Pre-clinical Studies of Allogeneic Anti-CD123 CAR-T Cells for the Treatment of Blastic plasmacytoid dendritic cell neoplasm (BPDCN)

#2625

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Abstract

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, aggressive hematologic malignancy. Data on the biology of BPDCN is limited, patient outcomes have historically been poor and there is no established standard of care. Nearly 100% of patients with BPDCN overexpress CD123, and considering the unmet medical need for these patients, targeting CD123 emerged as an attractive therapeutic target given its accessibility (cell surface) and differential expression.

UCART123 product candidate is based on genetically modified allogeneic T-cells (derived from healthy donors, so-called "off the shelf") containing an anti-CD123 CAR (CD123 scFv-41BB-CD3ζ) and a RQR8 depletion ligand that confers susceptibility to rituximab. To minimize the risk of graft-versus-host disease, the expression of the T-cell receptor αβ (TCRαβ) is abrogated through the inactivation of the *TCRα* constant (*TRAC*) gene, using Cellectis' TALEN® gene-editing technology.

The specific *in vitro* anti-tumor activity of UCART123 cells against an established BPDCN cell line (CAL-1) or CD123$^+$ primary BPDCN samples was demonstrated using several assays. The cytotoxicity assay showed that UCART123 cells eliminate CAL-1 cells (65.6±2.7%) at low E:T ratios (1:1) and induced specific lysis of all BPDCN samples (from 15.7% to 77.9%) (Fig. 1). This cytotoxic activity was confirmed by T-cell degranulation and the secretion of IFNγ and other cytokines (IL2, IL5, IL6, IL-13 and TNF-α) by UCART123 cells when cultured in the presence of BPDCN cells (Fig. 2).

To evaluate *in vivo* anti-tumor activity of UCART123 cells, we established three patient-derived xenografts (PDX1-3) from patients with relapsed BPDCN in NSG-SGM3 (NSG-S) mice. Upon engraftment, mice were randomized into five groups; and received either a single tail vein injection of vehicle, 10×10^6 TCRαβ KO control T-cells, or UCART123 cells (1×10^6, 3×10^6 or 10×10^6 cells). In PDX-1 model, all mice in vehicle-treated group died by D53, with high tumor burden in peripheral blood (PB, 30-65% engraftment), spleen and bone marrow (BM). UCART123 cells were detected in spleen (16.4% CAR+ cells) and BM (1.1% CAR+ cells) using human CD5 antibody or CD123-Fc protein, in a mouse injected with 10×10^6 UCART123 at D57 (Fig. 3). Six out of 9 (67%) mice in 10×10^6 UCART123 were alive and disease-free at the end of the study (D299), with remaining 3 mice dying without evidence of BPDCN (Days 155-280)

In PDX-2 model, UCART123 cells were injected upon confirmation of high tumor burden (75.0±21.1% engraftment in PB at D21). On D28, all the mice in 10×10^6 UCART123 group died. A high level of IFNγ was detected in PB from mice 2 days after treatment with UCART123 (Fig. 4A, right panel). On D31-32, mice in vehicle group died with tumor burden approaching 100%, indicating extremely aggressive disease. Cytokine storm and/or extremely high tumor burden may have contributed to the demise of UCART123 treated mice. We repeated this *in vivo* PDX-2 study and started treatment at an earlier time point (D14, when engraftment reached 1% in BM). On D34, all mice in vehicle group were sacrificed due to tumor progression. In contrast, all mice in 10×10^6 UCART123; 8 out of 9 mice in 3×10^6 UCART123; and 5 out of 9 mice in 1×10^6 UCART123 were still alive at the end of the study (Day 210), without evidence of detectable BPDCN by PB flow cytometry (Fig. 4B).

In PDX-3 model, 49 days after primary BPDCN sample injection, all the mice in vehicle were sacrificed due to high tumor burden in spleen and BM. In this model, we observed the emergence of CD123$^-$ BPDCN cells in all groups of UCART123-treated mice, causing relapse and early death after initial response (Fig. 5). Mechanisms of CD123 loss are under investigation.

