Table S1. Immunophenotyping of primary samples from healthy donors and AML patients

Cell Subpopulation	Identifying Markers
HSC	$CD34^+$, $CD38^-$, $CD90^+$, $CD45RA^-$
MPP	$CD34^+$, $CD38^-$, $CD90^-$, $CD45RA^-$
CLP	$CD34^+$, $CD10^+$
CMP	$CD34^+$, $CD38^+$, $CD10^-$, $CD135^+$,$CD45RA^-$
GMP	$CD34^+$, $CD38^+$, $CD10^-$, $CD135^+$,$CD45RA^+$
MEP	$CD34^+$, $CD38^+$, $CD10^-$, $CD135^-$,$CD45RA^-$
AML blast	SSC low-med, CD45 med
AML progenitors	SSC low-med, CD45 med, $CD34^+$, $CD38^+$
AML LSC	SSC low-med, CD45 med, $CD34^+$, $CD38^-$

HSC, hematopoietic stem cells; MPP, multipotent progenitors; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; GMP, granulocyte/macrophage progenitors; MEP, megakaryocyte erythroid progenitors; LSC, leukemic stem cells

Table S2. Binding affinities of anti-CD123 antibodies to HNT-34 cells, measured by flow cytometry.

Antibody	Kd, pM
7G3	3,000
9F5	1,000
6H6	2,000
CD123-6	80

Table S3. Binding affinities of G4723A, IMGN632, and X-ADC to HNT-34 cells, measured by flow cytometry

Compound	Kd, pM
G4723A	52
IMGN632	38
X-ADC	40

Table S4. Binding kinetics of G4723A and IMGN632, measured by ForteBio

Compound	K_{on}, (1/Ms)	K_{off} (1/s)	Kd, M
G4723A	2.00E+06	8.00E-05	6.00E-11
IMGN632	1.00E+06	9.00E-05	9.00E-11

