Targeting DLK1 improves survival in patient-derived xenograft models of myeloid leukemia associated with Down syndrome

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Key Points:

- We identified DLK1 as a novel target differentially expressed in myeloid leukemia associated with Down syndrome (ML-DS).
- DLK1-targeted antibody drug conjugate was effective in curbing leukemia progression in patient-derived xenograft models of refractory ML-DS

Abstract

Myeloid leukemia associated with Down syndrome (ML-DS) is a rare pediatric cancer. Current standard-of-care includes cytotoxic chemotherapy. 10-20% of patients develop relapsed leukemia or have refractory disease. These patients have a 3-year event-free survival less than 21%. Novel therapy options are needed to improve survival in these patients with relapsed or refractory disease. Our transcriptome analysis to identify transmembrane proteins that are overexpressed in ML-DS compared to normal bone marrow specimens revealed DLK1 as one of the topmost modulated targets. We show that DLK1 knockout in ML-DS cells significantly reduced proliferation *in vitro* and delayed engraftment *in vivo*. Furthermore, targeting DLK1 via antibody drug conjugates significantly prolonged survival compared to control isotype antibody drug conjugate in patient-derived xenograft models of refractory ML-DS. Taken together, targeting DLK1 may be a novel treatment option for refractory ML-DS patients.

Introduction

Although the risk of developing solid malignancies in individuals with Down syndrome (DS) is lower than the general population [1-3], they have a 500-fold increased incidence of leukemia of myeloid origin (ML-DS) [4,5]. ML-DS is often preceded by a preleukemic phase termed transient abnormal myelopoiesis (TAM) [6]. The 5-year event-free survival rates for patients with ML-DS have increased over the past 20 years and are now approaching 90% [7]. However, the 10-20% patients who do not respond to treatment or who exhibit early disease relapse have extremely poor 3-year event-free survival rate of less than 21% [8]. Therefore, novel therapy options are urgently needed to improve the outcome in ML-DS patients with refractory disease.

Delta-like non-canonical notch ligand 1 (DLK1), is known to be overexpressed in various solid tumors [9] and hematological malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [10,11]. DLK1 is an imprinted gene, usually expressed by the paternal allele [12]. Aberrant DLK1 expression in AML has been identified to be a result of biallelic expression [13]. However, there are no reports on DLK1 expression in ML-DS.

Although DLK1 is expressed during embryonic development, it's expression is almost undetectable in majority of healthy tissues postnatally [14] and in normal CD34+ cells [10], making it a good therapeutic target. DLK1 targeting modalities shown to be effective in curbing tumor growth include antibody-drug conjugate [15,16], chimeric antigen receptor T cells in preclinical models of hepatocellular carcinoma [17], and radioimmunotherapy using 125I-labeled anti-DLK1 antibody in lung cancer models [18]. Furthermore, given the role of DLK1 in promoting stemness in solid tumors [19,20], DLK1 targeted treatment options are expected to be effective in eradicatnig the leukemia stem cells.

In this study, we performed transcriptome analysis using cell line/PDX models of ML-DS in comparison with normal bone marrow-derived CD34+ cells to identify novel targets that are differentially regulated in ML-DS. We identified DLK1 as a novel cell surface target expressed in ML-DS patients with refractory disease. We observed that DLK1 knockout suppressed ML-DS cell proliferation *in vitro* and delayed engraftment *in vivo*, indicating a pro-tumoral role for DLK1 in ML-DS. We successfully targeted DLK1 using an antibody drug conjugate in patient-derived xenograft (PDX) models of ML-DS. Our data shows DLK1-specific cell death in a dose-dependent manner and proportional to DLK1 expression. Using a single dose of 3 mg/Kg DLK1-ADC, 66% of NTPL-386 xenografted mice achieved durable response. Thus, targeting DLK1 is beneficial in ML-DS.

Materials and Methods

Cell culture

CMK (ACC 392) cells were obtained from DSMZ and cultured in RPMI with 10% fetal bovine serum (FBS). For culturing PDX cells, SFEM media (StemCell Technologies, Ontario, Canada) supplemented with TPO 25ng/mL, IL3 10 ng/mL and GM-CSF 10 ng/mL (from R&D Systems, Minneapolis, MN) was used.

RNA-Sequencing

Four ML-DS PDX lines, one ML-DS cell line and primary normal human bone marrow-derived CD34+ cells from ATCC (American Type Culture Collection, PCS-800-012) were used for total RNA isolation using RNeasy Plus Micro kit (Qiagen, Germantown, MD). RNA sequencing library preparations, sequencing reactions, and preliminary bioinformatics analysis was conducted at GENEWIZ, LLC (South Plainfield, NJ). RNA-Sequencing was performed on 18 samples, 5 samples of ML-DS and 4 samples of normal cells in duplicates to generate 150 bp long paired-end reads. RNA-Seq analysis was performed as described previously [21], by aligning reads to hg38 using STAR and summarizing read counts per gene locus using featureCounts. Differentially expressed genes were identified using edgeR. UniProt list of membrane-associated proteins based on subcellular locations was used for filtering cell surface proteins.

RNA-Seq data from bone marrow specimens of normal individuals, and patients with TAM or ML-DS were obtained from the TARGET (Therapeutically Applicable Research to Generate Effective Treatments): Acute Myeloid Leukemia (AML) database, study ID phs000465.v23.p8.

Quantitative real time polymerase chase reaction (qRT-PCR)

Total RNA was isolated using RNeasy Plus Micro kit following manufacturer's protocol. cDNA was amplified from 1 μg total RNA using iScript™ cDNA Synthesis kit (BioRad, Hercules, CA). qRT-PCR reaction was performed using PowerUp™ SYBR™ Green Master Mix for qPCR (Applied Biosystems, Waltham, MA) in a QuantStudio 7 Real-Time PCR (Polymerase Chain Reaction) System (ThermoFisher Scientific, Waltham, MA). The relative quantification method using delta delta Ct calculations was used to determine the fold change in transcript levels.