In summary, UCART123 causes specific killing of BPDCN cells, associated with antigen-specific T-cell degranulation and robust levels of IFNγ production. Our preliminary data indicate long-term persistence of UCART123 cells *in vivo* in an NSG-S model of primary BPDCN, and confirm expected risk of deadly cytokine release syndrome at high tumor burden. Most importantly, UCART123 therapy results in BPDCN eradication and long-term disease-free survival in a subset of primary BPDCN PDX models. However, loss of CD123 expression may be anticipated and requires further studies. These results demonstrate pre-clinical proof-of principle of high anti-BPDCN activity of allogeneic UCART123 cells. A phase I trial of UCART123 in BPDCN is opened for enrollment (NCT03203369).

Cytotoxic activity of UCART123 cells against primary BPDCN cells *in vitro*

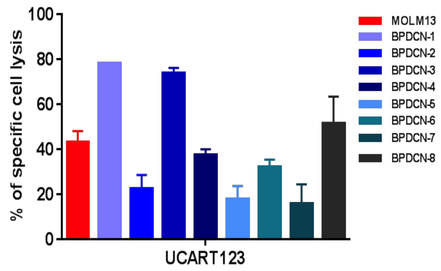


Figure 1. Cytotoxic activity of UCART123 cells against primary BPDCN cells *in vitro*. Specific cytotoxic activity against target cells for each of the CD123(+) cell line or patient samples upon 16h co-culture with either UCART123 cells or non-transduced TCRαβ-deficient T-cells (TCRαβ KO). Frozen UCART123 and NTD T-cells were thawed and immediately co-cultured with target cells. Each point represents the data obtained from triplicate experiments and the mean +/- SD value is shown.

IFNγ release assay

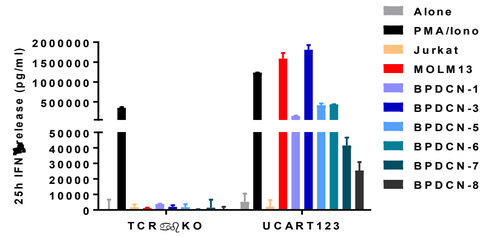
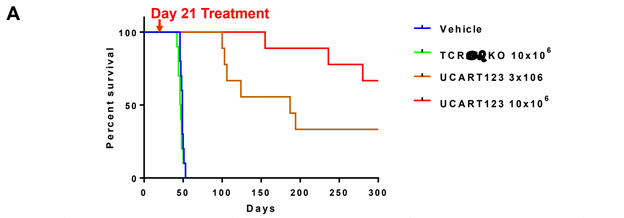


Figure 2. IFNγ release assay upon co-culture of UCART123 cells, or TCRαβ KO T-cells with CD123(-) or CD123(+) cells. IFNγ was examined using BioLegend's LEGENDplex™ assay. PMA/Ionomycin was used as a positive control.

UCART123 therapy results in BPDCN eradication and long-term disease-free survival in a primary BPDCN PDX model



B

	Engraftment at day 267 (CD123+ CD56+)	Engraftment at day 279 (CD123+ CD56+)
UCART123 3×10^6 groups	0.1%	0.0%
	0.0%	0.0%
	0.2%	0.0%
UCART123 10×10^6 groups	0.0%	0.1%
	0.1%	0.0%
	0.2%	0.0%
	0.1%	0.0%
	0.0%	0.0%
	0.2%	0.0%
	0.1%	0.0%

