopoietic Cell Transplant (HCT) [13]. Early preclinical data, obtained from murine models of HCT, demonstrated that the adoptive infusion of allogeneic CIK cells across MHC-barriers was associated with a lower risk of acute GVHD compared to conventional T cells. The main biological explanation for this beneficial effect is likely linked to the abundant production of IFN- $\gamma$ , by expanded CIK cells, and its known protective action against GVHD [18, 19]. Compared to conventional T lymphocytes, allogeneic CIK cells displayed a significant lower acquisition of homing molecules, required for the entry of inflamed and GVHD target organs ( $\alpha$ 4 $\beta$ 7, CCR9, E-selectin, CXCR3 and CCR5) and a higher susceptibility to apoptosis [20].

Even if murine studies confirmed a reduced GVHD potential, initial clinical trials infusing donor CIK cells after HLA identical HCT, reported a 36% of GVHD incidence [21]. These events were of low grade but sufficient however to raise concerns about a residual alloreactivity of CIK cells that might become clinically relevant when challenged across major HLA-barriers. Preclinical studies confirmed that human CIK cells retain a behavior similar to conventional T lymphocytes, being able of intense proliferation if stimulated across major HLA-barriers. Such alloreactive-driven proliferation is however mainly restricted to the CD3+CD56- subset [13]. The first important implication is that a depletion of the "alloreactive" (CD3+CD56-) CIK fraction from the expanded bulk population may further reduce their alloreactive and consequent GVHD potential. This approach may be of particular relevance in peculiar HCT settings with augmented degree of HLA-mismatch between donor and recipient (i.e. Haploidentical HCT) and high risk of GVHD. A second possible implication of CIKs' residual alloreactivity is its exploitation to ameliorate their ex-vivo expansion. A little modification in the standard CIKs' culture conditions, with the transient addition of allogeneic irradiated PBMC as stimulators, was shown to significantly increase the final expansion rate and augment the percentage of CD3+CD56+ cells without

negatively affecting the antitumor activity or increasing their alloreactivity versus third party [16].

In general these findings are in favor of a possible use of CIK cells as alternative Donor Lymphocyte Infusion (DLI) following allogeneic HCT, with the possibility of further decreasing the GVHD in high-risk settings by depleting the CD3+CD56- fraction before the infusion. A second-generation DLI with CIK cells might increase the overall antitumor activity of HCT and improve its safety profile. Such positive evolution might help new experimental perspectives of HCT like those exploring its application as immunotherapy treatment for solid tumors.

### Tumor killing ability of CIK cells

The ability to efficiently kill tumor cells is the ultimate basic ability requested to immune effectors candidate for adoptive immunotherapy. CIK cells are endowed with a MHC-independent tumor killing capacity against both solid and hematologic malignancies. The antitumor activity is mainly, even if not exclusively, associated with the CD3+CD56+ fraction and it is not due to the highest percentage of CD8+ cells compared to the CD3+CD56- subset [13]. The exact mechanism involved in tumor recognition and killing is not completely known but a main role seems to be played by the NKG2D molecule expressed on the membrane of CIK cells that interacts with MHC-unrestricted ligands on tumor cells [22]. The main targets recognized by NKG2D are MIC A/B (MHC class I related molecules) and proteins of the ULBP family (ULBPs 1,2 and 3) [23-25] while the final killing is perforin and granzyme mediated. NKG2D has a co-stimulatory role on conventional T lymphocytes, while on CIK cells it acquires a tumor-receptor function, following the ex-vivo activation with IL2 and upregulation of the DAP10 adaptor molecule [22]. MIC A/B are stress-induced proteins expressed by a wide range of malignant or transformed cells, their expression is MHC-unrestricted and not limited to a specific histotype [26]. The expression of such ligands may also vary within the same tumor type, according to variations in the biological behavior and aggressiveness of tumor phenotype. We recently reported, in a breast cancer preclinical model, that acquisition of resistance to treatment with Trastuzumab could associate with the increased expression of MIC A/B on tumor cells and consequent augmented susceptibility to immune-mediated killing by CIK cells [27]. The antitumor activity of CIK cells have been described in vitro against several hematologic and solid malignancies including lung, gastrointestinal and mesenchymal tumors [13, 28-31]. The tumor killing activity was confirmed in vivo with murine models of tumor

xenograft [32-34]. CIK cells are usually infused intravenously in the animal, they were shown to efficiently infiltrate tumor sites and demonstrated a superior activity compared to classic LAK cells against Non Hodgkin Lymphoma [2, 3, 35]. The majority of pre-clinical data were produced using allogeneic tumor cell lines as targets to demonstrate the efficacy of CIK cells against solid tumors. While cell lines may represent a useful tool to generate important proof of concepts, they may not fully account for the unique patient-specific biological and immunogenic characteristics of autologous tumor cells. Furthermore results may be influenced, at least in part, by HLA-mismatches between CIK cells and tumor targets. It is of great importance that future experiments may consider a design involving autologous tumor settings, to generate reliable patient-specific data that could serve as basis for subsequent clinical translation. In this direction first reports came by the Stanford group that showed activity of CIK cells against primary human ovarian cancer [33] and our group that recently reported data of intense tumor killing by patient-derived CIKs against autologous bone sarcoma cells [36]. An intriguing perspective for adoptive immunotherapy strategies with CIK cells is the emerging possibility to redirect and potentiate their antitumor activity with TAA bispecific antibodies [37] or their engineering to produce specific cytokines enhancing the cytotoxicity [38] or with tumor-receptor molecules [39] [40, 41].