Flow cytometry

Cells were resuspended in phosphate-buffered saline containing 1% FBS and stained in the dark for 15 min with APC-conjugated anti DLK1 antibody (R & D Systems, #FAB1144A). Samples were run on a Novocyte Quanteon flow cytometer (Agilent Technologies, Santa Clara, CA). Mouse blood was stained using CD45 mouse and human antibodies (BioLegend, CA) and run on flow cytometer following RBC lysis.

Western blot

Automated immunoblot analysis was performed using the Wes system (ProteinSimple, San Jose, CA) following manufacturer's instructions using a 12-230 kDa Separation Module (SM-W001) and the anti-rabbit Detection Module (DM-001) as described previously [21]. Cells were lysed in Minute Total Protein Extraction kit (Invent Biotechnologies, Plymouth, MN), sonicated and clarified via centrifugation 16000 x g for 15 min. The supernatant was collected and loaded into the capillary based on the number of cells lysed. Antibodies were purchased

from Cell Signaling Technology (Danvers, MA). Normalization to total protein was performed using the Total Protein Detection Module in Wes (DM-TP0010).

Knockout and knockdown

CRISPR guide sequence for DLK1 was chosen from one of the pre-optimized sequences provided by IDT (Coralville, IA). Alt-R CRISPR-Cas9 sgRNA with single RNA molecule comprising crRNA and tracrRNA was complexed with Alt-R Cas9-GFP nuclease (IDT) to form RNP complex, which was introduced into CMK cells using a 4D nucleofector system and P3 primary cell 4D-Nucleotransfector X Kit L (Lonza; Basel, Switzerland). Post transfection cells were cultured in 12-well plates. Individual wells were checked for DLK1 knockdown by flow cytometry. Clones with DLK1 expression overlapping with isotype control antibody were expanded and used for DNA isolation using a MicroDNA kit (Qiagen, Germantown, MD). PCR was used to amplify the region surrounding the CRISPR cut site in the genomic DNA. PCR products were Sanger sequenced and analyzed using a web-based tool (https://ice.synthego.com).

For DLK1 knockdown, pLV[shRNA]-EGFP:T2A:Puro-U6>DLK1 (VectorBuilder, Neu-Isenburg, Germany) shRNA lentiviral vector was used to generate lentiviral particles. CMK cells were spinoculated by centrifugation. Cells were cultured in puromycin to enrich the transduced cells.

Cell viability assay

Cells were incubated with varying concentrations of DLK1-ADC or isotype ADC and cultured for 72 hours. Cell viability was determined as the percentage of live cells based on forward and side scatter and/or propidium iodide negativity via flow cytometry.

ClickiT Edu cell proliferation assay

Cell proliferation was quantitated using the Click-IT EdU cell proliferation assay (Invitrogen, Waltham, MA). Cells were incubated with Click-IT EdU dye for 4 hours and samples were acquired on Quanteon flow cytometer.

Methocult colony forming assay

1000 cells were mixed with Isotype ADC or DLK1-ADC and resuspended in Methocult containing media. The cell suspension was plated in a 6-well plate and incubated in a 370C CO_2 incubator. The number of colonies were counted on day 10.

***In vivo* studies**

NSG-SGM3 mice (Stock#010636) purchased from Jackson Laboratories (Bar Harbor, ME, USA) were transplanted with leukemic cells ($1.0 - 2.5 \times 10^6$) by tail vein injections. Mice were maintained in the Nemours Life Science Center following the guidelines established by the Nemours IACUC. Following engraftment confirmation, mice were either left untreated or treated with indicated doses of DLK1-ADC or Isotype ADC via the tail vein. The leukemic burden was measured by flow cytometry of mouse peripheral blood drawn by submandibular bleeds as described previously [22,23]. Mice were monitored daily, and once mice reached experimental endpoints they were euthanized. Immunohistochemistry was performed as described previously [24].

