Università degli Studi di Firenze

hERG1 channels: from antitargets to novel therapeutic targets in oncology

Annarosa Arcangeli[1], Andrea Becchetti[2], Olivia Crociani, Massimo D'Amico[1], Luca Gasparoli[1], Marika Masselli, Serena Pillozzi[1], Kenneth Mugridge[3] and Wolfgang Tiedke[3].

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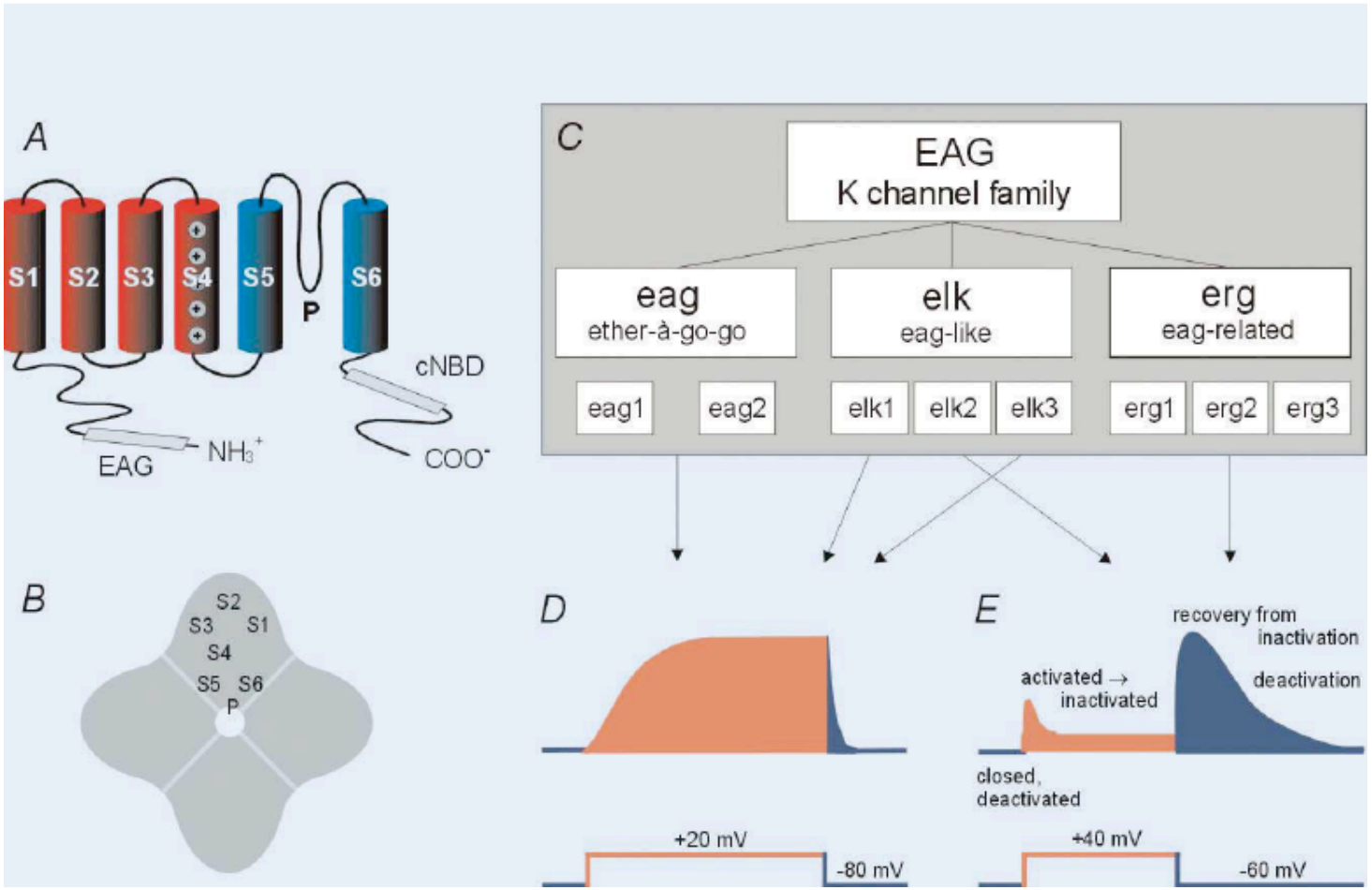
Disclosures

- No conflict of interest to declare

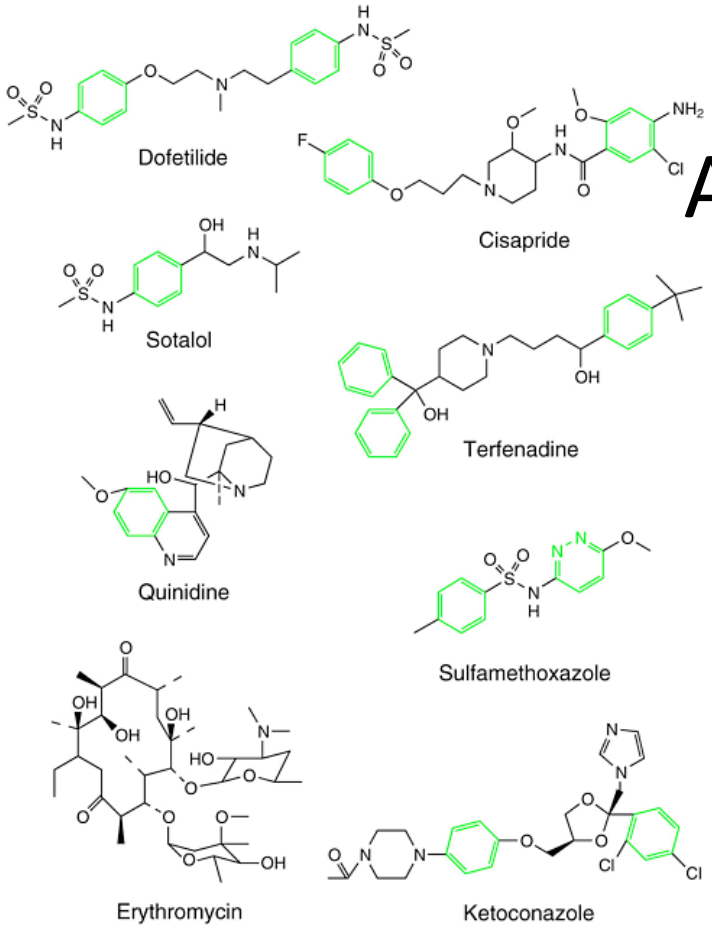
hERG1 POTASSIUM CHANNELS



Several drugs block hERG1 channels

drug-induced LQT2

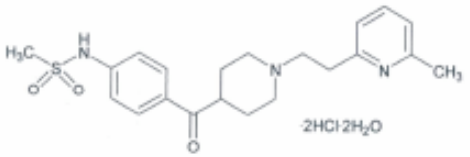
ANTITARGET



Specific hERG1 blockers

E4031

WAY 123,398



hERG1 is overexpressed in several types of human cancers

-Acute myeloid and lymphoblastic leukemias

Hofmann et al., J.Biol.Chem., 2001; Pillozzi et al., Leukemia, 2002; Pillozzi et al., Blood, 2007; Pillozzi et al., Blood, 2011