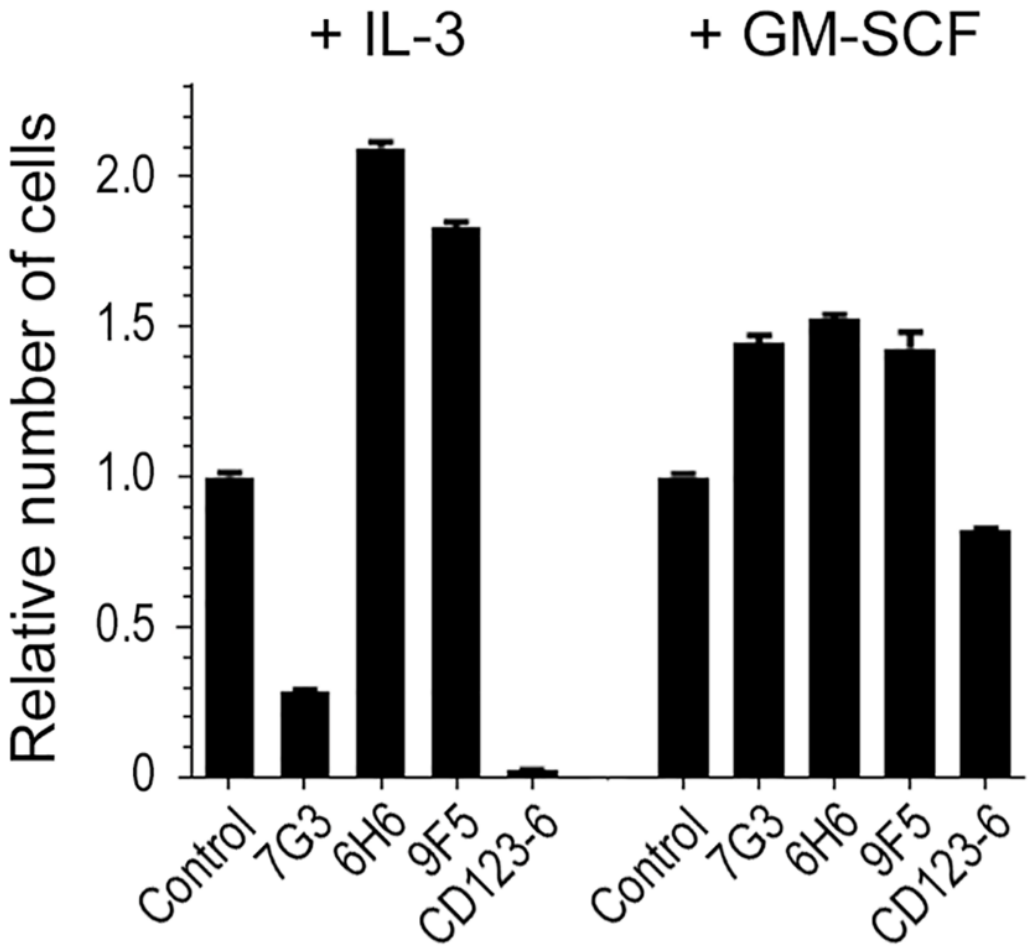

Figure S1. Effect of anti-CD123 antibodies on growth factor-mediated TF-1 cell proliferation. TF-1 cells were starved of IL-3 and GM-CSF overnight, then plated at 6,000 cells per well in either the presence or absence of 10 μg/mL of an anti-CD123 antibody, followed by addition of either IL-3 (1 ng/mL) or GM-CSF (2 ng/mL). Cells were incubated at 37°C for 3 days. The relative numbers of viable cells in each well were determined by WST-8 assay.