Results

DLK1 expression in ML-DS

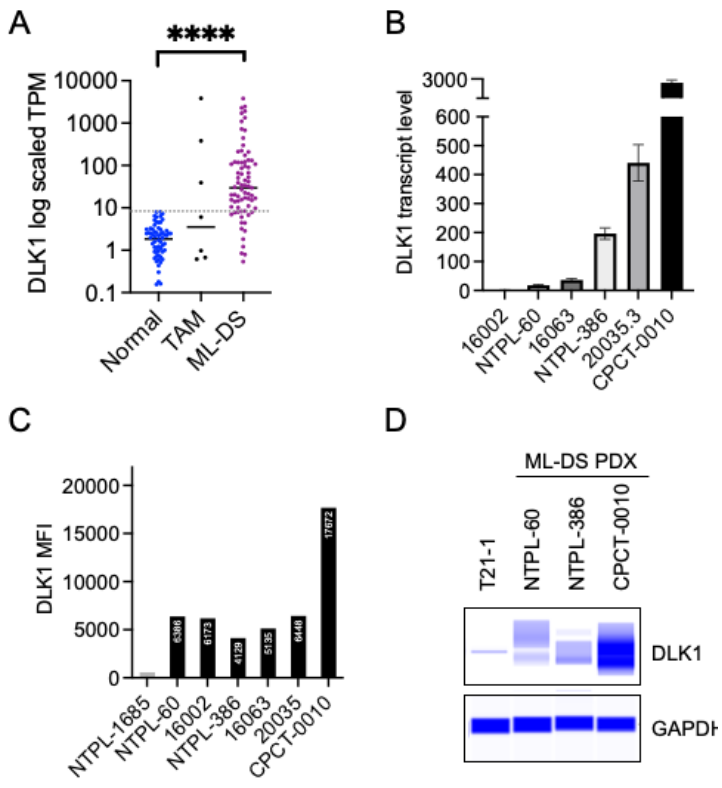

Fig. 1 DLK1 expression in ML-DS. A) DLK1 transcript levels in normal (n=68), TAM (n=8), and ML-DS (n=77) bone marrow specimens from AAML1531 and AAML1051. ****p <0.0001. B) DLK1 transcript level by qRT-PCR in ML-DS PDX lines. Fold change with respect to 16002 is plotted. Error bars denote SD of the Mean. Note: 78% of ML-DS samples have TPM values greater than the highest normal sample. C) DLK1 mean fluorescence intensities (MFI) in ML-DS PDX lines. D) Immunoblots showing the expression of DLK1 in ML-DS PDX lines using Wes automated immunoblotting. Total protein assay and GAPDH was used to ensure equal loading. Representative blots from 2 independent experiments are shown.

We performed transcriptome analysis to identify genes differentially expressed in ML-DS cell/PDX lines (n=5) compared to normal bone marrow-derived CD34+ cells (n=4) (Supplementary Figure 1). When genes were filtered to retain those genes encoding proteins resident on the cell surface, DLK1 was one of the top 10 hits identified (Supplementary Table 1). Analysis of a larger dataset from AAML1531 and AAML1051 also recognized DLK1 as one of the most differentially regulated cell surface proteins in ML-DS compared to normal bone marrow specimens (Fig. 1A). The mean DLK1 TPM scaled counts in ML-DS bone marrow samples (257.20 ±73.81, n=77) were signifcantly greater than the normal bone marrow specimens (2.23 ±0.24, n=68). Although the DLK1 TPM counts were higher in TAM bone

marrow samples (535.70 ±476.90, n=8) compared to ML-DS, this difference was not statistically significant, likely because of low number of TAM samples. We confirmed RNA-Seq data by quantitative RT-PCR and observed that the two ML-DS PDX lines derived from patients with chemotherapy responsive leukemia (NTPL-60 and 16002) had lower transcript levels compared to the 4 PDX lines derived from patients with refractory leukemia (Fig. 1B). Flow cytometry analysis showed DLK1 expression on the surface of ML-DS PDX samples, while it was below detectable levels in the bone marrow specimen from a patient with ML-DS who ultimately achieved remission (NTPL-1685) (Fig. 1C). Western blot analysis confirmed DLK1 expression in ML-DS PDX lines (Fig. 1D) in comparison with low expression in megakaryoblasts derived by hematopoieitic differentiation of induced pluripotent stem cells with trisomy 21 [25]. Taken together, these data indicate that DLK1 is differentially expressed in ML-DS.

DLK1 promotes proliferation *in vitro* and engraftment *in vivo*

To determine the role of DLK1 in ML-DS, we generated CMK (ML-DS cell line) cells with DLK1 knockdown and knockout using small hairpin RNA (shRNA) and CRISPR/Cas9 targeting respectively (Fig. 2A). CMK cells had high DLK1 expression on the cell surface, while it was reduced to 30% in DLK1 shRNA cells and was comparable to the background staining in the isotype control in the two DLK1 knockout clones (Fig. 2B). The percentage of proliferating cells measured by determining the number of EdU stained cells by flow cytometry was reduced by 19% in DLK1 knockdown cells, while it was substantially reduced (by 67% and 65% respectively) in each of the DLK1 knockout clones (Fig. 2C), suggesting that DLK1 promotes cell proliferation in ML-DS.

