



Evaluation of the human type-3 adenoviral dodecahedron as a vector to target acute myeloid leukemia

Benjamin Caulier, Gaëlle Stofleth, Dalil Hannani, Mélanie Guidetti, Véronique Josserand, David Laurin, Jadwiga Chroboczek, Pascal Mossuz, Dominique Plantaz

► To cite this version:

Benjamin Caulier, Gaëlle Stofleth, Dalil Hannani, Mélanie Guidetti, Véronique Josserand, et al.. Evaluation of the human type-3 adenoviral dodecahedron as a vector to target acute myeloid leukemia. *Molecular Therapy - Methods and Clinical Development*, 2020, <10.1016/j.omtm.2020.11.009>. <hal-03013800>

HAL Id: hal-03013800

<https://hal.science/hal-03013800v1>

Submitted on 17 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Evaluation of the human type-3 adenoviral dodecahedron as a vector to target acute myeloid leukemia

Benjamin Caulier¹⁻³, Gaëlle Stofleth^{2,4}, Dalil Hannani¹, Mélanie Guidetti³, Véronique Josserand³, David Laurin^{3,5}, Jadwiga Chroboczek¹, Pascal Mossuz^{2,3} and Dominique Plantaz⁴

¹Univ. Grenoble Alpes, CNRS, CHU Grenoble Alpes, Grenoble INP, TIMC-IMAG, 38000 Grenoble, France;

²Institute of Biology and Pathology, Laboratory of Cellular Hematology, Univ. Grenoble Alpes Hospital, Grenoble, France;

³Institute for Advanced Biosciences, INSERM U1209, CNRS UMR 5309, Univ. Grenoble Alpes, Grenoble, France;

⁴Department of Pediatric Onco-Immuno-Hematology, Univ. Grenoble Alpes Hospital, Grenoble, France;

⁵Etablissement Français du Sang Auvergne Rhône-Alpes, Grenoble, France

Corresponding author:

Benjamin Caulier, PhD

Institute of Biology and Pathology, Laboratory of Cellular Hematology, Univ. Grenoble Alpes Hospital, Grenoble, France

benjamin.caulier@gmail.com

Phone number: +33 4 76 76 94 93

Place of work: Grenoble, Isère, France

Short title: Adenoviral dodecahedron targets AML cells

Manuscript data:

- Text: 3618
- Abstract: 182
- Figure/table count: 4 figures + 2 tables (4 supplemental figures)
- Reference count: 49

Abstract

Intensive systemic chemotherapy is the gold standard of acute myeloid leukemia (AML) treatment and is associated with considerable off-target toxicities. Safer and targeted delivery systems are thus urgently needed. In this study, we evaluated a virus-like particle derived from the human type-3 adenovirus, called the adenoviral dodecahedron (Dd) to target AML cells. The vectorization of leukemic cells was proved very effective at nanomolar concentrations in a time- and dose-dependent manner, without vector toxicity. The internalization involved clathrin-mediated energy-dependent endocytosis and strongly correlated with the expression of $\alpha\beta_3$ integrin. The treatment of healthy donor peripheral blood mononuclear cells showed a preferential targeting of monocytes compared to lymphocytes and granulocytes. Similarly, monocytes but also AML blasts were the best-vectorized populations in patients while acute lymphoid leukemia blasts were less efficiently targeted. Importantly, AML leukemic stem cells (LSCs) could be addressed. Finally, Dd reached peripheral monocytes and bone marrow hematopoietic stem and progenitor cells following intravenous injection in mice, without excessive spreading in other organs. These findings reveal Dd as a promising myeloid vector especially for therapeutic purposes in AML blasts, LSCs and progenitor cells.

Keywords

Adenoviral dodecahedron, virus-like particles, acute myeloid leukemia, leukemic stem cells, delivery vector

Introduction

Acute myeloid leukemia (AML) has one of the lowest survival rates among childhood cancers with a 5-year prognostic survival of 60 to 75%.^{1,2} In adults, AML is the most common leukemia and patients under 60 years of age have a 5-year prognostic survival of 30% to 40% and this dramatically decreases below 15% above 60 years of age.³ For decades, AML was classified according to cell morphology using the French-British-American (FAB) classification (AML M0 to M7, *i.e.*: M3 acute promyelocytic leukemia). Since then, multiple revisions from the World Health Organization (WHO) led to 4 new classes: AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related AML and AML not otherwise specified.⁴

Although tremendous progress has been achieved over the past 40 years in the understanding of AML physiopathology, treatment has not substantially changed. Indeed, cytarabine and anthracyclines drugs, both impeding DNA replication, remain the gold standard of intensive chemotherapy treatment used in combination with hematopoietic stem cells (HSCs) transplantation but are associated with severe off-target toxicities and long-term sequelae, especially in children and elderly patients.^{2,5-7} The specific targeting of AML cells is thus a challenge of importance to enhance overall chemotherapy tolerability as well as for the delivery of new therapies.⁸

So far, many synthetic or bioinspired drug carriers are currently in development and trials but only few of them are in use with the most represented being liposomes and polymeric nanoparticles.^{8,9} These carriers improve bioavailability, toxicity and may ameliorate remission. For example, Vyxeos, a liposomal formulation of cytarabine and daunorubicin in a 5:1 molar ratio was approved by the FDA in 2017 and the EMA in 2018 for the treatment of adults with newly diagnosed high-risk secondary AML.^{10,11} Among bio-inspired delivery systems, virus-like particles (VLPs) are stable, self-assembled homogeneous nanoparticles, composed of virus capsid or envelope proteins.¹² They are easily producible, carry no

genetic material and represent a versatile tool for biomedical applications including cargo delivery for therapy.¹³

The capsid surface of the human serotype-3 adenovirus (Ad3) is composed of 3 structural proteins: the hexon, the fiber and the penton base (Pb). The hexon is the major building block of adenoviruses capsid but also the most immunogenic protein responsible for the bulk of neutralizing antibody production¹⁴, especially against the hypervariable loops that differ among adenovirus serotypes.¹⁵ The Ad3 fiber directs targeting properties through its specific attachment to cellular receptors such as ubiquitous complement regulatory protein CD46¹⁶, epithelial desmoglein-2¹⁷ and costimulatory molecules CD80/86¹⁸. The Ad3 Pb is meanwhile responsible for the entry through the interaction of its RGD (Arg-Gly-Asp) motifs within $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins present on cell membranes, which subsequently triggers endocytosis.^{19–21} In addition, an important feature of Ad3 (but not Ad2 or Ad5) is its ability to produce dodecahedral VLPs during infection, consisting of fiber and Pb.²² Otherwise, the recombinant expression of Ad3 Pb alone is sufficient to give rise to fibreless dodecahedron (Dd, commonly referred as Base (Bs)-Dd).^{23,24} This simplified VLP is ~30 nm in diameter and is exclusively composed of 12 copies of Ad3 Pb. Similarly to the Ad3 of origin, Dd shares the entry mechanism connecting Pbs to α_v integrins.²⁵ Therefore, this VLP has been efficiently used in the delivery of proteins^{26,27}, therapeutic compounds²⁸ and mRNA analogs.²⁹ Recently, a study on human healthy blood demonstrated Dd natural affinity for leukocytes, ~45-fold higher than for red blood cells (RBCs) or platelets.³⁰

We postulate that Dd can represent a promising vector for AML cells. We thus characterized its ability to target leukemic cells in vitro with attention for vector dose, subcellular localization, intracellular vector maintenance, endocytosis pathway and toxicity. We compared the internalization among peripheral blood mononuclear cells (PBMCs) in healthy and patient settings with a focus on cell type targeting. Further, we assessed AML and acute lymphoid leukemia (ALL) blasts targeting as well as the addressing of AML leukemic stem

cells (LSCs) ex vivo. Finally, we evaluated the in vivo biodistribution of Dd following IV injection, especially in peripheral blood and bone marrow.