-Endometrial Cancers

Cherubini et al., Br. J. Cancer, 2000

-Neuroblastomas

Arcangeli et al., J. Cell Biol., 1993; Arcangeli et al., J.Physiol., 1995; Arcangeli et al., Eur.J.Neurosci, 1997; Crociani et al., Mech.Devel., 2000; Crociani et al., J. Biol. Chem., 2003

-Colorectal and Gastric Cancers

Lastraioli et al., Cancer Res. 2004; Lastraioli et al., J.Cell.Physiol., 2006.

-Glioblastoma

Masi et al., Br. J. Cancer, 2005.

hERG1 regulates different biological functions in cancer cells

- Cell proliferation (myeloid leukemias)
- Drug-induced apoptosis (lymphoblastic leukemias)
- Tumor cell invasion and metastatic spread (colorectal cancers)
- Trans-endothelial migration and invasion of extra-medullary organs (myeloid leukemias)
- VEGF-A secretion and angiogenesis (astrocytomas; gastric and colorectal cancers)

hERG1:

new target in oncology?

Adverse cardiac effects!

Differences between “cardiac” and “tumour” hERG1

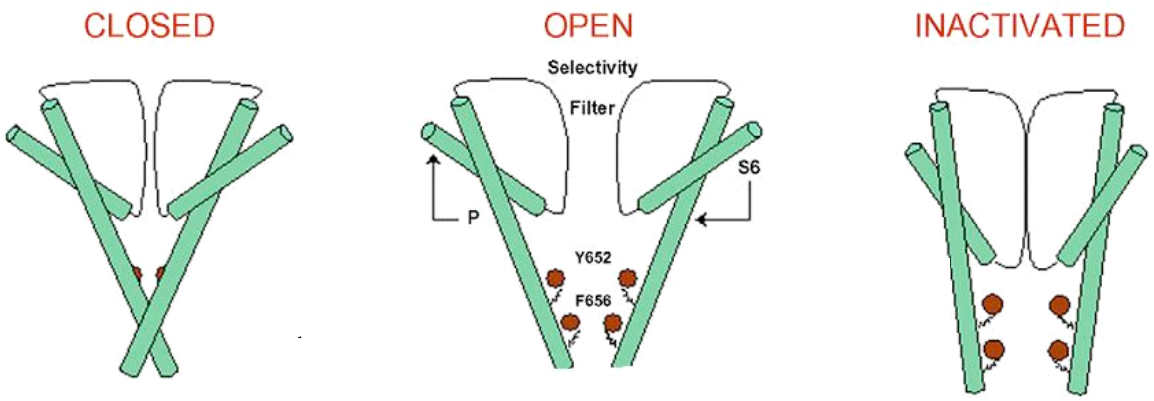
- Biophysical
- Molecular
- Functions

Biophysical characteristics of hERG1 channels

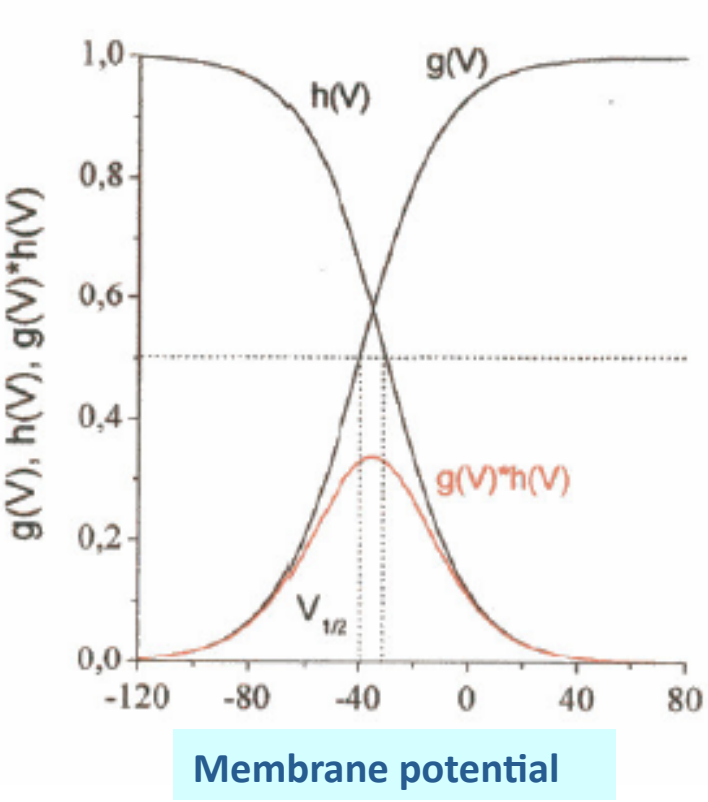
$I_{(K)ERG}$ quickly inactivates after opening at positive potentials

Inactivation is faster than activation

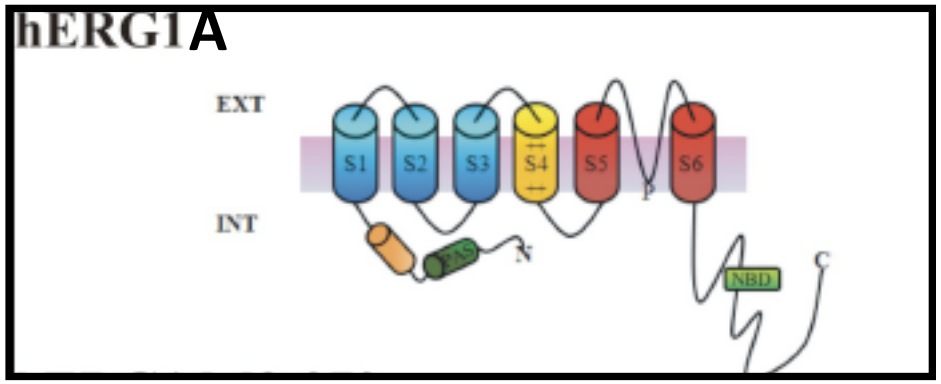
Recovery from inactivation is faster than deactivation