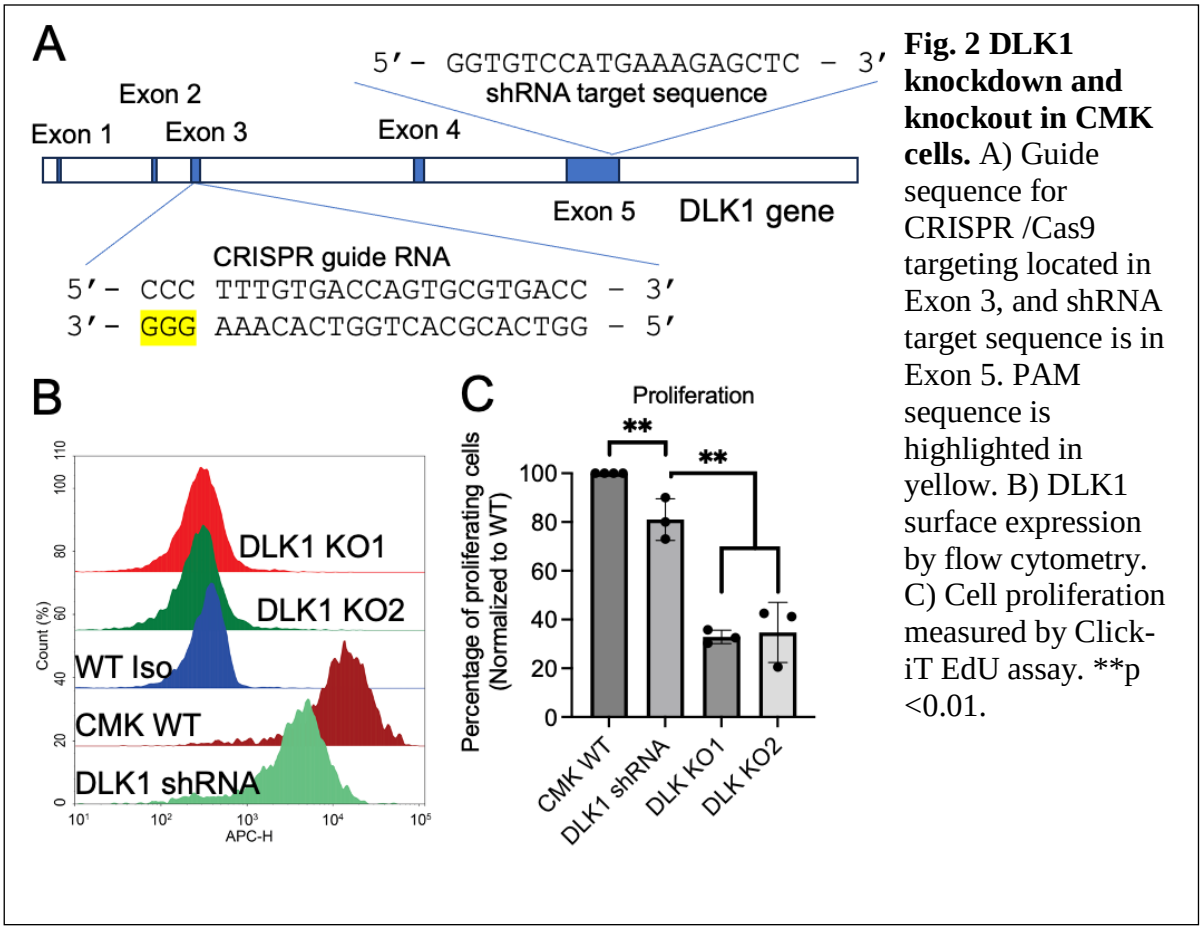

Fig. 2 DLK1 knockdown and knockout in CMK cells. A) Guide sequence for CRISPR /Cas9 targeting located in Exon 3, and shRNA target sequence is in Exon 5. PAM sequence is highlighted in yellow. B) DLK1 surface expression by flow cytometry. C) Cell proliferation measured by Click-iT EdU assay. **p <0.01.

To determine the effect of DLK1 on *in vivo* engraftment, we injected 3 million CMK or CMK DLK1 KO cells in male or female NSG-SGM3 mice via the tail vein. When the mice injected with CMK cells developed hind limb paralysis because of progressive disease (as is commonly seen in mice injected with CMK cells), we terminated the study and isolated femurs and spleen for flow cytometry and immunohistochemistry from 3 mice per group. The femurs and spleens of CMK WT (Wild Type) engrafted mice were packed with human cells, and spleen showed loss of normal morphology unlike DLK1 KO transplanted mice (Fig. 3A). While the percentage of human CD45+ cells (indicating CMK cells) was 63%, the mice injected with DLK1 KO cells showed less than 0.1% human cells in the bone marrow evaluated by flow cytometry (Fig. 3B).

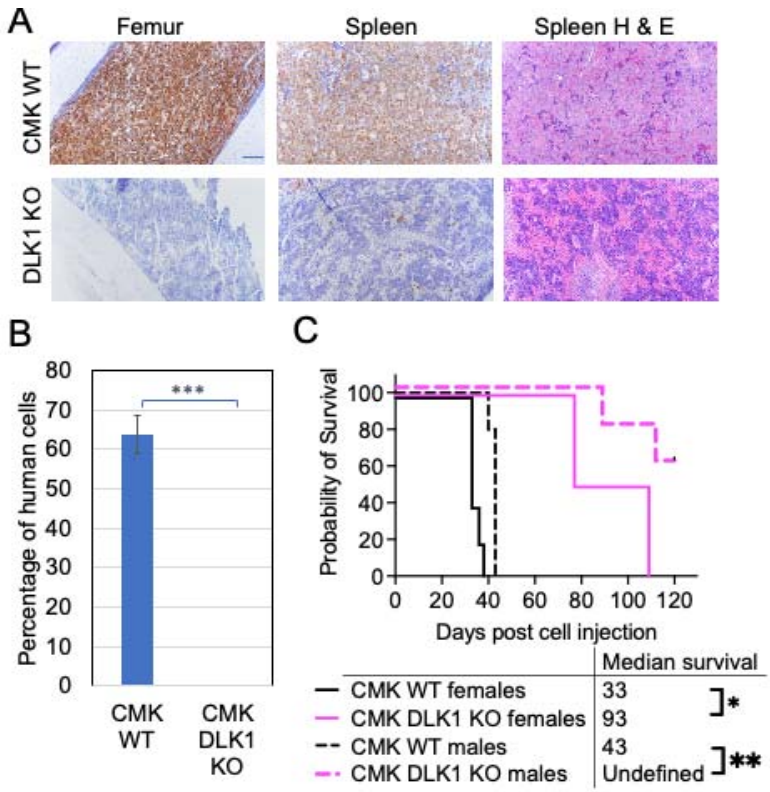

Fig. 3 CMK cells with DLK1 knockout show substantial reduction in engraftment. A) Immunohistochemistry showing the presence of human cells (detected by staining with anti-human mitochondrial antibody). Hematoxylin and eosin (H & E) staining of spleen sections. Representative images from n=3 each. Bar = 500 um. B) CMK cell percentage detected by flow cytometry in bone marrow cells harvested by flushing femurs following euthanasia (n=3 each). ***P<0.001. C) Kaplan-Meier survival estimates of male or female mice injected with CMK WT or DLK1 knockout (KO) cells. *p<0.05, **p <0.01, n=5 per group, except CMK DLK1 KO females (n=2).