Results

Dd efficiently vectorizes human leukemic cell lines

Dodecahedral VLPs were produced upon recombinant Ad3 Pbs expression in baculovirus-infected insect cells and purified by a two-step chromatography yielding soluble and stable nanoparticles (**Figure S1**).

In this study, we first evaluated the vectorization of leukemic cell lines during 1 hour with a labeled Dd (Dd-AF647). A significant increase of the percentage of Dd-AF647-positive cells was observed between 0.1 nM and 1 nM with a mean of $22\pm18\%$ and $94\pm9\%$, respectively (**Figure 1A**). At 5 nM Dd, that percentage was over 98% for all cell lines. In addition, the relative amount of vector in cells (MFI) rose concomitantly with increasing concentration above 0.1 nM. The MFI signal increased over incubation time (**Figure 1B**), until 24 hours ($P < .05$) and showed only a slow decline over 72 hours indicating a good internal maintenance of the AF647 cargo (**Figure 1C**).

The vector toxicity was assessed over a week with treatment every 48 hours (**Figure 1D**). We used 1 μM of doxorubicin as a positive cell death control (10 nM of doxorubicin had no effect, not shown) since it induced $85.7\pm7.8\%$ of dead cells in 24 hours (not shown). A metabolic assessment of the viability indicated that 1, 5 and 10 nM Dd had a minor toxic effect with $93.6\pm7.4\%$, $84.4\pm14.3\%$ and $83.1\pm13.9\%$ of alive cells at day 7, respectively. Higher doses of 25 and 50 nM displayed much more toxicity, with $76.3\pm11.5\%$ ($P < .001$) and $55.7\pm20.1\%$ ($P < .0001$) of alive cells at day 7.

Noteworthy, the AML cells vectorization featured high intracytoplasmic content of particles often close to the nucleus, that was not assigned to membrane adsorption of Dd (**Figure 1E**).

Taken together, these results demonstrate Dd as a dose- and time-dependent vector, efficiently targeting leukemic cell lines while displaying moderate toxicity.

The Dd vectorization involves clathrin-mediated energy-dependent endocytosis and strongly correlates with $\alpha_v\beta_3$ integrin expression in leukemic cells

We investigated the effect of temperature and ATP depletion on the cellular uptake of Dd-AF647 in leukemic cells. The vector accumulation was reduced upon treatment at 4°C, with 87% of reduction in Dd-AF647 MFI compared to 37°C ($P < .01$; **Figure 2A**). Similarly, when the cellular ATP pool was depleted by treatment with sodium azide and 2-deoxy-D-glucose, the vectorization was nearly abolished with 96% of reduction ($P < .01$). These results indicated that the vectorization is temperature- and energy-dependent.

To clarify which endocytosis pathways is involved in leukemic cells vectorization, we explored the effect of wortmannin, methyl- β -cyclodextrin (MBCD) and chlorpromazine that specifically inhibit micropinocytosis/phagocytosis, caveolae/raft- and clathrin-mediated endocytosis (CME).^{31–33} When cells were treated with wortmannin or MBCD, Dd-AF647 MFI slightly decreased while the treatment with chlorpromazine significantly reduced Dd uptake of ~92% compared to control ($P < .05$; **Figure 2B**). Noteworthily, phagocytic Mono-mac-6 and THP-1 cells as well as the chronic myeloid leukemia K-562 were less efficiently vectorized compared to others. These inhibition results showed that the active transport of Dd is carried out by CME.

Among integrins, $\alpha_v\beta_3$ is characterized as the entry receptor for Dd.²⁵ Therefore, we quantified its expression along Dd-AF647 internalization on leukemic cells. As expected, the MFI displayed a very close linear correlation between $\alpha_v\beta_3$ expression and the cellular level of Dd (**Figure 2C**). Conversely, although Dd was shown to interact with heparan sulfates³⁴, a competitive inhibition of entry using heparin did not reduce Dd uptake in leukemic cells (**Figure S2**).

Taken together, these results reveal the leukemic cells Dd-mediated vectorization as a temperature- & energy-dependent phenomenon involving CME in $\alpha_v\beta_3$ integrin expressing cells.

Dd vectorizes human healthy donor peripheral blood mononuclear cells, targeting preferentially monocytes

PBMCs were purified from 25 healthy donor bloods and treated with Dd-AF647. Monocytes appeared as the best-targeted cells followed by B lymphocytes with $96\pm4\%$ and $47.7\pm13.4\%$ of Dd-AF647-positive cells for 1 nM, respectively ($P < .0001$, **Figure 3A, left panel**). Comparatively, granulocytes, T lymphocytes and NK lymphocytes exhibited very close Dd penetration and were less efficiently vectorized. Importantly, T lymphocytes appeared to be the least-vectorized population, with $53.5\pm6.7\%$ of vector-positive cells at 10 nM ($P < .0001$, not displayed). Similar results were obtained for MFI except for granulocytes that showed a very low vector accumulation with a fold increase of 7 ± 2 at 10 nM (**Figure 3A, right panel**). Thus, Dd vectorizes PBMCs ex vivo, targeting preferentially monocytes.

Dd efficiently vectorizes AML blasts compared to ALL, targets monocytes and all subtypes of AML blasts among PBMCs and is able to enter AML leukemic stem cells

We next analyzed the targeting of 33 leukemic patient PBMCs. Among these samples, 8 were obtained from ALL pediatric patients and 25 from AML patients, among which 84% were adult and 16% were pediatric (**Table 1**). As expected, Dd preferentially vectorized AML blasts compared to ALL, at 1 and 10 nM, both in terms of Dd-positive cells and MFI (**Figure 3B**). We thus focused on AML samples (**Table 2**) and showed that the vector targeted blasts independently of the disease status (diagnosis versus relapse; **Figures S3 A-B**), the age (pediatric versus adult patients; **Figures S3 C-D**) or whether the biological sample was purified or not (**Figures S3 E-F**). These data suggest the Dd as a versatile vector for AML.

