Mechanism-Based Epigenetic Chemosensitization Therapy of Diffuse Large B Cell Lymphoma

Supplementary Figures

Figure S1

Figure S1. Normalized isobolograms (at GI90) for the combination of DAC and vincristine, mechlorethamine and dexamethasone for individually sensitive cell lines. Cell lines are represented with circles. Cells below the line show synergistic combination while cells above the line show no combinatorial effect or antagonism. Only individually sensitive cell lines are plotted.
Figure S2

A

0 h  24 h  48 h  48 h concurrent
5'-aza-dC  Doxorubicin  Viability

0 h  24 h  48 h  48 h sequential
5'-aza-dC  Doxorubicin  Viability

0 h  24 h  48 h  72 h  72 h sequential
5'-aza-dC  5'-aza-dC  Doxorubicin  Viability

B

48 h concurrent  48 h sequential  72 h sequential

\[ G_{50} \]
\[ G_{50} \]
\[ G_{50} \]

WSU-NHL
OCI-Ly7
OCI-Ly1

C

Combination
Doxorubicin
5'-aza-dC
Vehicle

OCI-Ly7
DoHH2
SU-DHL8
WSU-NHL

D

Fold Change in MFI (relative to vehicle treated and normalized to isotype control)

CD130  CD38

V  5A  D  C  V  5A  D  C  V  5A  D  C  V  5A  D  C

DoHH2  OCI-Ly7  WSU-NHL  SU-DHL8
Figure S2. Co-administration of DAC and doxorubicin in sensitive cells. A: Three different drug administration schedules were used to determine the effect of the sequence of administration on DLBCL cell viability over 48 h and 72 h. B: Isobolograms for 50% cell growth inhibition (ED50) for the combination of DAC and doxorubicin in two sensitive cell lines (WSU-NHL, blue dots and OCI-Ly7, red dots) and one relatively resistant cell line (OCI-Ly1, green dots). Each isobologram represents the result of a schedule of administration as shown in A. C: Cell cycle distribution at 12 h after treatment with DAC 100 nM, doxorubicin 500 nM, the combination of both drugs or vehicle (water) in four DAC sensitive cell lines. The number of cells in percentage is shown on the y axis. DNA content (DAPI) is shown on the x axis. Experiment representative of 4 replicates. Arrow indicates the difference in G2 for cells treated with drug or combination vs. vehicle. D: Percent of CD38+ and CD138+ cells in cell lines treated with vehicle (V), DAC (5A), doxorubicin (D) and combination of drugs (C ). Data obtain from flow cytometry analysis and represented as fold change in mean fluorescence intensity (MFI) normalized to isotype control and related to vehicle treated cells. Experiment carried out in duplicates.
Figure S3: Combinatorial effect in primary DLBCL samples. CD19+ single-cell suspensions from lymph node biopsies of 5 confirmed DLBCL specimens were co-cultured with HK dendritic cells in a dual chamber. CD19+ cells were exposed to DAC 100 nM (left) and 300 nM (right), doxorubicin 0.6 μM (left) and 1.2 μM (right) and the combination of the drugs. Cell viability at 48 h (represented as percentage of vehicle-treated cells) is shown on the y-axis. The experiment was carried out in duplicates for drug treated and 4 replicates for vehicle-treated cells. The dotted line represents 25% growth inhibition.
Figure S4

A

48 h exposure

H2AX gamma phosphorylation (fold to vehicle)

- Doxorubicin
- 5-aza-dC G1/20
- 5-aza-dC 100 nM

OCI-Ly1  Karpas422  OCI-Ly7

B

Bgal densitometry (to GAPDH)

- PARP
- cPARP
- BGAL1
- GAPDH

OCI-Ly1  Karpas422

d0  d3  d5  d0  d3  d5  d0  d3  d5

C

Karpas422  OCI-Ly1

Cell Number

CD38  CD138  CD38  CD138

- Staining control
- Vehicle
- 5-aza-dC 100 nM

D

OCI-Ly1  Karpas422

Vehicle  5-aza-dC 100nM

DNA Content

- Sub-G1
- G1
- S+G2/M

OCI-Ly1

Sub-G1: 1.1%  Sub-G1: 1.7%  Sub-G1: 27.1%  Sub-G1: 38.8%
G1: 64%  G1: 64%  G1: 50%  G1: 45.7%
S+G2/M: 33.5%  S+G2/M: 33.7%  S+G2/M: 22.2%  S+G2/M: 14.8%

Karpas422

Sub-G1: 0.8%  Sub-G1: 9.1%  Sub-G1: 16.9%  Sub-G1: 18%
G1: 68%  G1: 56.1%  G1: 56.3%  G1: 54.8%
S+G2/M: 30.5%  S+G2/M: 33.4%  S+G2/M: 25.9%  S+G2/M: 26.0%

Sub-G1: 72 h  Sub-G1: 120 h

E

Karpas 422  SC-1  OCI-Ly1

p21  p21/actin  p21  d0  d3  d5  Vehicle  Dac 100 nM
p21/actin  p16  p16  d0  d3  d5  d0  d3  d5
actin  actin  actin  1  2  1  2.6  1  10  1  2  1
Figure S4: Effect of DAC on doxorubicin resistant cells A: Percent of H2AX phosphorylation positive cells after exposure to doxorubicin GI50, DAC GI50 and DAC 100 nM for 48 h in OCI-Ly1 and Karpas422 cell lines. The effect of DAC GI50 (100 nM) for 48 h on OCI-Ly7 is shown for comparison. B: Immunoblot for PARP, cleaved-PARP and beta-galactosidase in DLBCL cells treated DAC 100 nM for 72 h and 120 h. The columns represent densitometry analysis normalized by GAPDH. C: Differentiation markers in DLBCL cells treated DAC. Plasmacytic differentiation markers (CD38 and CD138) determined by flow cytometry in Karpas422 (left panel) and OCI-Ly1 (right panel) cells after treatment with DAC 100 nM for 120 h. D: Cell cycle distribution in OCI-Ly1 and Karpas422 after exposure to vehicle (0 h) and DAC for 72 h (top) and 120 h (bottom). E: left: Immunoblot for p21 and p16 in Karpas422 and SC-1 cells treated with DAC 100 nM for 5 days. The numbers represent the ratio of band densitometry for the target antibody vs. loading control (actin); right: Immunoblot for p21 and p16 in OCI-Ly1 cells treated with vehicle or DAC 100 nM for 3 and 5 days. Actin was used as loading control.