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T cells expressing CD123 chimeric antigen receptors for treatment of acute myeloid leukemia

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Abstract

Purpose of review—The purpose of this article is to discuss the rationale of targeting CD123 using chimeric antigen receptor (CAR) T cells for the treatment of leukemia.

Recent findings—CD123 is a leukemia associated antigen that expresses at high levels in leukemic stem cells and leukemic blasts and low level in normal hematopoietic stem / progenitor cells. Immune-based therapies targeting CD123 are being developed. Preclinical data suggest that CD123 CAR T cells exhibit potent anti-leukemic activity and various impacts on normal hematopoiesis.

Summary—CD123 is an attractive surface target for novel anti-leukemic therapies. CD123 CAR T cell based immunotherapy is a promising treatment for patients with relapsed or refractory acute myeloid leukemia.

Keywords

CD123; AML; hematopoiesis; allogeneic stem cell transplant

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease group characterized by the uncontrolled clonal proliferation of myeloid precursors in the bone marrow and blood resulting in accumulation of leukemic blasts, and severe impairment of normal hematopoiesis [1,2]. AML is the most common acute leukemia in adults and has the highest death rate of all leukemias. An estimated 20,830 people in the United States will be diagnosed with AML and 10,460 deaths are projected to occur from AML in 2015 [2]. In the last 40 years, there was a only small increment in the five year relative overall survival for

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Conflicts of interest

None

AML patients from a dismal 6.3% between 1975 to 1980 to 23.9% between 2007 to 2012 (<http://seer.cancer.gov/statfacts/html/leuks.html>). Compared to other hematologic malignancies, the standard upfront treatment remains unchanged despite the advancement in our understanding of leukemic biology and emergence of new agents. Current induction chemotherapy can produce initial complete remission (CR) in almost 70% of young adult patients. However, 43% of patients will eventually relapse, and 18% never attain CR at frontline induction treatment [3**]. The 5-year overall survival rate for AML patients after the first relapse ranges from 7 to 12% [3,4]. Allogeneic hematopoietic stem cell transplantation (alloHSCT) provides the best chance to cure a patient with relapsed or refractory AML. It is the preferred treatment route following a second remission and can lead to 5-year disease-free survival in 40 to 50% of patients [5,6], highlighting the susceptibility of AML to immune-based therapy. Unfortunately, using current treatment strategies, the second CR rate is achieved in only about half of relapsed patients who previously attained a CR that lasted longer than 6 months, and is only 20% or fewer of patients with primary refractory disease and those with an initial CR lasting less than 6 months. In addition, the considerable complications of conventional salvage chemotherapy may worsen the performance status and organ function of the patient and decrease the chance of a successful alloHSCT. Therefore, novel therapeutics with higher efficacy and tolerable toxicity for patients with AML after relapse or induction failure are desperately needed.

DIFFERENTIAL EXPRESSION OF CD123 ON NORMAL HEMATOPOIETIC AND LEUKEMIC STEM CELLS

CD123 is a glycoprotein containing 360 amino acids. It is also known as the transmembrane α subunit of the interleukin-3 receptor (IL-3R α), which together with CD131 forms a high affinity IL-3R. Upon binding of IL-3, IL-3R promotes cell proliferation and survival. CD123 is normally expressed at high levels on plasmacytoid dendritic cells, and basophils, at low levels on monocytes, eosinophils and myeloid dendritic cells [7].

The last two decades have seen an increasing knowledge regarding the leukemic stem cells (LSCs). These cells are resistant to conventional chemotherapeutic treatment due to their quiescent nature and give rise to leukemic blasts [8]. This together with their self-renewing capacity renders them responsible for leukemic progression and recurrence. A successful anti-leukemic treatment that leads to long-term remission should include effective ways to eradicate LSCs. Identification of targets on the surface of LSCs are of particular interest in recent years as immune based therapies are on the rise.