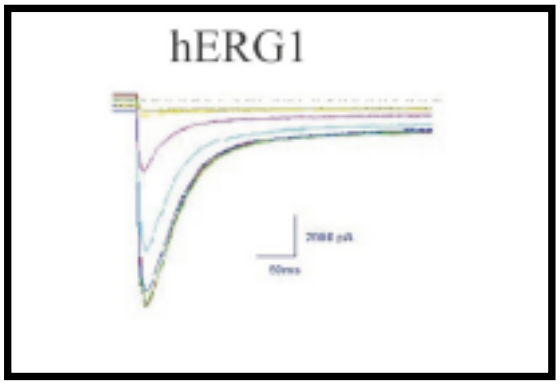


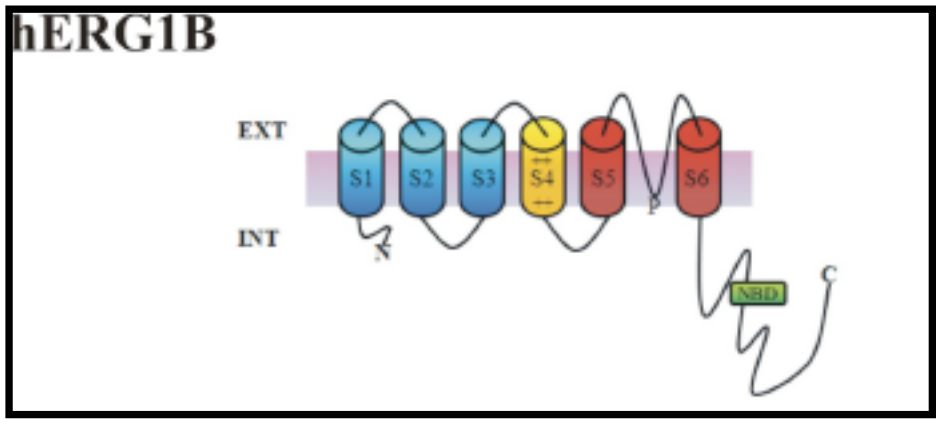
- **In the heart: hERG1 = Ikr**
- **hERG1 channels activate slowly and inactivate rapidly during the initial phases of the cAP; as repolarization begins, hERG1 channels rapidly recover from inactivation and generate an appreciable outward current.**
- **In cancer cells:**
- **hERG1 channels exibit a significant steady –state conductance at membrane potentials around -40 mV**

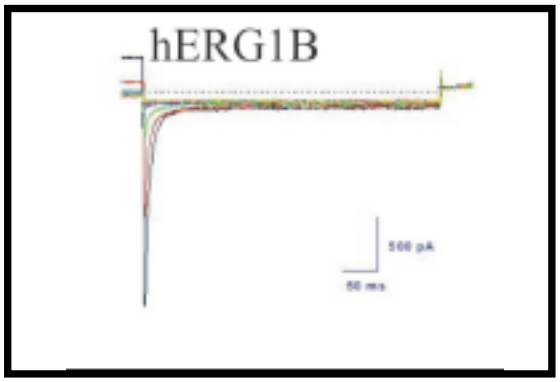


Molecular characteristics of hERG1 in cancer cells









Functions of hERG1 in cancer cells

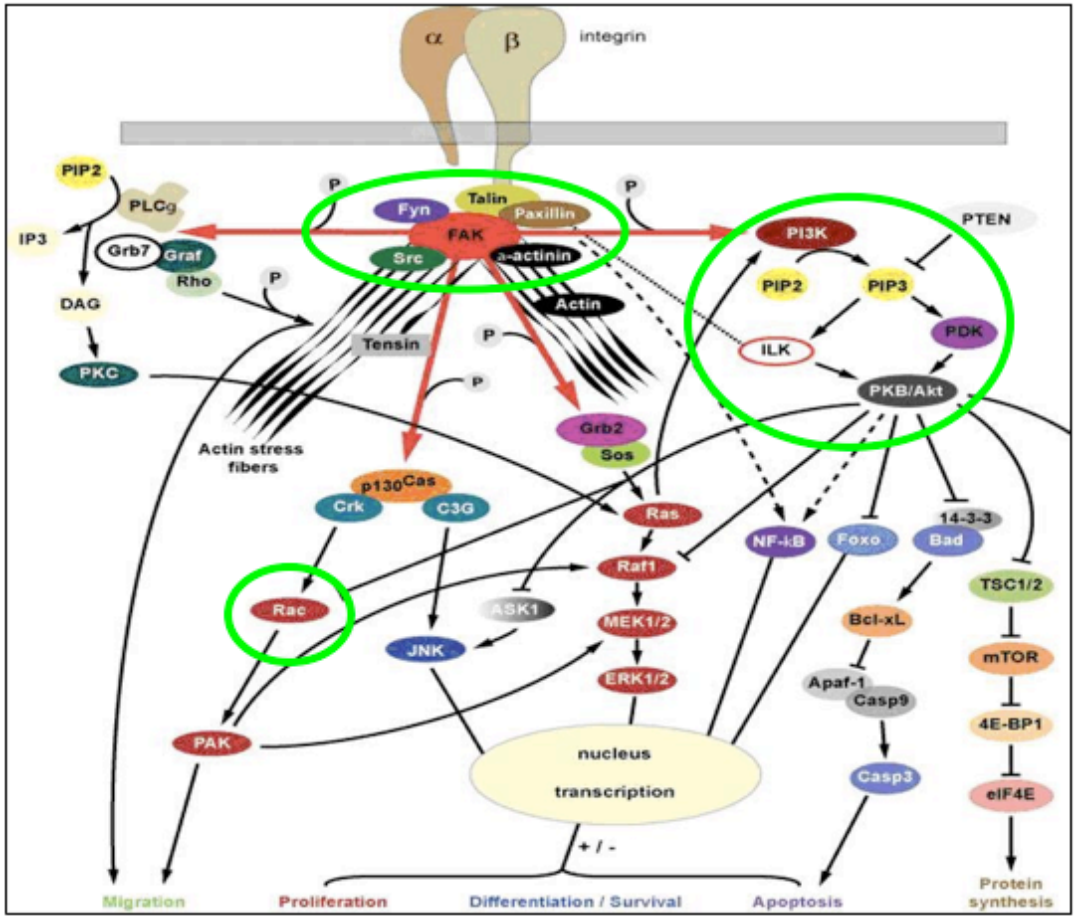
Review TRENDS in Cell Biology Vol.16 No.12

Complex functional interaction between integrin receptors and ion channels

Annarosa Arcangeli[1] and Andrea Becchetti[2]

[1] Department of Experimental Pathology and Oncology, University of Firenze, Viale G.B. Morgagni 50, 50134 Firenze, Italy
[2] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy

hERG1 channels co-assemble with integrins and modulate integrin-dependent signalling.