Lymphocytes, granulocytes and monocytes (LGM) were also monitored for Dd entry during vectorization of AML blasts. There was 60.5±30.5% of Dd-AF647-positive cells for AML blasts at 1 nM compared to 18±16% ($P < .0001$), 28±23% ($P < .0001$) and 81.7±19.9% ($P < .01$) for LGM, respectively (**Figure 3C, left panel**). A ten-fold concentrated dose of vector reduced these differences among populations. Quantitatively, data became statistically significant with the use of 10 nM Dd, with 313±301-fold increase of MFI compared to 54±47 ($P < .0001$), 29±22 ($P < .0001$) and 608±427 ($P < .0001$) for LGM (**Figure 3C, right panel**). It is thus noteworthy that Dd vectorizes massively AML blasts and monocytes compared to lymphocytes and granulocytes.

To go further, we analyzed the vectorization of AML subtypes according to cell morphological classification. All types of AML were vectorized with AML M4 displaying 802±322-fold increase of MFI at 10 nM compared to 351±263 ($P < .05$), 151±202 ($P < .01$), 143±154 ($P < .01$) and 130±192 ($P < .001$) for secondary AML, AML M0-1-2, M3 and M5, respectively (**Figures 3D**). Furthermore, we analyzed Dd uptake according to the 2016-revised WHO AML classification.⁴ There were no significant differences in Dd vectorization between these new AML subtypes nor for 9 AML classified with recurrent genetic abnormalities regarding *flt3*, *npm1* and *idh1/2* classical mutations (**Figure S4 A-B**). These results mean that the Dd vectorization is efficient in AML regardless of the morphological and the WHO classification.

A key feature of AML physiopathology is the presence of renewable LSCs particularly those found in granulocyte-macrophage progenitors (GMP-like LSCs).^{35,36} To explore the uptake of this particular subpopulation, whole marrow samples were treated with Dd before being enriched in CD34-positive cells. Overall, a similar number of Dd-AF647-positive cells and MFI was observed for total stem cells, myeloid precursors and GMP-like LSCs (**Figure 3E**).

Together, these results confirm the potential of Dd to vectorize AML blasts. Importantly, the vector is able to penetrate LSCs.

Dd reaches peripheral monocytes and bone marrow precursors in vivo

Finally, an in vivo biodistribution study was conducted to confirm Dd vectorization properties. Mice were IV injected with Dd-AF647 and sacrificed at 1-, 5- and 12-hours post-injection. Ex vivo fluorescence imaging was performed on isolated organs (**Figure 4A**). An important uptake was observed in the kidney at 1 and 5 hours and in the liver at the 3 time points while only a weak signal arose from other organs including heart, brain, skin, and spleen.

Additionally, peripheral blood and bone marrow were analyzed. Although we used a small injected amount of Dd, a qualitative analysis of targeted cells could be performed. Leukocytes were the major vectorized population with regard to platelets and RBCs at 1 hour and the signal decreased rapidly over 12 hours (**Figure 4B**). The study of leukocytes subsets revealed that monocytes were also preferentially targeted in vivo (**Figure 4C**).

Bone marrow hematopoietic stem and progenitor cells (HSPCs) showed interesting results (**Figure 4D**). The best-targeted population was the multipotent progenitors (MPPs) with a 3 time points-mean of $3.42 \pm 1.51\%$ of vector-positive cells, compared to $0.85 \pm 0.63\%$, $0.62 \pm 0.41\%$, $0.97 \pm 0.72\%$ and $0.55 \pm 0.36\%$ for the common-lymphoid progenitors (CLPs), the common-myeloid progenitors (CMPs), the GMPs and the megakaryocyte-erythrocyte progenitors (MEPs), respectively ($P < .001$). HSCs displayed a mean of $1.43 \pm 1.13\%$ of Dd-AF647-positive cells that was lower than for MPPs ($P < .01$). Comparisons between CLPs, CMPs, GMPs and MEPs were difficult as these populations showed similar but lower percentage of Dd penetration. Thus, all bone marrow-resident HSPCs were reached by Dd upon IV injection with MPPs accumulating more Dd than other cells

Taken together, these results recapitulate ex vivo observations and further demonstrate Dd ability to vectorize peripheral leukocytes in vivo, especially monocytes. Importantly, Dd reaches the bone marrow where it vectorizes progenitors, which is an important property for AML treatment delivery and targeting of LSCs.

Discussion

The targeted delivery of AML cells is crucial for the improvement of chemotherapy tolerability by reducing unspecific off-target effects and subsequent acute and chronic toxicities mediated by intensive treatment.^{5,6,8} Furthermore, myeloid delivery systems are scarce and urgently needed for the avenue of new therapies. In this study, we fully evaluated the use of a non-infectious VLP as a vector to target AML cells since it presents a natural affinity for leukocytes.³⁰ Dd highlights several advantageous properties to represent an interesting delivery system in AML:

The vector is only composed of Ad3 Pbs, and 12 copies are sufficient to trigger the assembly of a dodecahedral VLP that is massively produced upon recombinant expression.^{23,24} In addition, the vector is easily purifiable using size-exclusion followed by anion-exchange chromatography. Hence, it could be efficiently manufactured. Comparatively to the virus of origin, Dd is devoid of genome, hexon and fiber proteins thus simpler and non-infectious. Although pre-existing or de novo generation of neutralizing anti-Ad3 antibodies could mitigate efficient drug vectorization, it has been shown that the hexon was the main target of pre-existing antibodies as well as adenovirus specific CD4+ and CD8+ T cells.^{14,37,38} Moreover, Zochowska et al.²⁹ showed only a slow and moderate build-up of anti-Dd antibodies following 5 IV injections in rats. This suggest a limited vector neutralization risk at therapy initiation and overtime.

The Ad3 infects epithelial and hematopoietic cells using macropinocytosis as a major entry pathway.³⁹ Comparatively, the endocytic targeting of Dd mainly involved energy-dependent CME in tested leukemic cells. This behavior might be thus related to the simpler vector composition of only Pbs. The use of a fibreless Dd also restrains undesired cell tropism by reducing the number of attachment receptors.¹⁶⁻¹⁸ In this sense, it has been shown that the Ad3 enters platelets and granulocytes using fiber-mediated binding of desmoglein-2¹⁷ while, in this study, fibreless Dd showed only a slight internalization in these cells. Importantly, Dd keeps the ability to trigger specific endocytosis through the interaction of Pb RGD motifs with $\alpha_v\beta_3$ integrins.^{20,25} This feature confers, at least, a relative selectivity toward α_v integrins

expression and especially $\alpha_v\beta_3$ such as for highly expressing leukemic cell lines as well as monocytes^{40,41}, AML cells^{42–44} and bone marrow progenitors^{45–47} that were efficiently targeted. However, dedicated mechanistic studies could deeper characterized this $\alpha_v\beta_3$ -correlated, CME-mediated endocytosis in AML cells, since partial entrapment in lysosomes has been described and that could mitigate vectorized molecules effectiveness.²⁷ Besides, it was shown that Dd preferentially penetrates leukocytes than platelets or RBCs in healthy donor blood.³⁰ That distinct vectorization efficiency was confirmed and we further revealed monocytes as well as AML blasts to be better vectorized than ALL blasts, lymphocytes (T, B and NK) and granulocytes. Additionally, Dd slightly spread outside the bloodstream with most of filtration occurring in 12 hours, which support a rapid clearance after IV injection. It is noteworthy that Dd entered the heart only weakly, which is promising for AML to circumvent the cardiotoxicity induced by anthracyclines.⁶ The limited vector spreading is of great importance to allow optimal leukocytes targeting while reducing systemic toxicity. Altogether, these features strengthen the use of Dd to target leukemic cells compared to others carriers - with no selective cell delivery properties - such as liposomes, already evaluated in AML.^{10,11}