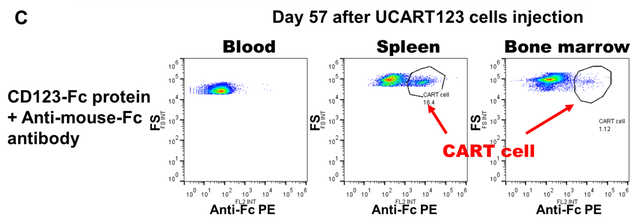


Figure 3. UCART123 treatment results in long-term survival in primary BPDCN PDX model. NSGS mice were injected with a primary BPDCN sample BPDCN-1 (68.4% CD123(+) cells, 2×10^6 tail veil injection at day 0). Circulating tumor burden was measured by flow cytometry in peripheral blood samples by retro-orbital bleeding after 21 days, after which mice were treated with a single injection of vehicle, 10×10^6 TCRαβ KO T-cells, 3×10^6 UCART123 or 10×10^6 UCART123 cells. (A) Treatment with UCART123 resulted in significant extension of mice survival. Three mice in 10×10^6 UCART123 died without evidence of BPDCN (Days 155-280) (B). UCART123 reduce or eliminate circulating tumor (BPDCN cells collected on the indicated days (post tumor injection), using 9F5 monoclonal CD123-PE antibody (555644, BD Pharmingen) and B159 monoclonal CD56-APC antibody (555518, BD Pharmingen) gating on viable cells (DAPI(-)). (C). Analysis of the presence of UCART123 cells in one mouse of 10×10^6 UCART123 group on D78 sacrificed for flow cytometry analysis without clinical sign of tumor progression.

Increased levels of IFNγ production after treatment of mice presenting high tumor burden

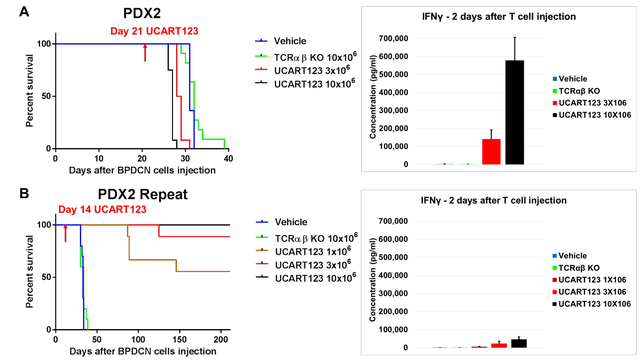


Figure 4. PDX2 model. NSG-S mice were injected with a primary BPDCN2 sample (PDX2). When engraftment was confirmed, mice were randomized into cohorts of 11 as following: vehicle; 10×10^6 TCRαβ KO control T cells; 3×10^6 UCART123 cells or 10×10^6 UCART123 cells. Top, treatment was started on day 21 (75.0±21.1% circulating BPDCN); Bottom, treatment was started on day 14 (0% circulating BPDCN, 1.0% engraftment in BM). Cytokine levels were evaluated 2 days after injection of T-cells in plasma of all mice and are graphed on the right panels. Mice in UCART-treated group started at high tumor burden died earlier than controls (A). On the contrary, UCART123 extended survival and cured a significant fraction of mice when treatment was initiated at low tumor burden (B).

Loss of CD123 leads to escape from UCART123 therapy and causes early relapses

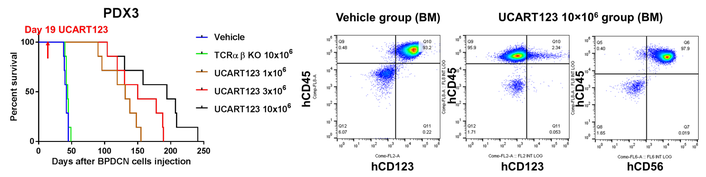


Figure 5. PDX3 model. NSG-S mice injected with a primary BPDCN3 sample were randomized on Day 19 upon engraftment (0% engraftment in PB, 13.9% and 33.8% engraftment in BM from 2 mice) to receive vehicle; 10×10^6 TCRαβ KO control T cells; 1×10^6 UCART123 cells, 3×10^6 UCART123 cells or 10×10^6 UCART123 cells. Left, survival of mice in different therapy groups was estimated using Kaplan-Myer method. Right, expression of CD123 on tumor cells isolated from bone marrow of diseased vehicle-treated or CART-treated mice upon sacrifice from disease, using CD123-PE antibody, CD45-APC-Cy7 antibody and gating on viable cells (DAPI(-)). Tumor cells from UCART123-treated mice were CD123(-) but remained CD56(+).

Conclusions

- UCART123 causes specific killing of BPDCN cells, associated with antigen-specific T-cell degranulation and robust levels of IFNγ production.
- UCART123 allogeneic therapy results in BPDCN eradication and long-term disease-free survival in primary BPDCN PDX model.

Acknowledgement

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