The role of the mineralocorticoid receptor in steroid-induced cytotoxicity in pediatric acute lymphoblastic leukemia

Authors

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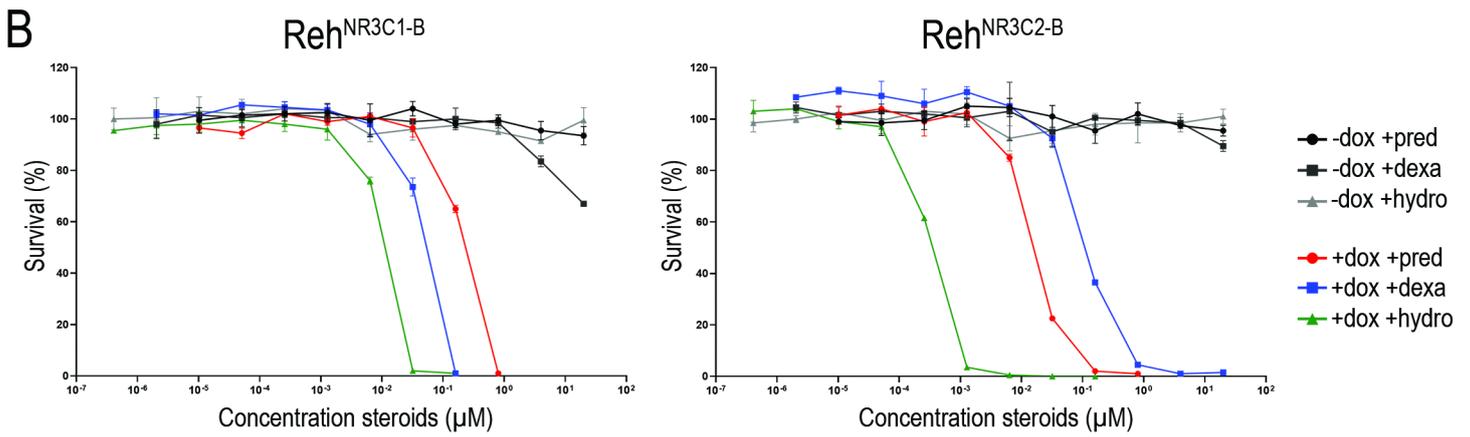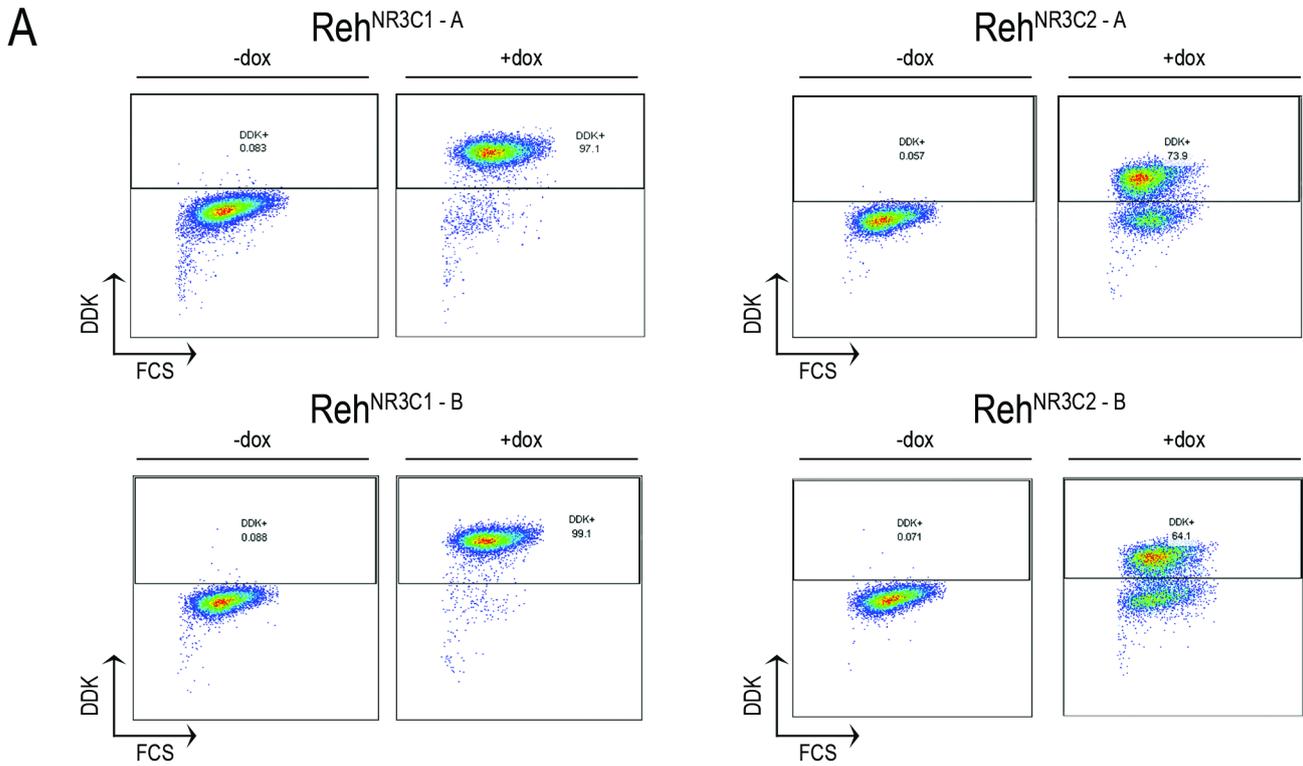A. M. VAN HULST - a.vanhulst@prinsesmaximacentrum.nl