The relevance of LSCs has been thoroughly described in AML³⁶ but novel directed systems do not always evaluate the vector ability to reach these cells. The vector presented here is able to target human GMP-like LSCs ex vivo which, along with lymphoid-primed multipotent progenitors LSCs, coexist in 80% of patients.³⁵ Furthermore, upon IV injection in mice, the vector reached the bone marrow and HSPCs, among which MPPs were the best-vectorized. Since 10 to 15% of AML cases are characterized by a dominant population resembling MPPs³⁵, that population would be potentially targeted by Dd. These features emphasize the use of Dd to target HSPCs and by extension the place where LSCs reside.

The vectorization of model leukemic cells revealed an efficient targeting at low nanomolar concentration as well as a good intracytoplasmic maintenance of the fluorescent cargo. These points are of importance since intensive chemotherapy treatment induces efflux-mediated multidrug resistance (MDR) mechanisms that greatly contribute to AML relapses.⁴⁸

Of note, the vectorization of AML blasts obtained from relapsed patients was as effective as the vectorization of patient blasts at diagnosis. Thus, the enhanced retaining time of cargo provided with low concentration of Dd could circumvent those hurdles, similarly to doxorubicin in the context of human MDR sarcoma cells.³⁰ Since our data also indicate a moderate toxicity of Dd, its utilization as a vector might be safe in vivo. Hence, Dd and chemotherapy could be associated to enhance the effect of free therapeutic form, thereby reducing off-targets and side effects. Using this strategy, the effective cytotoxic concentration of bleomycin delivered with Dd to human cervical cancer cells was 100 times lower than that of free drug.²⁸ Thus, the vector is effective from very low molecular concentrations, supporting its use as an efficient enhancer for therapeutics in AML cells also via improvement of cell retention while cytotoxicity of Dd remains acceptable.

In conclusion, these results highlight promising features of Dd vector for myeloid and monocytes cell delivery applications as well as for AML therapy-aiming at targeting preferentially blasts and HSPCs. Further studies should now consider a therapeutic form of Dd such as Dd bearing doxorubicin and assess its full potential in AML engrafted mice models in comparison to free-drug doxorubicin or another reference therapy.

Materials & Methods

Dd labelling

Dd was conjugated to AlexaFluor 647 (Dd-AF647) using AF647 Protein Labeling Kit (Molecular Probes) and the structure was verified by nanoparticle tracking analysis on a NanoSight NS300 (Malvern Panalytical).

Dd cell vectorization

Leukemic cell lines, PBMCs, bone marrow mononuclear cells and whole blood/bone marrow were incubated with 0.01 to 10 nM Dd-AF647 for up to 7 days at 37°C. Untreated cells or whole blood/bone marrow were used as controls. After incubation, purified cells were treated with 0.5% Trypsin-EDTA (Gibco) during 5 min at 37°C to remove surface-bound Dd. For

LSCs vectorization, CD34 enrichment was performed using CD34 MicroBeads kit (autoMACS Pro, Miltenyi Biotech).

Cytotoxicity assays

The Dd toxicity was assessed on cell lines using a metabolic assessment of cell viability by a chromogenic assay. Cells were quantified using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) on a Varioskan Flash (Thermo Scientific). Doxorubicin (Accord) was used as a positive control for cell death.

Confocal microscopy

Cells were treated with PBS or 10 nM Dd-AF647 for 2h at 37°C. After incubation, cells were fixed with 1x FACS lysing solution (BD Biosciences). Nuclei were stained with DAPI (1:3000; Sigma) and membranes with AlexaFluor 555-conjugated wheat germ agglutinin (WGA; 1:200; Invitrogen) during 10 min at room temperature. Images were acquired after sequential laser excitation using an LSM 710 NLO laser-scanning microscope (Zeiss) onto optical sectioning of 1 µm and analyzed with ImageJ software.

Drug treatment

Cells were pre-incubated during 1h prior to Dd addition in PBS containing the following drugs: 100 nM wortmannin, 30 µM chlorpromazine hydrochloride, 500 µM MBCD, 10 mM 2-deoxy-D-glucose and 10 mM sodium azide (Sigma).

Flow cytometry

Human and mouse antibodies were purchased from BD Biosciences. In all experiments, cells were selected by a viability labelling using Zombie Aqua Fixable Viability Kit (BioLegend). The percentage of Dd-AF647 positive cells and AF647 mean fluorescence intensity (MFI) were measured for each population. The threshold percent gate was set-up by cell type for background fluorescence of untreated cells. The MFI of Dd-AF647-treated cells was compared to control by dividing (fold increase) or subtracting the MFI of the corresponding

untreated cells. For measurement of $\alpha_v\beta_3$ integrin expression, cells were labeled with anti- $\alpha_v\beta_3$ -PE monoclonal antibody. Data were acquired on FACS Canto II & FACS LSR II (Beckton Dickinson) and analyzed with FlowJo V10 software.

In-vivo biodistribution in blood and bone marrow

The project was authorized by the French ministry of research with the permit number: APAFIS#18922-2019013112124432v4. Twelve 5-weeks old C57Black6 female mice were purchased (Janvier Laboratory) and housed for 1 week before the day of experiment. Mice were injected in the tail vein with 100 μ g Dd-AF647 in 200 μ L of PBS (n = 9) or with 200 μ L of PBS alone (n = 3). Three mice were anesthetized per time point at 1, 5 and 12h post-injection, 500 μ L of blood were collected and mice were sacrificed for organs and bone marrow sampling.⁴⁹

Statistics

Data are reported as the mean values \pm standard deviation (SD). Student *t*-test was used in univariate analysis and Pearson *r* coefficient was calculated for correlation. One-way or two-way ANOVA tests were performed for multivariate analysis and *P*-value were conservatively adjusted for multiple comparisons using a Bonferroni correction. *P*-value $<.05$ was considered significant and the abbreviations were: n.s., not significant; *, $P \leq .05$; **, $P \leq .01$; ***, $P \leq .001$; ****, $P < .0001$. Statistics were calculated using GraphPad Prism V7 software.