A separate cohort of mice was used to determine median survival, based on the time when the mice were euthanized because they met one of the pre-determined experimental endpoints. The median survival of CMK WT cells injected female and male mice was 33 and 43 days respectively. The difference in the engraftment rate between male and female mice was significant (p<0.01) consistent with prior reports [26]. Mice injected with DLK1 KO cells survived significantly longer compared to those injected with CMK WT cells in both female and male mice. The female mice injected with DLK1 KO cells survived 60 days longer compared to those injected with CMK WT cells (p<0.05). Only 2 of 5 DLK1 KO cell-injected male mice met

experimental endpoints so far (Fig. 3C). These data indicate that DLK1 knockout significantly hinders engraftment.

DLK1-targeting antibody drug conjugate kills DLK1 expressing ML-DS cells in a dose-

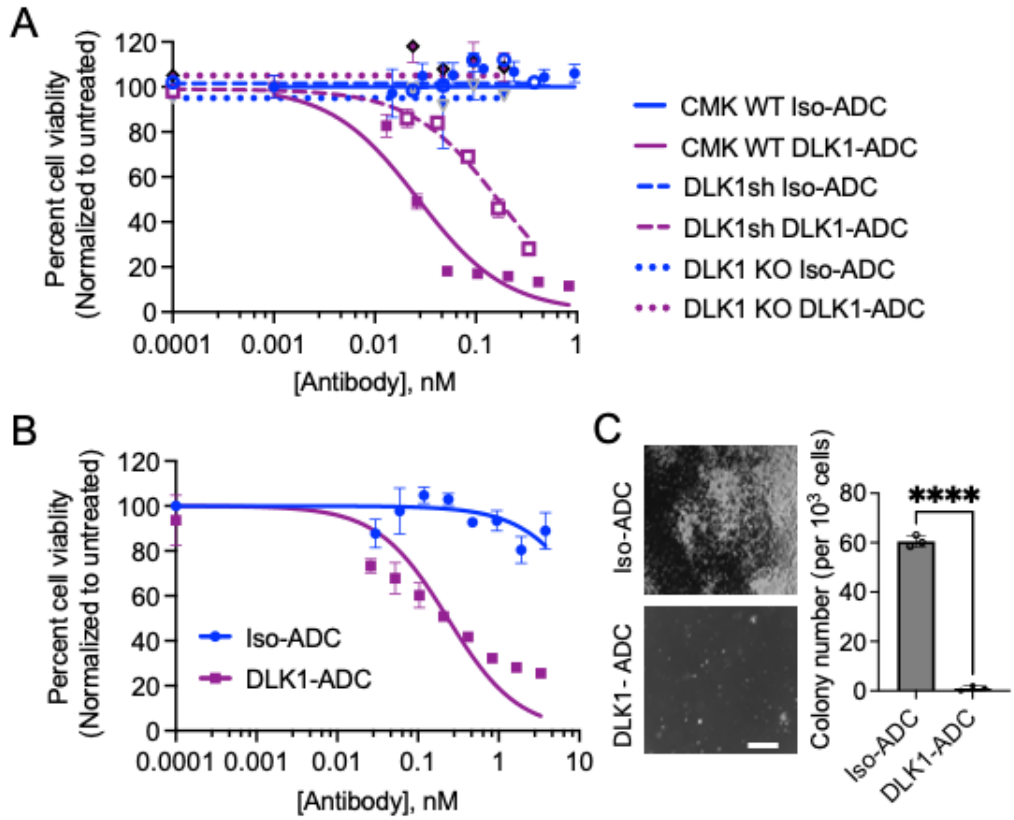

Fig. 4 Targeting DLK1 using antibody drug conjugate ADCT-701. A) *In vitro* viability assay in CMK wild type (WT), CMK DLK1 shRNA knockdown (DLK1sh) and CMK DLK1 knockout (DLK1 KO) cells. B) Viability of CPCT-0010 cells treated with varying concentrations of isotype ADC (Iso-ADC) and DLK1-ADC. C) Effect of DLK1-ADC (2.67 nM) on CPCT-0010 colony formation in Methocult. Representative images are shown. Scale bar =100 um. Data from two independent experiments in triplicates is plotted. ****p<0.0001.

dependent manner

DLK1-targeting antibody drug conjugate (ADCT-701, here referred as DLK1-ADC) comprising anti-DLK1 antibody coupled with a DNA intercalating agent payload

pyrrolbenzodiazepine and the isotype control antibody (B12-PL1601 or Iso-ADC) conjugated with the same payload were obtained from ADC Therapeutics SA, Switzerland. While the DLK1-ADC IC50 for CMK WT cells was 0.027 nM, that of DLK1 knockdown cells (DLK1sh) was 0.172 nM, highlighting the expression-dependent reduction in the DLK1-ADC's ability to mediate cell death (Fig. 4A). The DLK1-ADC failed to induce cell death in DLK1 KO cells, indicating its specificity in killing DLK1 expressing cells. Iso-ADC did not show any activity in the given concentration range.

The *ex vivo* IC50 of DLK1-ADC in CPCT-0010, a PDX model of refractory ML-DS with high DLK1 expression was 0.234 nM (Fig. 4B). DLK1-ADC treatment failed to form colonies