The discovery of CD123 as an overexpressed cell surface molecule on AML blasts by Jordan et al. [9] marked the beginning of studying, and targeting, this receptor in AML and other hematologic malignancies. This pioneering study demonstrated that CD123 is expressed at substantially higher levels in leukemic cells compared to their normal hematopoietic counterparts. Its expression was observed on AML blasts and LSCs in 75 to 89% of AML patients [10*]. This is in sharp contrast to the low or not detectable expression on normal hematopoietic stem cells (HSCs). The biological significance of CD123 expression in primary AML centers on the signal transducer and activator of transcription 5

(STAT5). For example, primary AML samples have spontaneous phosphorylated STAT5 which can lead to IL-3 independent proliferation and resistance to apoptosis. Additionally, CD123 expression in primary AML has been correlated with poor prognosis, and FMS-related tyrosine kinase 3-internal tandem duplication (FLT3-ITD) and nucleophosmin 1 (NPM1) mutations. Apart from AML, CD123 overexpression was identified on a variety of hematologic malignancies including B cell lineage acute lymphoblastic leukemia, chronic myeloid leukemia, plasmacytoid dendritic cell neoplasm, and hairy cell leukemia [11].

TARGETING CD123 IN HEMATOLOGIC MALIGNANCIES

Unlike CD33, which expresses at high levels on both leukemic and normal hematopoietic cells [12], CD123 expression on normal HSCs is low to negligible [9]. The differential expression of CD123 on normal HSCs and LSCs makes CD123 an attractive target for AML treatment.

SL-401 was one of the first, and most examined, immunotherapies developed targeting CD123 [13]. SL-401 is composed of the catalytic and translocation domain of diphtheria toxin fused to IL-3. Upon interaction with CD123 on leukemic cells, SL-401 is internalized and as a result new protein synthesis of the leukemic cells is inhibited. In pre-clinical studies, the activity of SL-401 has correlated with IL-3 receptor beta chain expression level [14]. Unlike CD123, IL-3 receptor beta is not commonly expressed in AML. In a phase I clinical trial, seven of 9 patients with blastic plasmacytoid dendritic cell neoplasm achieved major response to a single course of SL-401 treatment including 5 complete responses and 2 partial responses. However the median duration of response was only 5 months [10].

Several antibody-based therapies have been developed targeting CD123. An anti-CD123 monoclonal antibody (7G3, CSL360) has completed phase I clinical trials with one of 27 patients achieving a durable complete response [15], indicating neutralizing IL3R function is ineffective in treating AML. CSL362 is a humanized, Fc receptor engineered, and affinity-matured version of CSL360 with enhanced ADCC activity. Potent anti-leukemic effect was observed in mouse model [16]. It has begun early phase clinical studies in AML patients who achieved currently in CR and at high risk for relapse [17*]. Several bispecific antibody based reagents targeting CD123 have recently been developed [18*]. A dual affinity retargeting (DART) molecule (MGD006) targeting CD123 and CD3 demonstrated anti-leukemic activity in preclinical evaluations and has been infused in patients with relapsed/refractory AML [19]. Finally, a bispecific antibody (XmAb14045) targeting CD123xCD3 has completed nonhuman primate toxicology studies and exhibited clearance of normal CD123 expressing cells in the blood and bone marrow [20]. To date, the CD123 targeting therapies to be used in early phase clinical trials, have been shown to be safe with no major adverse effects on hematopoiesis reported. Their anti-leukemic activities in humans are still being investigated.

PRE-CLINICAL CD123 CAR T CELL DEVELOPMENT

An alternative therapy for the treatment of relapsed/refractory AML utilizes T cells expressing a chimeric antigen receptor (CAR) that redirects T cell specificity towards CD123, in an MHC-independent manner [21,22]. Unlike conventional chemotherapeutics

and antibody-based therapies, CAR T cells have the potential to proliferate and persist in humans for many years, establishing long-term immunity [23,24]. In most cases, CARs consist of a single-chain variable fragment (scFv) from a monoclonal antibody fused to the signaling domain of CD3 ζ , and may also contain a co-stimulatory endodomain [21,22]. Recently, groups from Memorial Sloan-Kettering Cancer Center, the Fred Hutchinson Cancer Research Center, the University of Pennsylvania and National Cancer Institute have reported on the achievement of complete remissions in patients with relapsed or refractory acute lymphoblastic leukemia treated with CD19-specific CAR T cells, demonstrating the therapeutic potential of this immunotherapy in the setting of acute leukemia [25–27].