A

Reh$^{NR3C1 - A}$

-dox   +dox

DDK+
0.083

DDK+
97.1

DDK

FCS

Reh$^{NR3C2 - A}$

-dox   +dox

DDK+
0.057

DDK+
73.9

DDK

FCS

Reh$^{NR3C1 - B}$

-dox   +dox

DDK+
0.088

DDK+
99.1

DDK

FCS

Reh$^{NR3C2 - B}$

-dox   +dox

DDK+
0.071

DDK+
64.1

DDK

FCS

B

Reh$^{NR3C1-B}$

Reh$^{NR3C2-B}$

Survival (%)

Concentration steroids (µM)

- -dox +pred
- -dox +dexa
- -dox +hydro

- +dox +pred
- +dox +dexa
- +dox +hydro

Supplemental Figure 1.
(A), Reh cells were transduced with a doxycycline-inducible *NR3C1* or *NR3C2* construct. The inducibility of the NR3C1- or NR3C2- constructs was measured through flow cytometry following intracellular DDK staining. Doxycycline exposure induced the expression of DDK-tagged *NR3C1* (referred to as Reh^{NR3C1}) or *NR3C2* (referred to as Reh^{NR3C2}) in Reh single cells that intrinsically lack *NR3C1* and *NR3C2* expression. (B), Cell toxicity screening of our Reh$^{NR3C1-B}$ and Reh$^{NR3C2-B}$ cell lines with (color) and without (gray-scales) doxycycline induction and after treatment with prednisolone, dexamethasone or hydrocortisone. These secondary cell lines showed the same pattern of steroid sensitivity as our primary cell lines.