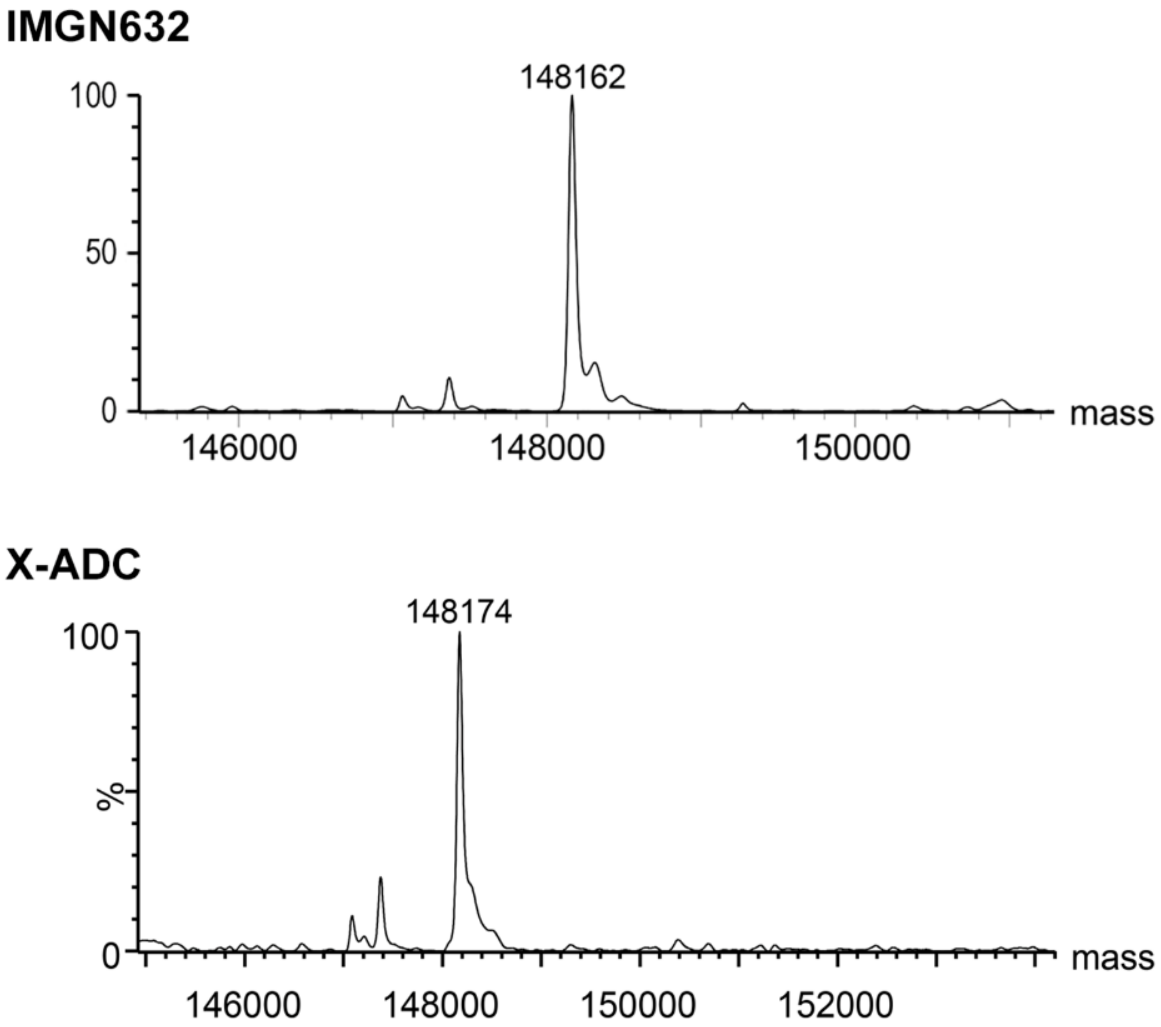

Figure S2. Homogeneity of drug loading on IMGN632 and X-ADC. ADCs were evaluated by mass spectrophotometric analysis. The major peak represents G4723A antibody conjugated with 2 payload molecules.