### Clinical Studies

The clinical translation of adoptive immunotherapy with CIK cells as treatment for patients with solid tumors is currently the object of clinical trials and their number has increased in the very recent years. The first clinical experience included 10 patients with metastatic renal carcinoma, colorectal cancer and lymphoma. Circulating CIK cells persisted for up to 2 weeks after the infusion, no relevant associated toxicities were reported. One patient with lymphoma obtained a complete response while six patients had progressive diseases and three did not experience any change. In this first study, CIK cells were engineered to auto-generate IL-2 [38]. Other clinical trials subsequently confirmed the safety and feasibility of this immunotherapy approach along with demonstration of initial clinical activity. In a phase I study reported on 12 patients with advanced non-Hodgkin's lymphoma (NHL), metastatic renal cancer or hepatocellular carcinoma (HCC), the adoptive infusion of 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 responding patients received the simultaneous subcutaneous injection of low dose IL-2 and IFN- $\alpha$  respectively [42]. The above mentioned are, to our knowledge, the only two trials with CIK cells for solid tumors that have currently been published in Europe or U.S. A higher number of clinical studies have been recently performed in Asia with very interesting clinical results. In the setting of hepatocellular carcinoma, adjuvant infusions of autologous CIK cells after surgical resection, showed a significant increase in disease-free survival [43, 44]; an interesting observation was the reduction of HBV viral load associated with CIK treatments [45]. The adoptive infusion of CIK cells produced interesting clinical responses in patients with lung and gastric cancers with a reported positive impact on survival in association with chemotherapy [46-49]. A summary of clinical trials with CIK cells for the treatment of solid tumors is reported in table 1.

Overall these studies confirmed the high safety of adoptive immunotherapy with CIK cells with important indications suggestive for a clinical activity; the heterogeneity in methods and criteria for response assessment make still early and difficult to draw definitive statements concerning tumor response or impact on survival. Recently it has been created an

International Registry on CIK Cells (IRCC) with the aim of collecting data worldwide and setting standard criteria to report results from clinical trial with CIK cells [50]. Similar initiatives are very important as they could help a rational development of clinical research in the field and provide useful tools to analyze past and future results. These issues are crucial in order to derive objective and reliable conclusions on the efficacy of CIK cells as adoptive immunotherapy for cancer.

## Conclusions

CIK cells represent a promising tool in the scenario of cancer adoptive immunotherapy strategies. Their easy and inexpensive ex-vivo expansion, along with the MHC-unrestricted tumor killing ability may overcome some crucial problems that have limited the diffusion and clinical translation of other immunotherapy approaches. The reduced alloreactivity across major HLA-barriers may open new perspective applications of CIK cells as alternative to conventional DLI after HCT, helping the extension of HCT to other experimental settings like the treatment of solid tumors.

**Table 1.** Clinical trials of adoptive immunotherapy with CIK cells as treatment for solid tumors.

Disease	Patients	Type of CIKs	Toxicity	Clinical Responses	Ref.
Colorectal and renal carcinoma; NHL	10	Autologous	Fever: 3	CR (1) SD (3)	[38]
NHL; Renal Carcinoma; HCC	12	Autologous	Fever: 2	CR(3); SD (2)	[42]
Advanced NSCLC	59 (randomized)	Autologous (+ Chemotherapy)	None relevant	Improved PFS and OS	[46]
Resected HCC (adjuvant setting)	127 (randomized)	Autologous	Fever: 5	Improved DFS	[43]
HCC (adjuvant setting)	85 (randomized)	Autologous	None relevant	Reduced recurrence rate	[44]
Gastric cancer (Stage IV)	57 (randomized)	Autologous (+ chemotherapy)	None Relevant	Reduced Tumor Markers; Improved QOL	[47]
HCC	13	Autologous	Transient Fever (most of pts)	Reduced tumor volume (3); Improved symptoms; Decreased HBV-DNA load	[45]
Various (NLSLC; gastrointestinal)	40 (randomized)	Allogeneic (CB-derived)	None Relevant	Improved PFS and OS	[49]
Gastric Cancer	156 (randomized)	Autologous (+ Chemotherapy)	None Relevant	Improved Survival	[48]



## Conflict of Interest

The author has declared that no conflict of interest exists.

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