Potential approaches to improve the efficacy and safety of hERG1 channel targeting in oncology:

- Use of “non-torsadogenic” hERG1 blockers (e.g.erythromycin, sertindole).

-Use of compounds which bind hERG1 channels in the open state (D-Roscovitine).

-Use/development of compounds which selectively inhibit tumour-specific hERG1 isoform(s)

- Development of compounds (antibodies, peptides) which disassemble the ion channel/ integrin complex.

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ELSEVIER

Cardiovascular Research 58 (2003) 32–45

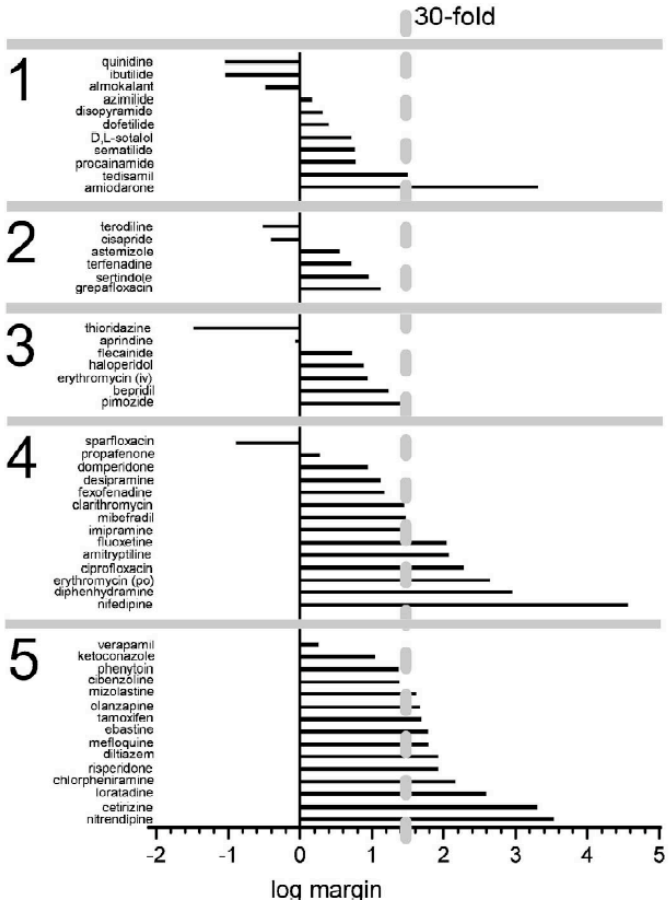
Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development☆

W.S. Redfern[a], L. Carlsson[b], A.S. Davis[c], W.G. Lynch[d], I. MacKenzie[e], S. Palethorpe[a], P.K.S. Siegl[f], I. Strang[a], A.T. Sullivan[g], R. Wallis[h], A.J. Camm[i], T.G. Hammond[a,*]



Erythromycin
Sertindole

State-dependent block of HERG potassium channels by *R-roscovitine:*
implications for cancer therapy

Ganapathi SB et al., *Am J Physiol Cell Physiol 296: C701–C710, 2009.*

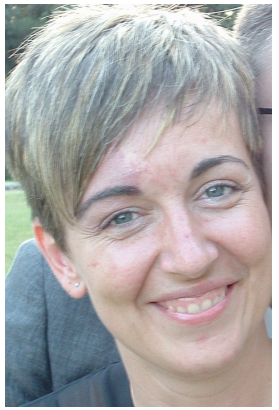
R-Roscovitine

blood

Prepublished online Nov 3, 2010;
doi:10.1182/blood-2010-01-262691

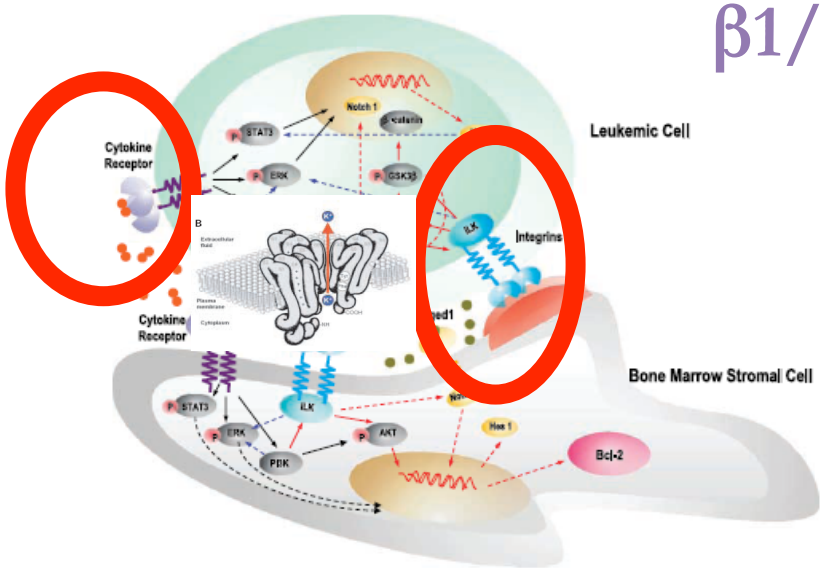
Chemotherapy resistance in acute lymphoblastic leukemia requires hERG1 channels and is overcome by hERG1 blockers

Serena Pillozzi, Marika Masselli, Emanuele De Lorenzo, Benedetta Accordi, Emanuele Cilia, Olivia Crociani, Amedeo Amedei, Marinella Veltroni, Massimo D'Amico, Giuseppe Basso, Andrea Becchetti, Dario Campana and Annarosa Arcangeli



Serena Pillozzi

β1/hERG1/CXCR4 complex



SURVIVAL (ILK, AKT)

The complex is relevant to drive survival signals

which is the effect of blocking hERG1 channels on drug-induced apoptosis?