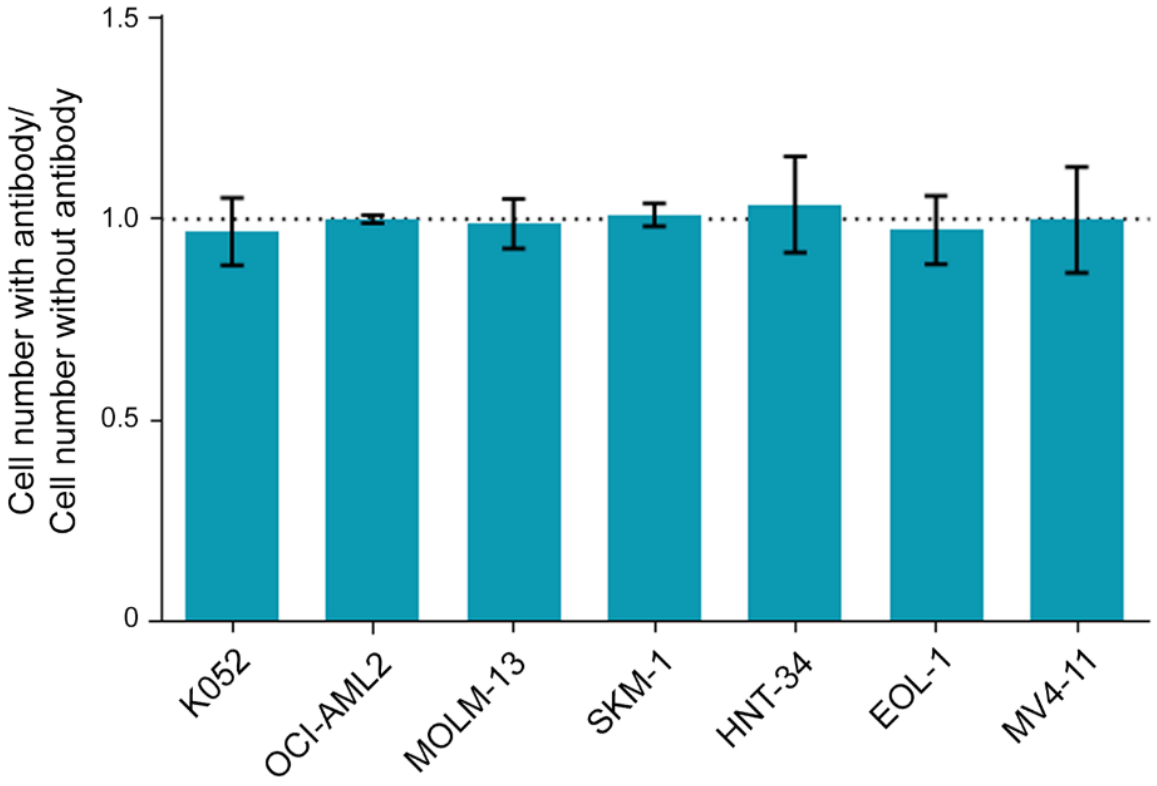

Figure S3. Effect of G4723A on proliferation of AML cell lines. Cells were incubated with 1 μM of an antibody that is identical to G4723A, but lacking engineered cysteines. Four to seven days later, cell viability was determined using WST-8 assays and compared to that of the cells grown in the absence of the antibody.