We are the first to generate and test T cells expressing second generation CD123 CAR with a CD28 costimulatory domain (CD123-28 CAR) in treating AML in a preclinical study [28**]. Our group described two second-generation CD123 CARs with CD28 derived costimulation. When expressed in primary human T cells, both of the CD123 CAR T cell products exhibited anti-leukemic activity *in vitro* and *in vivo* in a xenogeneic mouse model of AML. Furthermore, when expressed in T cells from AML patients, these CD123 CARs could redirect patient-derived T cell cytolytic activity against their autologous leukemic blasts. When CD34⁺ cells from normal cord blood were cocultured with CD123 CAR T cells, normal progenitor colony formation was not abolished, suggesting preservation of normal hematopoietic progenitors from CD123 CAR T cell targeting. In line with our results, Tettamanti et al [29] using cytokine-induced killer (CIK) cells expressing a first generation CD123 CAR, a CAR without any costimulatory domain, demonstrated robust cytolytic activity against AML cell lines and primary AML patient samples *in vitro*. Additionally, the group demonstrated that CD123-CIK cells did not eliminate primary and secondary colony formation by hematopoietic stem and progenitor cells *in vitro*. The same group also generated and tested a third generation CD123 CAR with CD28 and OX40 as costimulatory domain (CD123-28OX40 CAR) [30**]. These CAR CIK cells exhibited potent anti-leukemic activity. More importantly, they only had limited killing on normal cord blood or human bone marrow derived hematopoietic stem or progenitor cells in xenograft models. However, works from Gill et al. [31**] have raised serious safety concerns of targeting CD123 for AML patients. Using T cells transduced with a second generation CD123 CAR containing a 4-1BB costimulatory domain (CD123-41BB CAR), they reported effective elimination of AML cells in a patient derived xenograft model of AML and severe impairment of normal hematopoiesis in NSG mice using CD34-selected fetal liver cells as a source of hematopoietic progenitors. It is worth pointing out that the CD123 expression level is higher on human fetal liver CD34⁺ cells than CD34⁺ cells derived from cord blood or human bone marrow [32].

While it is tempting to compare the potency and on-target off-tumor activity of these CD123 CARs, the multitude of differing variables between the studies described should deter from such comparisons. While the benefits of incorporating at least one costimulatory domain to the CAR constructs are well established, the choice of scFv, and extracellular spacer domain, the T cell subsets, and the manufacturing process remain an area of intense research. The optimal CD123 CAR design, like all other CAR configurations, will have to be determined empirically.

CLINICAL TRANSLATION OF CD123 CAR T CELL IMMUNOTHERAPY

We have recently successfully developed a clinical manufacturing platform to produce patient derived CD123 CAR T cells using clinical grade lentivirus encoding the CD123-28 CAR and have validated the specific cytolytic activity of the engineered CD123-CAR T cells against autologous patient leukemic blasts. The FDA on 5/26/2015 approved our CD123 CAR IND. A first-in-human phase 1 clinical trial using this CAR for patients with relapsed or refractory AML is currently in preparation (Clinicaltrials.gov identifier NCT02159495). In this trial, autologous patient derived CD123 CAR T cells will be used as a salvage therapy and serve as a “bridge-to-transplant” in an effort to achieve maximal disease reduction in patients prior to their receiving an allogeneic hematopoietic stem cell transplant. To address the potential concern of “on-target off-tumor effect” of targeting CD123 on normal hematopoiesis, this study has included multiple safe guards to circumvent such an occurrence. First, all patients before receiving autologous CD123 CAR T cell infusion, must have an identified allogeneic stem cell donors or donor source such as cord blood. This will be used as a means to rescue hematopoiesis should there is CD123 CAR related irreversible cytopenia. Second, the use of a second generation CAR with an IgG spacer and CD28 costimulatory domain will likely limit *in vivo* persistence of the CD123 CAR T cells as previously demonstrated for other CAR products incorporating the similar domains [33]. This will reduce the risk of prolonged cytopenia and further minimize the risk of the potential cytolytic activity of the CD123 CAR T cells on allogeneic CD34+ stem cells during the ensuing alloHCT. Third, our vector construct contains a truncated Epidermal Growth Factor Receptor (EGFRt). Besides its role as a selectable, tracking marker for transduced T cells, EGFRt may also act as a suicide switch upon administration of cetuximab [34]. Preclinical work from our group has demonstrated effective elimination of transduced T cells mediated by interaction between EGFRt and cetuximab. However, we acknowledge that the kinetics of this conditional suicide gene remains to be determined in humans.