A

B

no treatment | +RU
prednisolone | pred +RU
dexamethasone | dexa +RU
hydrocortisone | hydro +RU
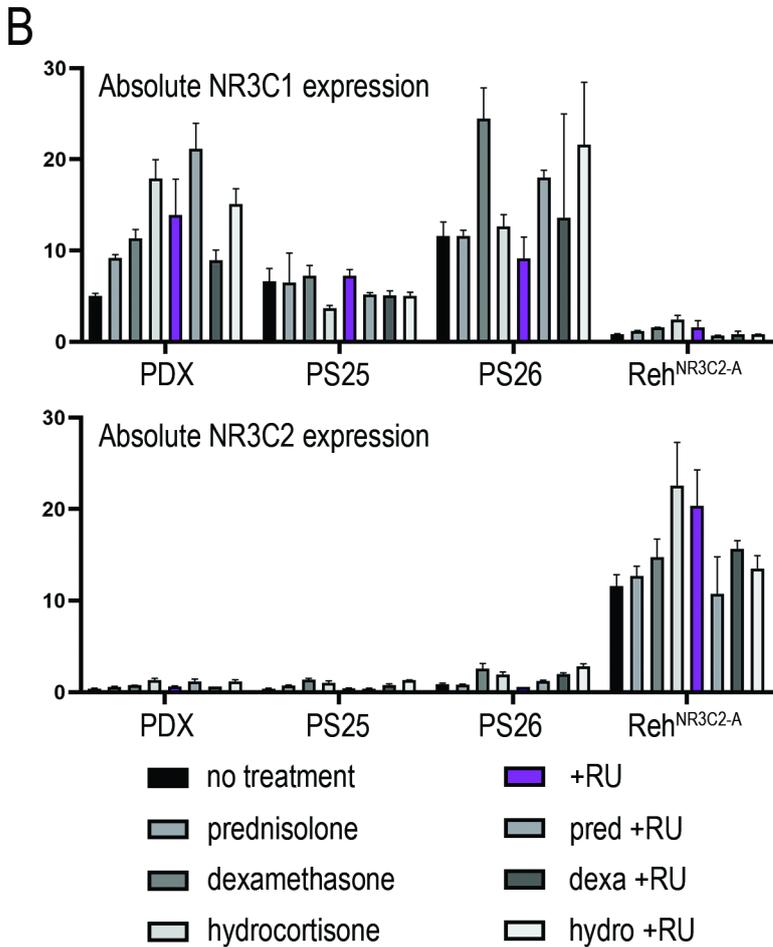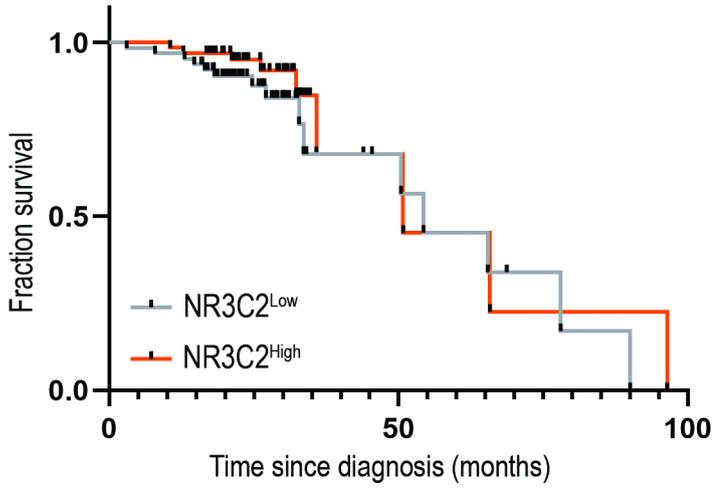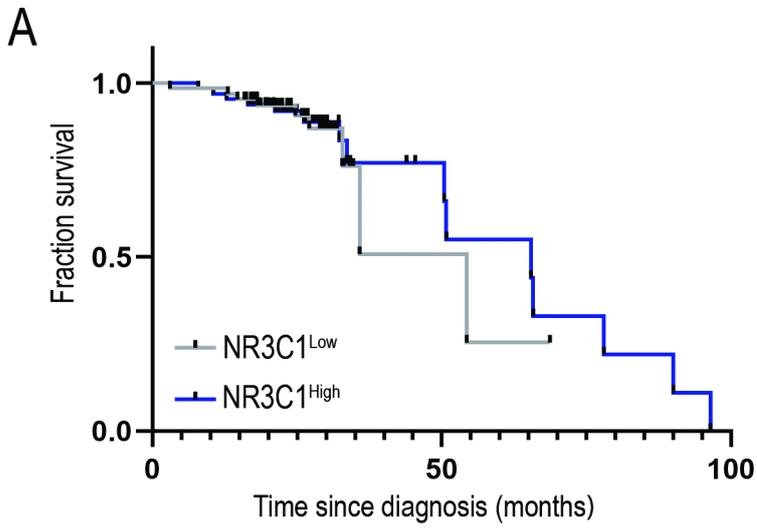
Supplemental Figure 2.
(A), Event free survival (EFS) for the 131 ALL patients with either high or low *NR3C1* or *NR3C2* expression. Low or high MR and GR expression levels were defined with the median as cut-off value. EFS estimated with Kaplan Meier's methodology. (B), Transcriptional steroid response of two primary patient samples (PS25 and PS26), one PDX sample and doxycycline-induced Reh[NR3C2]. Patient and PDX cells were treated with 0.05µM prednisolone, 0.05µM dexamethasone or 2µM hydrocortisone, Reh[NR3C2] cells were treated with 0.032µM prednisolone, 0.16µM dexamethasone or 0.0028µM hydrocortisone, all in presence or absence of MR antagonist RU28318. Absolute *NR3C1* (upper panel) and *NR3C2* (lower panel) expression was measured.