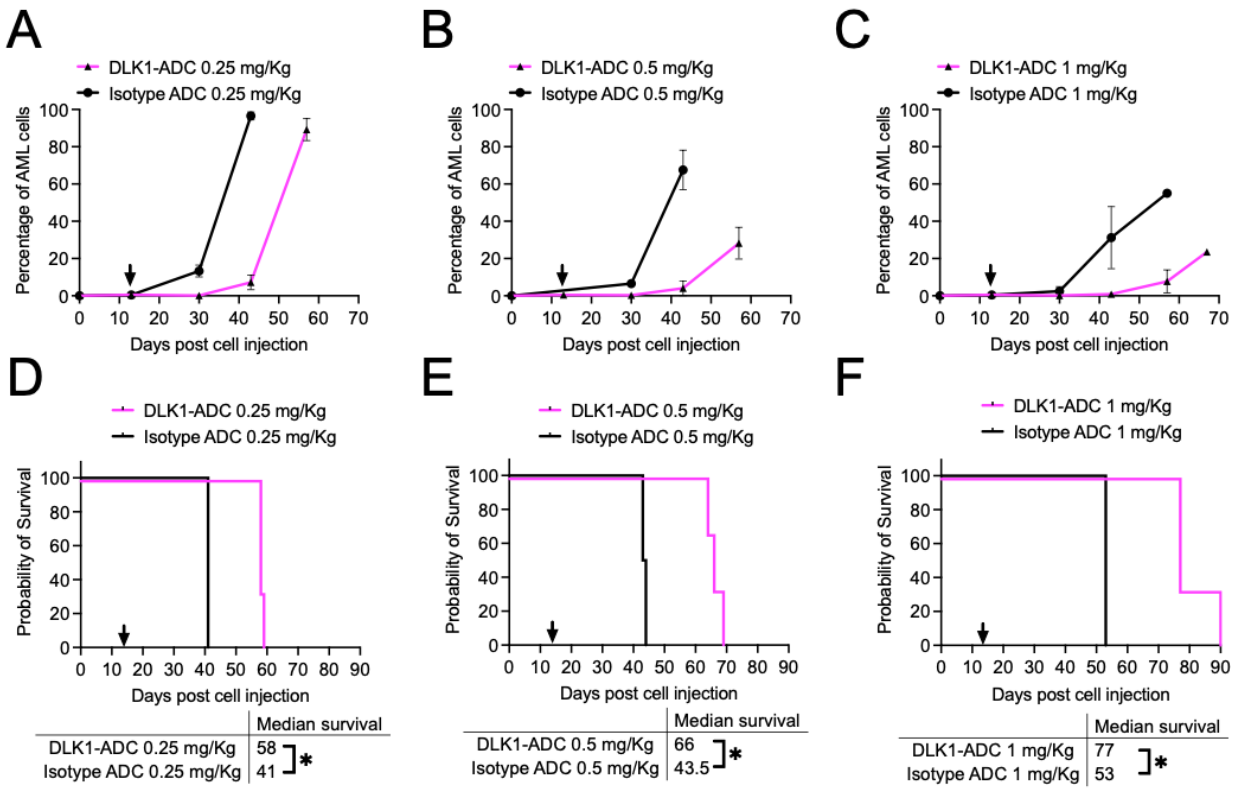

Fig. 5 *In vivo* evaluation of antibody drug conjugate ADCT-701 in CPCT-0010 PDX. A, B and C) Growth curve showing the rise in the percentage of AML cells in peripheral blood in mice transplanted with CPCT-0010. D, E and F) Kaplan-Meier survival estimates of mice treated with isotype control antibody drug conjugate B12-PL1601 (Isotype ADC) or DLK1-targeting ADCT-701 (DLK1-ADC). Arrows indicate time when ADC was administered intravenously. *p <0.01, n=3 per group.

when CPCT-0010 cells were cultured in Methocult (Fig. 4C), suggesting that targeting DLK1 affects stem cell potential.

DLK1-ADC is effective in ML-DS PDX models *in vivo*

We evaluated the *in vivo* efficacy of DLK1-ADC in CPCT-0010 PDX model. A single dose of DLK1-ADC or Isotype ADC was administered 14 days post cell injection. The percentage of human cells in mouse blood indicative of leukemia burden was higher in the mice treated with Isotype ADC compared to DLK1-ADC treated mice at every time point evaluated (Fig. 5A, B,