Author Contributions

Conceptualization, B.C, D.H, D.L and J.C; Methodology, B.C, D.H and J.C; Investigation, B.C G.S, D.H and M.G; Validation, Formal Analysis, Visualization and Writing – Original Draft, B.C; Writing – Review & Editing, B.C, D.H, V.J, D.L, J.C, P.M and D.P; Funding Acquisition, D.L, J.C and D.P; Resources, J.C, P.M and D.P; Supervision, B.C, P.M and D.P.

Conflict of interest

The authors declare no competing interests.

Acknowledgment

This work was funded by Société Française de lutte contre les Cancers et les leucémies de l'Enfant et de l'adolescent, La Ligue contre le Cancer 38, Association Grenobloise pour le Développement d'Etudes et de Recherche en Médecine Infantile, Association Espoir, GEFLUC Grenoble Dauphiné-Savoie and IDEX Univ. Grenoble Alpes. The authors thanks Sylvie Berthier of the Institute of Biology and Pathology (Grenoble) for access and technical assistance with flow cytometers, Alexei Grichine and Mylène Pezet of the Institute for Advanced Biosciences (Grenoble) for technical assistance with confocal microscopes and flow cytometers, Gautier Szymancki, Claire Vettier, Christine Lefebvre and the technicians of the Laboratory of Cellular Hematology (Grenoble), for providing patient samples, Pascal Fender of the Institut de Biologie Structurale (IBS, Grenoble) for helpful advice and discussions on the adenoviral Dd, Marta Jedynak and Ewa Szolajska of the Institute of Biochemistry and Biophysics (Warsaw, Poland) for materials and discussions on the adenoviral Dd, Karine Laulagnier from Remy Sadoul group of the Grenoble Institute of Neuroscience (Grenoble), for access to Nanosight and advice on particle analysis, Daphna Fenel and Guy Schoehn of the electron microscopy (EM) IBS platform (Grenoble). This work used the EM facilities at the Grenoble Instruct-ERIC Center (ISBG; UMS 3518 CNRS CEA-UJF-EMBL) with support from the French Infrastructure for Integrated Structural Biology (FRISBI; ANR-10-INSB-05-02) and the Grenoble Alliance for Integrated Structural Cell Biology (GRAL; ANR-10-LABX-49-01) within the Grenoble Partnership for Structural Biology. The IBS EM platform is supported by the Rhône-Alpes Region, the Fonds Feder, the Fondation pour la Recherche Médicale and GIS-IBiSA.

385 **References**

- 386 1. Rasche, M., Zimmermann, M., Borschel, L., Bourquin, J.-P., Dworzak, M., Klingebiel, T.,
387 Lehrnbecher, T., Creutzig, U., Klusmann, J.-H., and Reinhardt, D. (2018). Successes and
388 challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the
389 AML-BFM trials from 1987 to 2012. *Leukemia* 32, 2167–2177.
- 390 2. Creutzig, U., van den Heuvel-Eibrink, M.M., Gibson, B., Dworzak, M.N., Adachi, S., de Bont, E.,
391 Harbott, J., Hasle, H., Johnston, D., Kinoshita, A., et al. (2012). Diagnosis and management of
392 acute myeloid leukemia in children and adolescents: recommendations from an international
393 expert panel. *Blood* 120, 3187–3205.
- 394 3. Döhner, H., Estey, E.H., Amadori, S., Appelbaum, F.R., Buchner, T., Burnett, A.K., Dombret, H.,
395 Fenaux, P., Grimwade, D., Larson, R.A., et al. (2010). Diagnosis and management of acute
396 myeloid leukemia in adults: recommendations from an international expert panel, on behalf of
397 the European LeukemiaNet. *Blood* 115, 453–474.
- 398 4. Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., Bloomfield, C.D.,
399 Cazzola, M., and Vardiman, J.W. (2016). The 2016 revision to the World Health Organization
400 classification of myeloid neoplasms and acute leukemia. *Blood* 127, 2391–2405.
- 401 5. Döhner, H., Weisdorf, D.J., and Bloomfield, C.D. (2015). Acute Myeloid Leukemia. *New England*
402 *Journal of Medicine* 373, 1136–1152.
- 403 6. McGowan, J.V., Chung, R., Maulik, A., Piotrowska, I., Walker, J.M., and Yellon, D.M. (2017).
404 Anthracycline Chemotherapy and Cardiotoxicity. *Cardiovasc Drugs Ther* 31, 63–75.
- 405 7. Leung, W., Hudson, M.M., Strickland, D.K., Phipps, S., Srivastava, D.K., Ribeiro, R.C., Rubnitz,
406 J.E., Sandlund, J.T., Kun, L.E., Bowman, L.C., et al. (2000). Late Effects of Treatment in Survivors
407 of Childhood Acute Myeloid Leukemia. *Journal of Clinical Oncology* 18, 3273–3279.
- 408 8. Briot, T., Roger, E., Thépot, S., and Lagarce, F. (2018). Advances in treatment formulations for
409 acute myeloid leukemia. *Drug Discovery Today* 23, 1936–1949.
- 410 9. Torchilin, V.P. (2014). Multifunctional, stimuli-sensitive nanoparticulate systems for drug
411 delivery. *Nature Reviews Drug Discovery* 13, 813–827.
- 412 10. Krauss, A.C., Gao, X., Li, L., Manning, M.L., Patel, P., Fu, W., Janoria, K.G., Gieser, G., Bateman,
413 D.A., Przepiorka, D., et al. (2019). FDA Approval Summary: (Daunorubicin and Cytarabine)
414 Liposome for Injection for the Treatment of Adults with High-Risk Acute Myeloid Leukemia. *Clin*
415 *Cancer Res* 25, 2685–2690.
- 416 11. European public assessment reports (2018). Vyxeos liposomal (previously known as Vyxeos).
417 European Medicines Agency. [https://www.ema.europa.eu/en/medicines/human/EPAR/vyxeos-](https://www.ema.europa.eu/en/medicines/human/EPAR/vyxeos-liposomal)
418 [liposomal](https://www.ema.europa.eu/en/medicines/human/EPAR/vyxeos-liposomal).
- 419 12. Yoo, J.-W., Irvine, D.J., Discher, D.E., and Mitragotri, S. (2011). Bio-inspired, bioengineered and
420 biomimetic drug delivery carriers. *Nature Reviews Drug Discovery* 10, 521–535.
- 421 13. Koudelka, K.J., Pitek, A.S., Manchester, M., and Steinmetz, N.F. (2015). Virus-Based
422 Nanoparticles as Versatile Nanomachines. *Annu. Rev. Virol.* 2, 379–401.
- 423 14. Sumida, S.M., Truitt, D.M., Lemckert, A.A.C., Vogels, R., Custers, J.H.H.V., Addo, M.M., Lockman,
424 S., Peter, T., Peyerl, F.W., Kishko, M.G., et al. (2005). Neutralizing antibodies to adenovirus
425 serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *J.*
426 *Immunol.* 174, 7179–7185.