Supplemental Table 1. Patients' characteristics for survival analyses and estimated hazard ratio with 95%-CI for any first event			
Patients	Number	131	
	Median follow up (95%-CI) *months*	26.1 (23.8-28.4) *Reverse Kaplan Meier*	
Events	Induction failure n(%)	1 (0.8)	
	Relapse n(%)	22 (16.8)	
	Secondary malignancy n(%)	2 (1.5)	
	Death n(%)	8 (6.1) *NB: all second events*	
		n (%)	**Hazard ratio (95%-CI)**
Age at diagnosis *years*	Median (range)	5 (1-18)	
	1-4	63 (48.1)	
	5-9	24 (18.3)	0.38 (0.11-1.28)
	10-14	24 (18.3)	0.93 (0.33-2.63)
	15-18	20 (15.3)	0.62 (0.14-2.85)
Sex	Female	50 (38.2)	
	Male	81 (61.8)	0.86 (0.37-1.99)
ALL subclass	B-cell ALL	114 (87)	
	T-cell ALL	17 (13)	2.17 (0.72-6.53)
Cytogenetic subtype[1]	ETV6-RUNX1	28 (21.4)	
	Other	103 (78.6)	2.45 (0.57-10.51)
CNS involvement[2]	No	122 (93.1)	
	Yes	4 (3.1)	*7.73 (1.70-35.15)*
	Unknown	5 (3.8)	0.62 (0.14-2.81)
Leucocytes at diagnosis	Median (range)	11.2 (0.8-705)	
	<10	59 (45.0)	
	10-25	29 (22.1)	1.38 (0.52-3.66)
	25-50	14 (10.7)	0.73 (0.15-3.58)
	50-100	15 (11.5)	1.51 (0.32-7.20)
	>100	14 (10.7)	1.91 (0.51-7.17)
NCI risk group	NCI standard risk	71 (54.2)	
	NCI high risk	60 (45.8)	1.33 (0.58-3.03)
Prednisone response day 8[3]	Good	114 (87)	
	Poor	14 (10.7)	2.34 (0.66-8.25)
Risk group stratification[4]	SR	24 (18.3)	
	MR	95 (72.5)	2.10 (0.59-7.54)
	HR	12 (9.2)	*10.33 (2.19-48.71)*
MRD day 33[5]	Negative	30 (22.9)	
	Positive	97 (74)	2.59 (0.74-9.06)
MRD day 79[6]	Negative	80 (61.1)	
	Positive	*45 (34.4)*	*4.22 (1.57-11.28)*
GR (NR3C1) expression[7]	Median (range)	199.6 (41.9-567.1)	
	Low	65 (49.6)	
	High	66 (50.4)	0.96 (0.40-2.30)
MR (NR3C2) expression[7]	Median (range)	5.4 (0.2-122.3)	
	Low	65 (49.6)	
	High	66 (50.4)	0.57 (0.24-1.33)

Hazard ratio is depicted for the first occurring event. NCI-risk category: standard risk= <10 years and white cell count at diagnosis <50x109/L, high risk= other.

Abbreviations: CI=confidence interval, ALL=acute lymphoblastic leukemia, CNS=central nervous system, NCI=National Cancer Institute, SR=standard risk, MR=medium risk, HR=high risk, MRD=minimal residual disease, GR=glucocorticoid receptor, MR=mineralocorticoid receptor

[1] *Groups too small: only 2 events in ETV6-RUNX1 group*

[2] *Groups too small: only 2 events in group with CNS involvement*

[3] *Landmark analysis, starting at day 8: 1 event (induction failure) lost*

[4] *Groups too small: only 3 events in the standard risk group*

[5] *Landmark analysis, starting at day 33: 1 event (induction failure) lost*

[6] *Landmark analysis, starting at day 79: 1 event (induction failure) lost*

[7] *MR and GR expression levels were categorized as low or high, with the median as cut-off value*