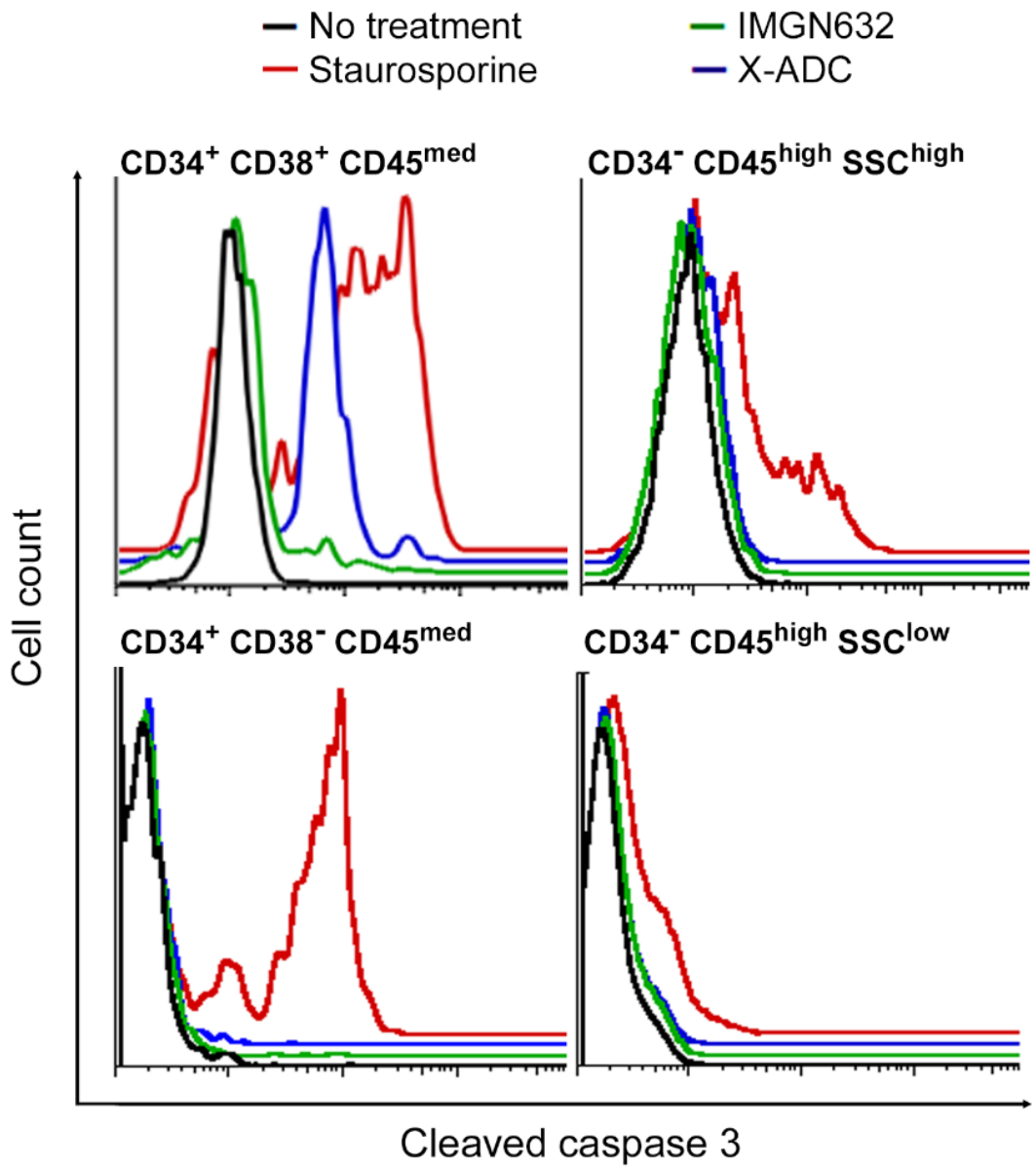

Figure S4. X-ADC, but not IMGN632, induces apoptosis of $CD34^{+}CD38^{+}$ bone marrow cells. Bone marrow mononuclear cells from a healthy volunteer were treated with 5 μM of staurosporine for 4 hours or 100 pM of either IMGN632 or X-ADC for 72 hours at 37°C. Cells were harvested and stained for markers of cell lineage (CD45, CD34, CD38) and apoptosis (cleaved caspase-3) using multiparametric flow cytometry and analyzed with ModFit or FlowJo software.

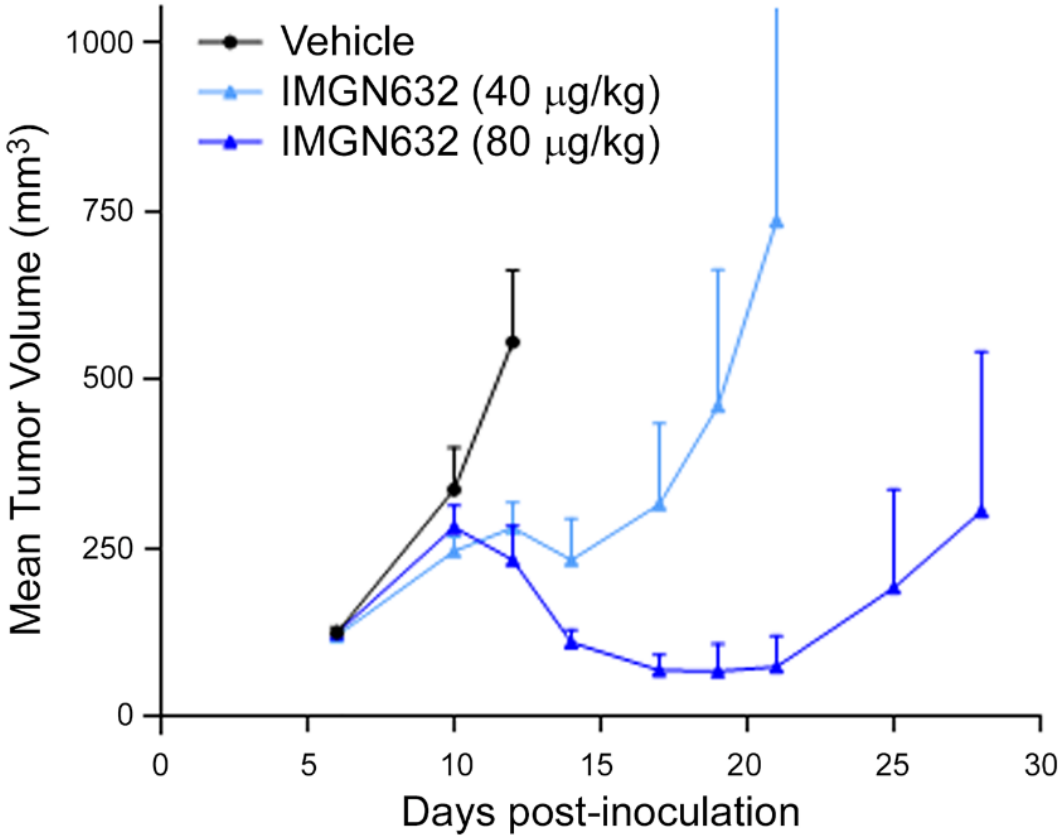

Figure S5. Low, single dose IMGN632 is active in EOL-1 subcutaneous xenograft AML model. Nude mice bearing EOL-1 xenografts received a single dose of vehicle or IMGN632 (40 μg/kg or 80 μg/kg), as indicated.