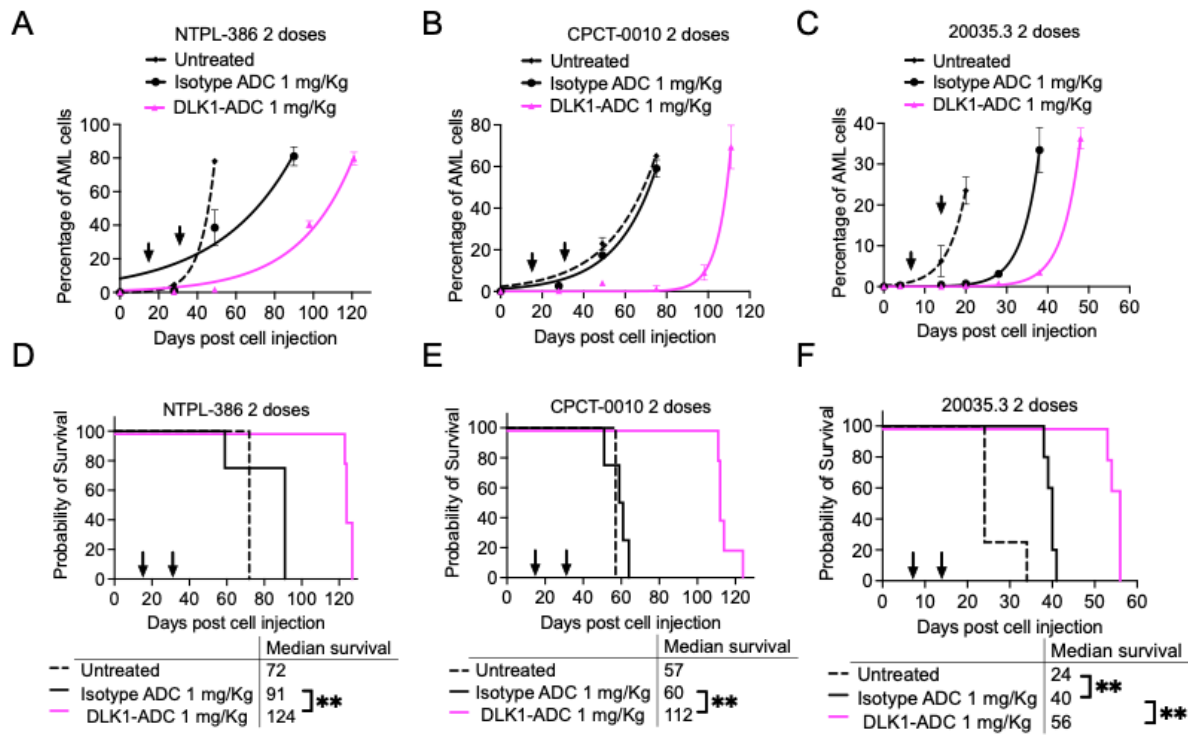

Fig. 6 In vivo evaluation of antibody drug conjugate ADCT-701 in ML-DS PDX models. A, B and C) Growth curve showing the rise in the percentage of AML cells in peripheral blood in mice transplanted with NTPL-386, CPCT-0010, and 20035.3 respectively. D, E and F) Kaplan-Meier survival estimates of mice treated with isotype control antibody drug conjugate B12-PL1601 (Isotype ADC) or DLK1-targeting ADCT-701 (DLK1-ADC). Arrows indicate time when ADC was administered intravenously. *p <0.01, n=4-5 per group.

C). There was a statistically significant improvement in median survival in mice treated with 0.25, 0.5 or 1 mg/Kg DLK1-ADC which survived 17, 22.5 and 24 days longer than those treated with Isotype ADC at the same concentrations (Fig. 5D, E, F). Thus, we observed a dose-dependent effect of DLK1-ADC in prolonging survival and slowing leukemia progression in CPCT-0010 engrafted mice.

We next tested the effect of DLK1-ADC in three ML-DS PDX models derived from patients with refractory leukemia with varying levels of DLK1 expression (Fig. 6). Following cell injection and engraftment, mice were dosed twice with 1 mg/Kg DLK1-ADC or Iso-ADC. In the NTPL-386 model, DLK1-ADC prolonged survival by 33 days compared to Isotype ADC treated mice (Fig. 6D). The DLK1-ADC showed a 52-day prolonged survival in CPCT-0010 with this treatment regimen (Fig. 6E), while there was a 16-day improvement in median survival in mice engrafted with 20035.3 model (Fig. 6F). Although the Isotype ADC increased median survival by 19 days and 3 days compared to untreated mice in NTPL-386 and CPCT-001 respectively, these differences were not statistically significant. On the other hand, the Isotype ADC had a significant impact on median survival compared to the untreated mice engrafted with 20035.3.

In an attempt to optimize DLK1-ADC dosing, we tested the efficacy of a single dose of 3 mg/Kg DLK1-ADC in NTPL-386 model. While all 3 of the Isotype ADC treated mice showed a rapid growth in AML cell percentage in peripheral blood, 2 of 3 DLK1-ADC treated mice have less than 1% of AML cells (Fig. 7A). The third DLK1-ADC treated mouse showed significant delay in leukemia progression compared to Isotype ADC treated mice. Accordingly, 2 of 3 of DLK1-ADC treated mice were cured (Fig. 7B), while all 3 of the Isotype ADC treated mice succumbed to disease. A single dose of DLK1-ADC 3 mg/Kg in NTPL-386 model significantly delayed leukemia progression and induced remission, highlighting the benefit of targeting

DLK1. Taken together, these data indicate that targeting DLK1 is beneficial in controlling ML-DS progression.