- 427 15. Fausther-Bovendo, H., and Kobinger, G.P. (2014). Pre-existing immunity against Ad vectors:
428 humoral, cellular, and innate response, what's important? *Hum Vaccin Immunother* 10, 2875–
429 2884.
- 430 16. Gaggar, A., Shayakhmetov, D.M., and Lieber, A. (2003). CD46 is a cellular receptor for group B
431 adenoviruses. *Nat Med* 9, 1408–1412.
- 432 17. Wang, H., Li, Z.-Y., Liu, Y., Persson, J., Beyer, I., Möller, T., Koyuncu, D., Drescher, M.R., Strauss,
433 R., Zhang, X.-B., et al. (2011). Desmoglein 2 is a receptor for adenovirus serotypes 3, 7, 11 and
434 14. *Nat. Med.* 17, 96–104.
- 435 18. Short, J.J., Pereboev, A.V., Kawakami, Y., Vasu, C., Holterman, M.J., and Curiel, D.T. (2004).
436 Adenovirus serotype 3 utilizes CD80 (B7.1) and CD86 (B7.2) as cellular attachment receptors.
437 *Virology* 322, 349–359.
- 438 19. Wickham, T.J., Mathias, P., Cheresch, D.A., and Nemerow, G.R. (1993). Integrins alpha v beta 3
439 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* 73, 309–
440 319.
- 441 20. Cuzange, A., Chroboczek, J., and Jacrot, B. (1994). The penton base of human adenovirus type 3
442 has the RGD motif. *Gene* 146, 257–259.
- 443 21. Xiong, J.-P. (2002). Crystal Structure of the Extracellular Segment of Integrin alpha Vbeta 3 in
444 Complex with an Arg-Gly-Asp Ligand. *Science* 296, 151–155.
- 445 22. Fender, P., Boussaid, A., Mezin, P., and Chroboczek, J. (2005). Synthesis, cellular localization,
446 and quantification of penton-dodecahedron in serotype 3 adenovirus-infected cells. *Virology*
447 340, 167–173.
- 448 23. Fender, P., Ruigrok, R.W.H., Gout, E., Buffet, S., and Chroboczek, J. (1997). Adenovirus
449 dodecahedron, a new vector for human gene transfer. *Nature Biotechnology* 15, 52–56.
- 450 24. Szolajska, E., Burmeister, W.P., Zochowska, M., Nerlo, B., Andreev, I., Schoehn, G., Andrieu, J.-
451 P., Fender, P., Naskalska, A., Zubieta, C., et al. (2012). The Structural Basis for the Integrity of
452 Adenovirus Ad3 Dodecahedron. *PLoS ONE* 7, e46075.
- 453 25. Fender, P., Schoehn, G., Perron-Sierra, F., Tucker, G.C., and Lortat-Jacob, H. (2008). Adenovirus
454 dodecahedron cell attachment and entry are mediated by heparan sulfate and integrins and
455 vary along the cell cycle. *Virology* 371, 155–164.
- 456 26. Garcel, A., Gout, E., Timmins, J., Chroboczek, J., and Fender, P. (2006). Protein transduction into
457 human cells by adenovirus dodecahedron using WW domains as universal adaptors. *The*
458 *Journal of Gene Medicine* 8, 524–531.
- 459 27. Sumarheni, Gallet, B., and Fender, P. (2016). The Use of Adenovirus Dodecahedron in the
460 Delivery of an Enzymatic Activity in the Cell. *Biotechnology Research International* 2016, 1–11.
- 461 28. Zochowska, M., Paca, A., Schoehn, G., Andrieu, J.-P., Chroboczek, J., Dublet, B., and Szolajska, E.
462 (2009). Adenovirus Dodecahedron, as a Drug Delivery Vector. *PLoS ONE* 4, e5569.
- 463 29. Zochowska, M., Piguet, A.-C., Jemielity, J., Kowalska, J., Szolajska, E., Dufour, J.-F., and
464 Chroboczek, J. (2015). Virus-like particle-mediated intracellular delivery of mRNA cap analog
465 with in vivo activity against hepatocellular carcinoma. *Nanomedicine: Nanotechnology, Biology*
466 *and Medicine* 11, 67–76.
- 467 30. Jedynak, M., Laurin, D., Dolega, P., Podsiadla-Bialoskorska, M., Szurgot, I., Chroboczek, J., and
468 Szolajska, E. (2018). Leukocytes and drug-resistant cancer cells are targets for intracellular
469 delivery by adenoviral dodecahedron. *Nanomedicine: Nanotechnology, Biology and Medicine*
470 14, 1853–1865.