The addition of hERG1 blockers shortcomes the protective effect of Bone Marrow Stromal Cells on Doxorubicin (Prednisone, Methotrexate, L-asparaginase)- induced apoptosis

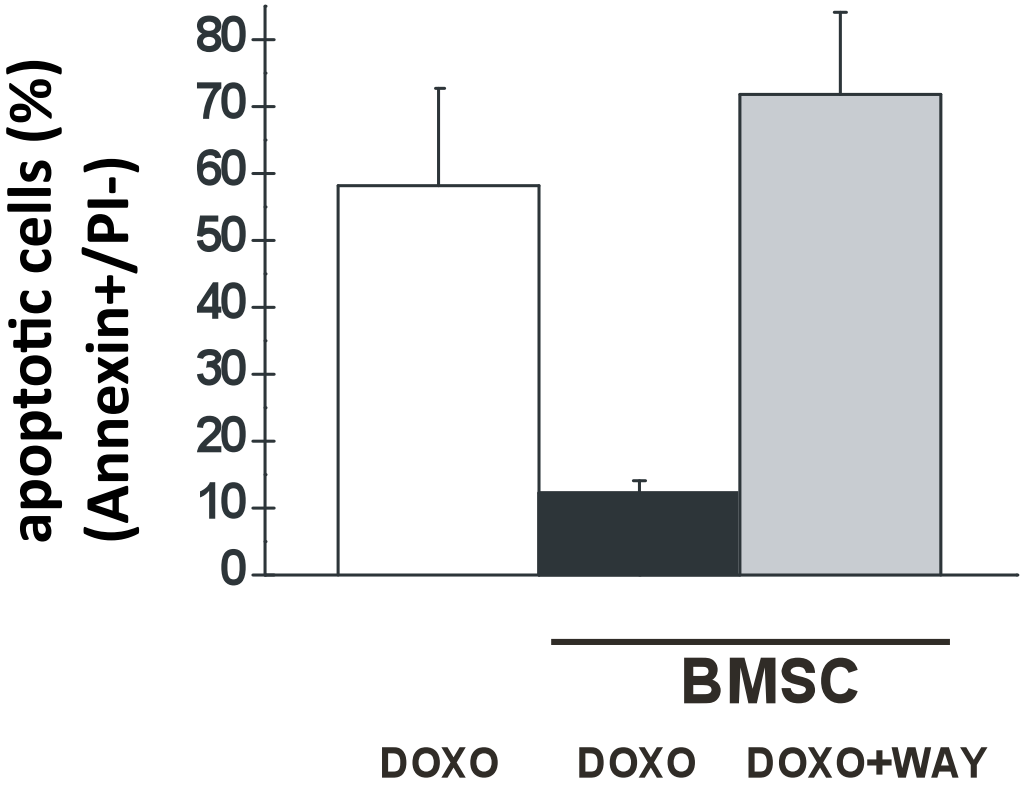
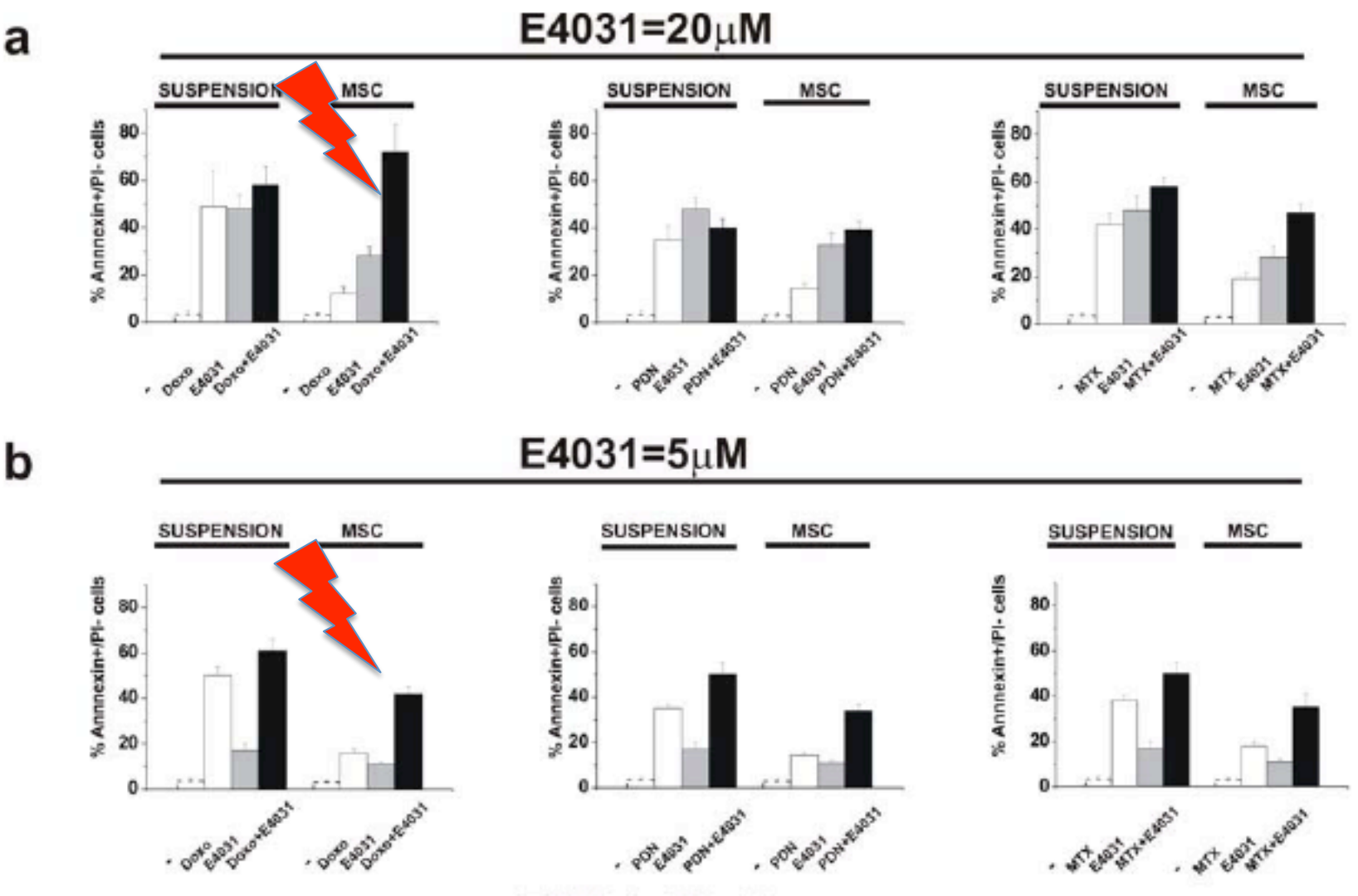
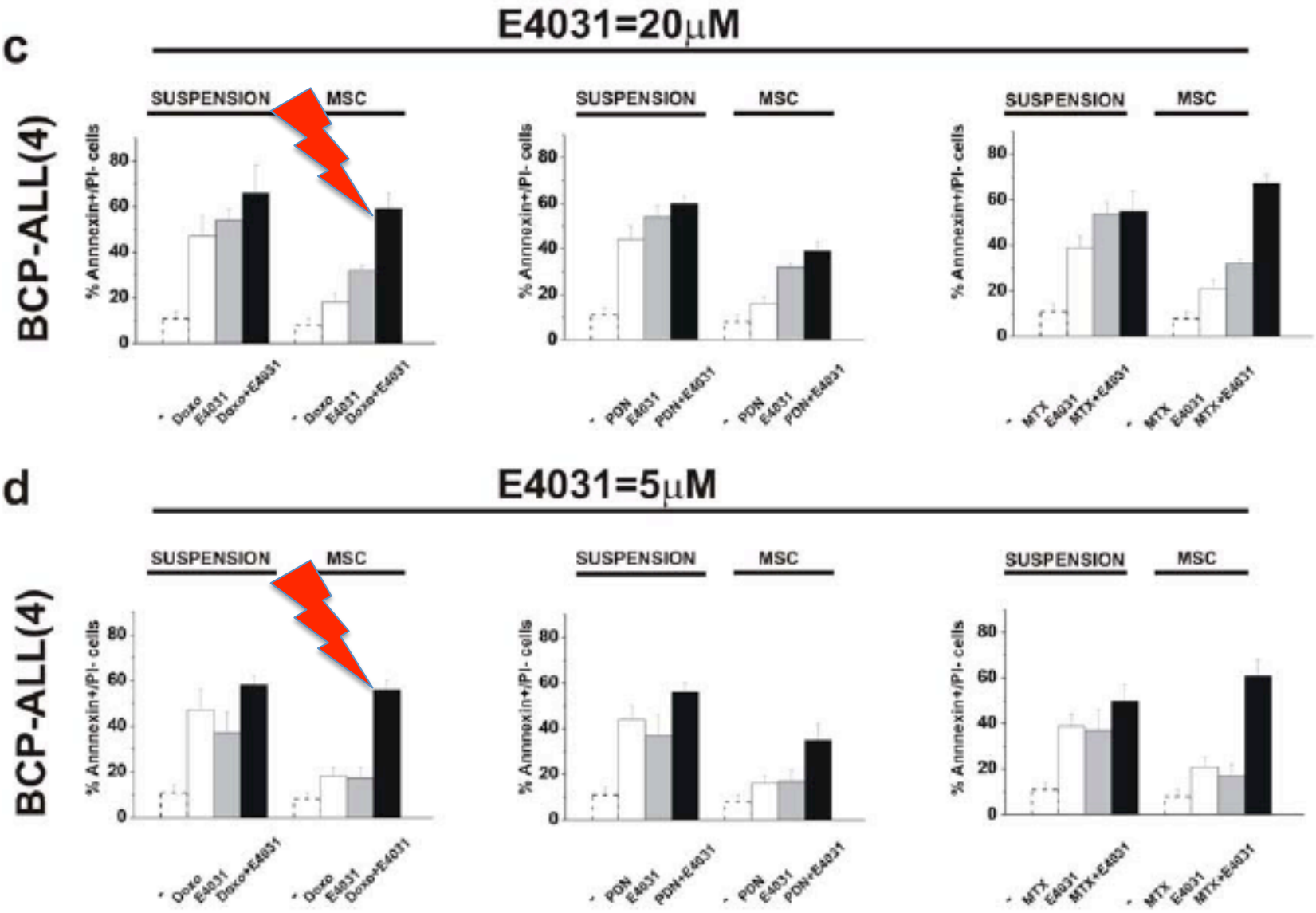