At present time, after achieving complete remission upon CD123 CAR T cell therapy, patients are strongly encouraged to proceed to allogeneic stem cell transplant, which remains the current standard of care for patients with relapsed or refractory leukemia in second remission. Whether CAR T cell therapy can replace alloHCT in this patient population requires extensive prospective clinical trial testing. AlloSCT is also necessary in the event of profound cytopenia due to elimination of normal hematopoietic cells by the CD123 CAR T cells. This raises a potential application of incorporating CD123 CAR T cells into a myeloablative conditioning regimen preceding the alloSCT.

Another group of patients who might potentially benefit from CD123 CAR T cell therapy is patients with relapsed or refractory BPDCN. BPDCN is an aggressive blood cancer with initial skin involvement and rapid progression to leukemic phase [35]. The prognosis of this disease is extremely poor [36]. A unique feature of this malignancy is the uniformly high expression of CD123 on the tumor cell surface. In theory, a T cell based therapy has the potential advantage of delivering a deeper remission state and leading to prolonged remission duration. It is therefore, of great interest to test CD123 CAR T cells in these patients.

Serious cytokine release syndrome, neurotoxicity and treatment related mortality have been reported in ALL patients treated with CD19 CAR T cells [26,27,37]. A recent CD33 CAR CIK therapy in a single AML patient also reported CRS [38]. Hence it is reasonable to expect that AML patient responders to CD123 CAR T cell therapy are at risk for the abovementioned toxicities, especially the ones with high blast counts. These patients should be closely monitored by experienced immunotherapists once T cells are infused. We have also developed a working algorithm to manage these toxicities. Whether CD123 CAR T cell therapy will elicit similar acute adverse events seen in CD19 CAR T cell trials remains to be defined. This trial provides a unique learning opportunity to expand our knowledge on conducting CAR T cell therapy beyond CD19 CARs.

CONCLUSION

There is considerable evidence that CD123 has emerged as an attractive immunotherapeutic target for AML in recent years. The differential expression of CD123 on leukemic stem cells and normal hematopoietic cells makes CD123 a therapeutically important tumor associated antigen. Preclinical studies testing various CD123 CARs have demonstrated the potency of T cell based immunotherapy in eradicating AML and built a solid foundation for translating this therapy to clinical setting. CD123 CAR-based T cell adoptive immunotherapy have the potential to change the paradigm in AML treatment. Detailed response assessment, carefully designed correlative studies, toxicity documentation from the soon-to-open phase 1 clinical trial will shed light on the impact of CD123 CAR T cells on leukemic cells and normal hematopoiesis. Additional studies combining CD123 CAR T cell therapy with other immune based therapies such as immune inhibitory checkpoint blockade might further improve the anti-leukemic response rate and result in a better survival outcome.

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*of special interest

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KEY POINTS

- The current treatment for AML is unsatisfactory. Novel therapy is urgently needed.
- CD123 is an excellent leukemia associated antigen given its differential expression in leukemic stem cells and leukemic blasts and normal hematopoietic stem / progenitor cells.
- CD123 CAR T cells exhibited potent anti-leukemic activity and various impacts on normal hematopoiesis in preclinical studies
- A Phase 1 clinical trial using CD123 CAR T cells for patients with relapsed or refractory AML is underway.