- 471 31. Araki, N. (1996). A role for phosphoinositide 3-kinase in the completion of macropinocytosis
472 and phagocytosis by macrophages. *The Journal of Cell Biology* 135, 1249–1260.
- 473 32. Nabi, I.R., and Le, P.U. (2003). Caveolae/raft-dependent endocytosis. *The Journal of Cell Biology*
474 161, 673–677.
- 475 33. Wang, L.H. (1993). Mis-assembly of clathrin lattices on endosomes reveals a regulatory switch
476 for coated pit formation. *The Journal of Cell Biology* 123, 1107–1117.
- 477 34. Vivès, R.R., Lortat-Jacob, H., Chroboczek, J., and Fender, P. (2004). Heparan sulfate
478 proteoglycan mediates the selective attachment and internalization of serotype 3 human
479 adenovirus dodecahedron. *Virology* 321, 332–340.
- 480 35. Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford,
481 K.A., Rout, R., et al. (2011). Coexistence of LMPP-like and GMP-like Leukemia Stem Cells in
482 Acute Myeloid Leukemia. *Cancer Cell* 19, 138–152.
- 483 36. Thomas, D., and Majeti, R. (2017). Biology and relevance of human acute myeloid leukemia
484 stem cells. *Blood* 129, 1577–1585.
- 485 37. Serangeli, C., Bicanic, O., Scheible, M.H., Wernet, D., Lang, P., Rammensee, H.-G., Stevanovic, S.,
486 Handgretinger, R., and Feuchtinger, T. (2010). Ex vivo detection of adenovirus specific CD4+ T-
487 cell responses to HLA-DR-epitopes of the Hexon protein show a contracted specificity of
488 T(HELPER) cells following stem cell transplantation. *Virology* 397, 277–284.
- 489 38. Leen, A.M., Christin, A., Khalil, M., Weiss, H., Gee, A.P., Brenner, M.K., Heslop, H.E., Rooney,
490 C.M., and Bollard, C.M. (2008). Identification of hexon-specific CD4 and CD8 T-cell epitopes for
491 vaccine and immunotherapy. *J. Virol.* 82, 546–554.
- 492 39. Amstutz, B., Gastaldelli, M., Kälin, S., Imelli, N., Boucke, K., Wandeler, E., Mercer, J., Hemmi, S.,
493 and Greber, U.F. (2008). Subversion of CtBP1-controlled macropinocytosis by human
494 adenovirus serotype 3. *EMBO J* 27, 956–969.
- 495 40. Weerasinghe, D., McHugh, K.P., Ross, F.P., Brown, E.J., Gisler, R.H., and Imhof, B.A. (1998). A
496 role for the alphavbeta3 integrin in the transmigration of monocytes. *J. Cell Biol.* 142, 595–607.
- 497 41. Brilha, S., Wysoczanski, R., Whittington, A.M., Friedland, J.S., and Porter, J.C. (2017). Monocyte
498 Adhesion, Migration, and Extracellular Matrix Breakdown Are Regulated by Integrin $\alpha V\beta 3$ in
499 *Mycobacterium tuberculosis* Infection. *J.I.* 199, 982–991.
- 500 42. Al-Asadi, M.G., Brindle, G., Castellanos, M., May, S.T., Mills, K.I., Russell, N.H., Seedhouse, C.H.,
501 and Pallis, M. (2017). A molecular signature of dormancy in CD34+CD38- acute myeloid
502 leukaemia cells. *Oncotarget* 8, 111405–111418.
- 503 43. Miller, P.G., Al-Shahrour, F., Hartwell, K.A., Chu, L.P., Järås, M., Puram, R.V., Puissant, A.,
504 Callahan, K.P., Ashton, J., McConkey, M.E., et al. (2013). In Vivo RNAi Screening Identifies a
505 Leukemia-Specific Dependence on Integrin Beta 3 Signaling. *Cancer Cell* 24, 45–58.
- 506 44. Johansen, S., Brenner, A., Bartaula-Brevik, S., Reikvam, H., and Bruserud, Ø. (2018). The
507 Possible Importance of $\beta 3$ Integrins for Leukemogenesis and Chemoresistance in Acute Myeloid
508 Leukemia. *IJMS* 19, 251.
- 509 45. Umemoto, T., Matsuzaki, Y., Shiratsuchi, Y., Hashimoto, M., Yoshimoto, T., Nakamura-Ishizu, A.,
510 Petrich, B., Yamato, M., and Suda, T. (2017). Integrin $\alpha V\beta 3$ enhances the suppressive effect of
511 interferon- γ on hematopoietic stem cells. *EMBO J.* 36, 2390–2403.
- 512 46. Kräter, M., Jacobi, A., Otto, O., Tietze, S., Müller, K., Poitz, D.M., Palm, S., Zinna, V.M., Biehain,
513 U., Wobus, M., et al. (2017). Bone marrow niche-mimetics modulate HSPC function via integrin
514 signaling. *Sci Rep* 7, 2549.

- 515 47. Wang, J., Liu, Z., Zhang, S., Wang, X., Bai, H., Xie, M., Dong, F., and Ema, H. (2019). Lineage
516 marker expression on mouse hematopoietic stem cells. *Experimental Hematology* 76, 13-23.e2.
- 517 48. Zhang, J., Gu, Y., and Chen, B. (2019). Mechanisms of drug resistance in acute myeloid
518 leukemia. *OTT Volume 12*, 1937–1945.
- 519 49. Liu, X., and Quan, N. (2015). Immune Cell Isolation from Mouse Femur Bone Marrow. *Bio*
520 *Protoc* 5.
- 521

Figures Legends

Figure 1. The adenoviral Dd efficiently vectorizes human leukemic cells, displaying robust intracellular maintenance and low toxicity.

KG-1a, THP-1, Mono-Mac-6, SupB15, KG-1, OCI-AML-3, HEL, K-562, Jurkat, U-937 and HL-60 cells were incubated with Dd-AF647. The corresponding untreated cells were used as negative control. All measures were done in triplicates for each cell lines and the mean \pm SD is indicated. *P*-values were determined by one-way ANOVA (C) or two-way ANOVA (D). ns, not significant; *, *P* \leq 0.05; **, *P* \leq 0.01; ***, *P* \leq 0.001; ****, *P* \leq 0.0001. (A) Effect of Dd concentration on the 11 cell lines during 1 h. In blue, cells positive (%) for Dd-AF647. In red, fold increase in Dd-AF647 MFI compared to untreated. (B) Effect of incubation time. KG-1a, THP-1, Mono-Mac-6, SupB15, KG-1, OCI-AML-3 and Jurkat were treated with 1 nM Dd-AF647 up to 180 minutes. (C) Persistence of Dd-AF647 inside treated cells. Cells were treated with 1 nM Dd-AF647 and analyzed at 2, 24, 48 and 72 h for AF647 MFI. (D) Effect of Dd on cell viability. The 11 cell lines were treated with repetitive doses of unlabeled-Dd every 24 h and assessed for viability by measurement of cell metabolism at days 0, 3, 5 and 7. Doxorubicin (DOX; 1 μ M) was used as a cell death control. Left panel: DOX, 1, 5 and 10 nM Dd. Right panel: 25 and 50 nM Dd. (E) Cellular localization of Dd. HEL & OCI-AML-3 cells were incubated with 10 nM Dd-AF647 (red signal) during 2 h. Membranes were labeled with AF555-conjugated wheat germ agglutinin (green signal) and nuclei counterstained with DAPI (blue signal). Visualization was performed on a Zeiss LSM710 NLO microscope with a 63x/1.4 oil-immersion objective. Signals were collected after sequential laser excitation onto optical sectioning of 1 μ m. Scale bars represent 10 μ m.

Figure 2. The vectorization is an energy-dependent phenomenon, involving clathrin-coated pit-mediated endocytosis and strongly correlating with $\alpha_v\beta_3$ integrin highly expressing cells.

Leukemic cell lines were either pre-incubated at 4°C or with inhibitor drugs at 37°C in complete medium during 1 h before incubation with 1 nM Dd-AF647 during 1 h. Control cells were directly exposed to Dd without inhibitors at 37°C and untreated cells were used as negative control. Measures were done in triplicates for each cell lines and the mean \pm SD is indicated. *P*-values were determined by one-way ANOVA. ns, not significant; *, *P* \leq 0.05; **, *P* \leq 0.01; ***, *P* \leq 0.001; ****, *P* \leq 0.0001. **(A)** Effect of low temperature (4°C) and cellular ATP depletion (w/o ATP) on the internalization of Dd-AF647 (MFI). **(B)** Effect of endocytic inhibitors (chlorpromazine, wortmannin and methyl- β -cyclodextrin) on the internalization of Dd-AF647 (MFI). **(C)** Scatterplot showing linear correlation between Dd-AF647 MFI and $\alpha_v\beta_3$ integrin MFI. The 7 cell lines were treated with 1 nM Dd-AF647 during 1 h at 37°C (without inhibitors) and then labeled with anti- $\alpha_v\beta_3$ -PE monoclonal antibody. Pearson *r* coefficient of correlation and *P*-value (two-tailed) are indicated.