Table 1. LD_{50} values for doxorubicin, prednisone, and methotrexate in 697 cells cultured with or without MSCs (suspension) in combination or not with the LD_{50} dose of the hERG1 inhibitor E4031 in B-ALL*

	Suspension	MSC
Doxorubicin	0.13 ± 0.04 μg/mL	0.42 ± 0.06 μg/mL
Doxorubicin + E4031	0.08 ± 0.04 μg/mL	0.05 ± 0.01 μg/mL
Prednisone	4.96 ± 1.01 μM	23.82 ± 8.63 μM
Prednisone + E4031	3.77 ± 0.93 μM	3.48 ± 0.97 μM
Methotrexate	2.16 ± 1.33 μM	15.04 ± 2.56 μM
Methotrexate + E4031	1.20 ± 0.09 μM	2.12 ± 0.65 μM
E4031	22.01 ± 2.34 μM	75.32 ± 5.68 μM
Roscovitine	29.13 ± 3.67 μM	34.57 ± 3.51 μM


a
E4031=20μM
SUSPENSION
MSC
% Annnexin+/PI- cells
0
20
40
60
80
- Doxo
E4031
Doxo+E4031
- PDN
PDN+E4031
- MTX
MTX+E4031
b
E4031=5μM

Primary B-ALL



E4031 synergizes with chemotherapeutic drugs ……on MSC

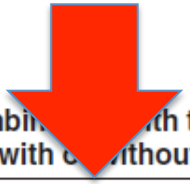


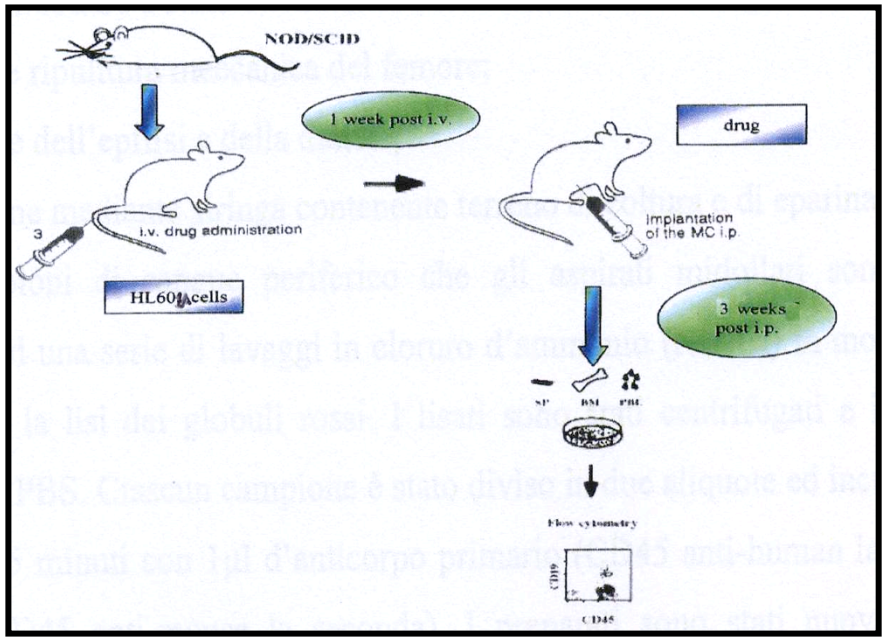
Table 2. CI values for doxorubicin, prednisone, and methotrexate (at the LD_{50} dose) in combi… …th the hERG1 inhibitor E4031 (at both 20μM and 5μM) in 697 and primary BCP-ALL cells, BCP-ALL(4) and BCP-ALL(3), cultured with … …ithout MSCs (suspension)*

	Suspension	*P* (suspension vs MSC)	MSC	*P* (E4031 20μM vs E4031 5μM)
697 cell line				
Doxorubicin + E4031 20μM	> 1	0.002	0.368 ± 0.037	0.039
Doxorubicin + E4031 5μM	0.605 ± 0.082	0.037	0.294 ± 0.020	
Prednisone + E4031 20μM	> 1	0.041	0.722 ± 0.112	0.002
Prednisone + E4031 5μM	0.887 ± 0.103	0.0001	0.298 ± 0.075	
Methotrexate + E4031 20μM	> 1	0.003	0.317 ± 0.081	0.021
Methotrexate + E4031 5μM	0.563 ± 0.096	0.023	0.224 ± 0.076	
BCP-ALL (4)				
Doxorubicin + E4031 20μM	> 1	0.008	0.679 ± 0.087	0.003
Doxorubicin + E4031 5μM	> 1	0.001	0.345 ± 0.033	
Prednisone + E4031 20μM	> 1		> 1	0.042
Prednisone + E4031 5μM	> 1	0.043	0.854 ± 0.096	
Methotrexate + E4031 20μM	> 1	0.002	0.446 ± 0.081	0.022
Methotrexate + E4031 5μM	> 1	0.000	0.203 ± 0.073	
BCP-ALL (3)				
Doxorubicin + E4031 20μM	0.865 ± 0.105	0.036	0.487 ± 0.086	0.031
Doxorubicin + E4031 5μM	0.824 ± 0.043	0.025	0.398 ± 0.075	
Prednisone + E4031 20μM	> 1		> 1	0.004
Prednisone + E4031 5μM	> 1	0.037	0.876 ± 0.056	
Methotrexate + E4031 20μM	> 1	0.005	0.865 ± 0.056	
Methotrexate + E4031 5μM	> 1	0.033	0.849 ± 0.021	

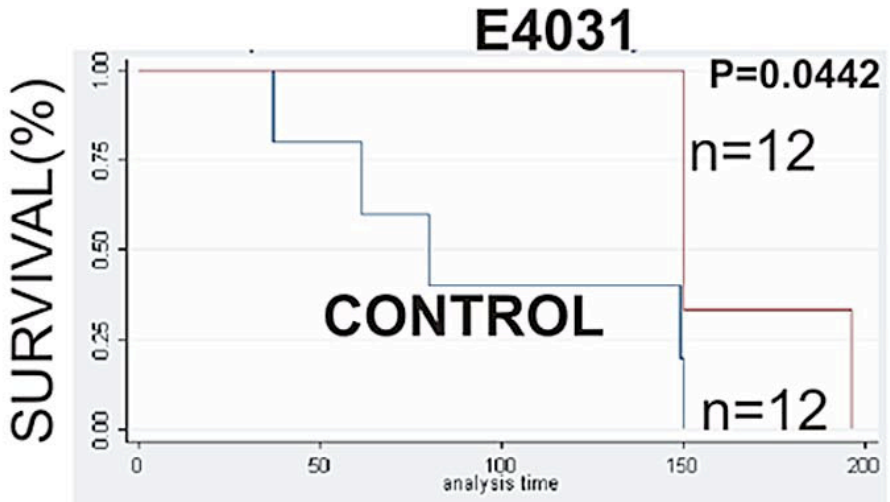
*Original data are from Figure 4 and supplemental Figure 7c. CI values were calculated using CalcuSyn software Version 2 (Biosoft). span lang=IT

CI > 1, antagonisms; CI = 1, additivity; CI < 1, synergy. The statistical analysis was performed using the Student *t* test comparing either CI values in suspension versus CI values on MSC, for each drug combination, or CI values in the presence of 20mM E4031 versus CI values in the presence of 5μM E4031. Only statistically significant *P* values are reported.