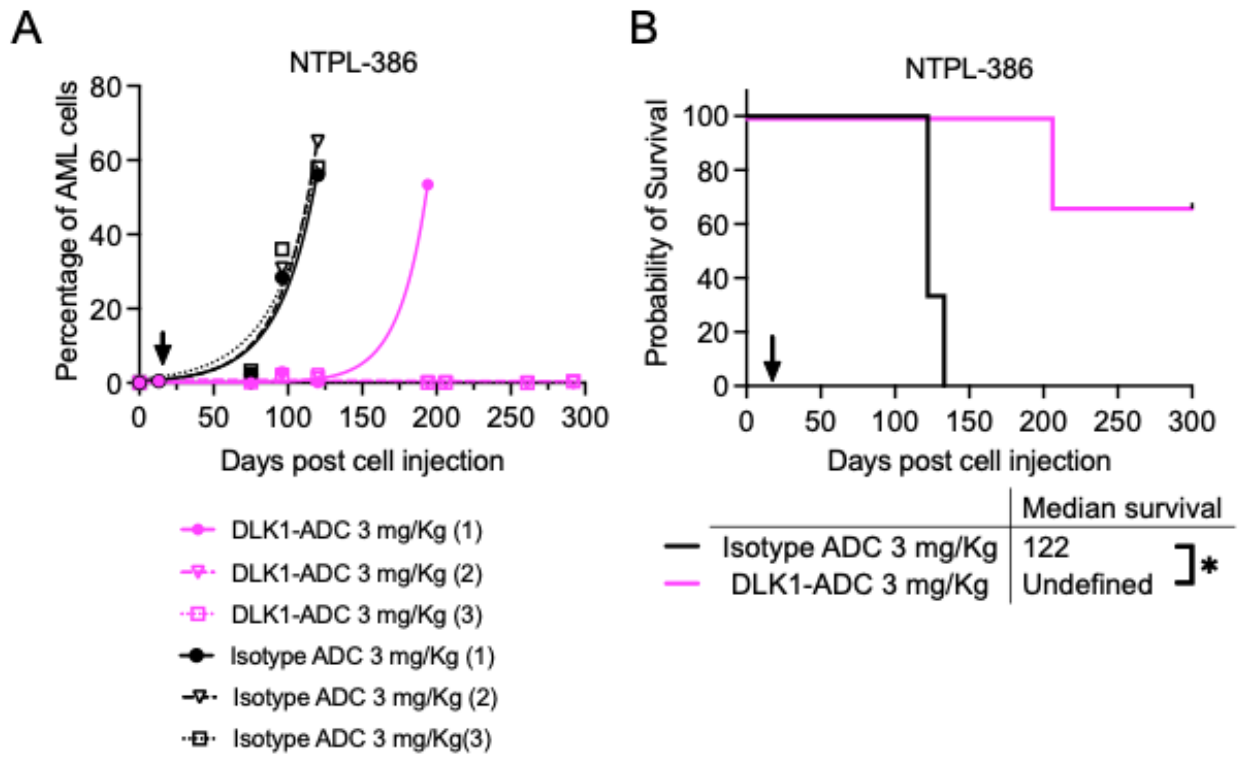

Fig. 7 *In vivo* evaluation of antibody drug conjugate ADCT-701 in NTPL-386 PDX. A) Growth curve showing the rise in the percentage of AML cells in peripheral blood in mice transplanted with NTPL-386. Note, that each line represents individual mouse. B) Kaplan-Meier survival estimate of mice treated with isotype control antibody drug conjugate B12-PL1601 (Isotype ADC) or DLK1-targeting ADCT-701 (DLK1-ADC). Arrows indicate time when ADC was administered intravenously. *p <0.01, n=3 per group.

Discussion

Although the genomic landscape of ML-DS has been well characterized [27-30], there are no studies profiling ML-DS gene expression. Using transcriptome analysis to recognize novel ML-DS targets, we identified DLK1 as a transmembrane protein that is differentially expressed in ML-DS cell/PDX lines compared to CD34+ cells isolated from normal bone marrow specimens. DLK1 knockout reduced ML-DS cell proliferation *in vitro* and delayed engraftment *in vivo*. An antibody drug conjugate targeting DLK1 killed DLK1 expressing cells in a dose-dependent manner, also correlating with DLK1 expression. Targeting DLK1 eliminated colony formation ability in Methocult indicating that it abolished the stem cell population. Furthermore, DLK1-ADC significantly prolonged survival and greatly suppressed leukemia progression in PDX models of refractory ML-DS. Thus, our data show that targeting DLK1 is a novel therapeutic option for refractory ML-DS.

DLK1 or Delta-like non-canonical Notch ligand 1, as the name suggests, is a Notch ligand that binds to Notch1 receptor through EGF-like repeats and inhibits Notch signaling unlike canonical ligands of Notch. [31,32]. Notch signaling regulates megakaryocytic differentiation [33] and can play both tumor suppressor [34] and oncogenic roles in myeloid leukemia [35]. In addition to suppression of Notch signaling, DLK1 also has Notch-independent functions mediated by the cleavage of the intracellular domain of DLK1 [20]. Whether DLK1 mediates its effect on ML-DS cell proliferation and engraftment via Notch dependent or independent function remains to be determined.

DLK1 is overexpressed in MDS and AML [10,11], and is associated with significantly shorter overall survival in adult AML patients [36]. DLK1 overexpression is associated with delayed differentiation and increased proliferation of precursors [20,37]. Its role has been implicated in

cancer stem cell function in solid tumors [19,20]. Our data showing significantly delayed engraftment of DLK1 KO cells in NSG-SGM3 mice supports DLK1 role in ML-DS leukemia stem cell function. Furthermore, suppression of colony formation potential in ML-DS cells treated with DLK1-ADC suggests that targeting DLK1 is also beneficial in suppressing leukemia stem cell viability.

DLK1-ADC showed a strong dose-dependent response in both ML-DS PDX models tested. In CPCT-0010, we saw a 17-, 22.5-, 24- and 52-day improvement in survival when dosed with 0.25, 0.5, 1 and 2 (cumulative dose) mg/Kg in comparison to equivalent doses of the Isotype ADC. Similarly, the response in NTPL-386 engrafted mice improved from a 33-day survival enhancement to durable response in 2/3 mice when dosed with 2 mg/Kg (cumulative dose) versus 3 mg/Kg respectively. In addition, we observed a correlation between DLK1 expression and DLK1-ADC effect. *In vitro,* CMK cells had 6.4-fold lower IC50 for DLK1-ADC compared to CMK cells with DLK1 knockdown. The extension in median survival in CPCT-0010 PDX model with high DLK1 expression was 52 days compared to 33-day or 16-day prolongation in survival of NTPL-386 and 20035.3 mice respectively with moderate DLK1 expression. Taken together, these results highlight the dose-dependence and correlation with DLK1 protein expression of DLK1-ADC. Thus, our data validate the preclinical efficacy and support the development of DLK1-targeted therapies for treating refractory ML-DS in the clinic.

Author Contributions and Disclosures

SPB designed and performed experiments, analyzed data and wrote the manuscript. MT designed and performed experiments, and collected and analyzed data for some of the *in vitro* studies. AS, RER, SM collected and analyzed data. JKH and YP provided PDX models. PHvB

provided antibody drug conjugates. PHvB, JKH, AS, YP EAK edited the manuscript. AG designed the research study, performed experiments, analyzed the data, and wrote the manuscript. Patrick van Berkel is employed by ADC Therapeutics. None of the other authors has a relevant conflict of interest to disclose.

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Role of the funding source

The funders of the study had no role in study design, data interpretation, or manuscript writing. RNA-Seq data are deposited as GSE271179.

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