Figure 3. Dd targets preferentially monocytes among PBMCs and blasts from AML compared to ALL, addressing all AML subtypes with a higher enrichment in acute myelomonocytic leukemia and is able to enter AML LSCs.

Healthy donor (A) and leukemic patient (B, C, D) PBMCs or whole bone marrow obtained from AML patients (E) were incubated with Dd-AF647 during 2h. Cells positive (%) for Dd-AF647 (A, C left panel; B, E in blue). Fold increase in Dd-AF647 MFI (A, C right panel; B in red; D) or Dd-AF647 MFI (E in red). Results represent the mean \pm SD and the corresponding number of samples is indicated. *P*-values were determined by Student *t*-tests (B) or two-way ANOVA (A, C, D). ns, not significant; *, *P* \leq 0.05; **, *P* \leq 0.01; ***, *P* \leq 0.001; ****, *P* \leq 0.0001. **(A)** Dd penetration within subpopulations of healthy donor PBMCs. Min/Max values (external lines) are also indicated. Cells were identified as follow: CD45⁺ CD66b⁺ for remaining granulocytes after PBMCs purification (blue); CD45⁺ CD3⁺ for T lymphocytes (red); CD45⁺ CD3⁻ CD19⁻ CD56⁺ for NK lymphocytes (purple); CD45⁺ CD19⁺ for B lymphocytes (green) and CD45⁺ CD33⁺ CD14⁺ for monocytes (orange). **(B)** Vectorization of SSC^{low} CD45^{low} blasts from acute lymphoblastic leukemia (ALL, n = 8) versus acute myeloblastic leukemia

(AML, n = 25). **(C)** Dd penetration within subpopulations of 25 AML PBMCs. Min/Max values (external lines) are also indicated. Subpopulations were identified as follow: SSC^{low} CD45^{high} CD3+ and CD19+ and CD56+ for lymphocytes (red); SSC^{high} CD45+ CD66b+ for remaining granulocytes after PBMCs purification (blue); SSC^{int} CD45+ CD33+ CD14+ for monocytes (orange) and SSC^{low} CD45^{low} distinct population for AML blasts (grey). **(D)** Vectorization of SSC^{low} CD45^{low} AML blasts according to the morphological French-American-British (FAB) classification (n = 25). Single patient values are displayed and the mean is indicated by a black line. Secondary AML represent AML contracted after a myelodysplastic syndrome, myeloproliferative syndrome, chronic myelomonocytic leukemia and chemotherapy. FAB M0-1-2 define undifferentiated AML or with minimal differentiation/maturation; FAB M3 = acute promyelocytic leukemia; FAB M4 = acute myelomonocytic leukemia; FAB M5 = acute monocytic leukemia and FAB M7 = acute megakaryoblastic leukemia. **(E)** Vectorization of stem cells with 1 nM Dd-AF647. Cells were enriched for CD34 expression after Dd incubation using magnetic sorting. Total stem cells = CD34+, Myeloid precursors = CD34+ CD33+ and GMP-like LSCs = Lin- CD34+ CD38+ CD123+ CD45RA+.

Figure 4. Dd demonstrates limited vector spreading outside the bloodstream and reach peripheral monocytes and bone marrow precursors in vivo.

Mice were injected IV with 100 µg Dd-AF647 in 200 µL of PBS (n = 9) or with 200 µL of PBS alone (n = 3). Three mice were sacrificed at 1-, 5- and 12-hours post-injection (p.i). Organs and cells from PBS-injected mice were used as vehicle control. Results represent the mean (n = 3) ± SD. *P*-values were determined by Student *t*-tests (B) or two-way ANOVA (C-D). ns, not significant; *, *P* ≤ .05; **, *P* ≤ .01; ***, *P* ≤ .001; ****, *P* < .0001. **(A)** 2D fluorescence imaging performed ex vivo on isolated organs, displayed in [photon/sec]/cm². Insert: a sample image of a mouse sacrificed 1 h p.i. **(B)** Principal blood constituents positive (%) for Dd-AF647. Platelets, red blood cells and leukocytes were gated by their specific expression of CD41, TER-119 (Ly-76) and CD45, respectively. **(C)** Lymphoid and myeloid lineages positive (%) for Dd-AF647. Subpopulations were identified with the following markers: CD45^{high} and CD3+

601 for T lymphocytes or CD335+ (NKp46) for NK lymphocytes or CD19+ for B lymphocytes;
602 CD45+ CD11c- MHCII- GR1+ (Ly-6G) for granulocytes and CD45+ CD11c^{low/neg} MHCII+
603 F4/80+ (Ly-71) for monocytes. **(D)** Stem cells and early precursors positive (%) for Dd-
604 AF647. Subpopulations were identified as follow: Lin- Sca1+ c-Kit+ CD135- CD34+ for
605 hematopoietic stem cells (HSCs); Lin- Sca1+ c-Kit+ CD135+ CD34+ for multipotent
606 progenitors (MPPs); Lin- CD127+ Sca1+ c-Kit+ for common lymphoid progenitors (CLPs);
607 Lin- CD127- Sca1- c-Kit+ CD16/32- CD34+ for common myeloid progenitors (CMPs); Lin-
608 CD127- Sca1- c-Kit+ CD16/32+ CD34+ for granulocyte-monocyte progenitors (GMPs) and
609 Lin- CD127- Sca1- c-Kit+ CD16/32- CD34- for megakaryocyte-erythrocyte progenitors
610 (MEPs).

611

612 Tables

613 **Table 1. Description of Acute Lymphoid Leukemia (ALL) and Acute Myeloid**
 614 **Leukemia (AML) patient population.**

	ALL (n, %)	AML (n, %)
NUMBER OF SAMPLES	8 (16)	25 (84)
SEX (male %)	62,5	68
AGE AT DIAGNOSIS		
<2	0	2 (8)
2-10	5 (62,5)	1 (4)
10-18	3 (37,5)	1 (4)
18-60	N/A	9 (36)
>60	N/A	12 (48)

615

616 **Table 2. Classification of pediatric and adult AML patient population.**

	PEDIATRIC (n, %)	ADULT (n, %)
AML at		
Diagnosis	4 (100)	21 (71)
Relapsing	0	6 (29)
FAB CLASSIFICATION		
M0	1 (25)	5 (24)
M1	0	2 (10)
M2	1 (25)	7 (33)
M3	0	1 (5)
M4	0	3 (14)
M5	1 (25)	3 (14)
M6	0	0
M7	1 (25)	0
WHO CLASSIFICATION		
AML with recurrent genetic abnormalities	2 (50)	7 (33)
<i>flt3</i>	1 (25)	3 (14)
<i>npm1</i>	0	2 (10)
<i>idh1/2</i>	0	2 (10)
<i>Others</i>	1 (25)	0
AML with myelodysplasia related changes	0	8 (38)
therapy-related AML	1 (25)	0
AML not otherwise specified	1 (25)	6 (25)

617