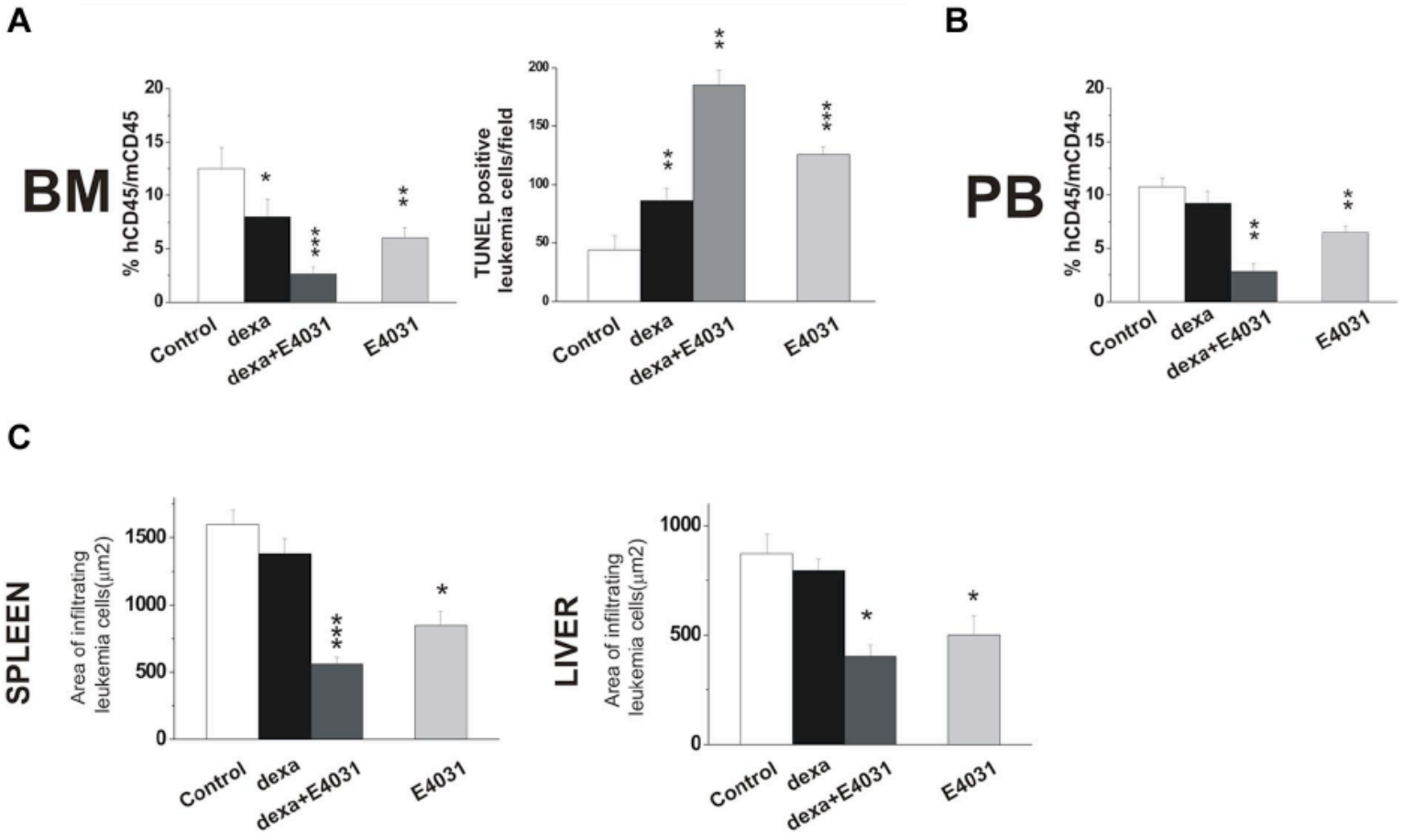
Effects of the hERG1 blocker E4031 *in vivo*



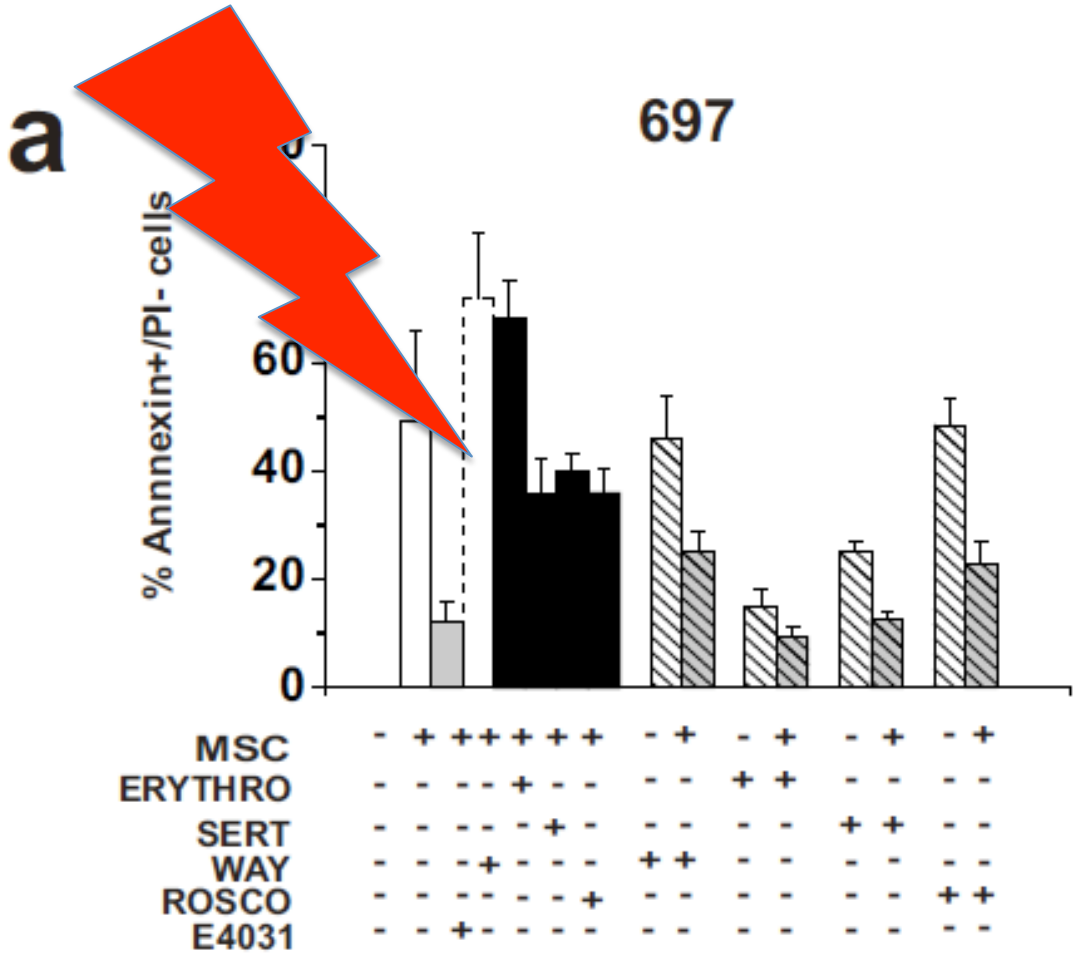
E4031 has a therapeutic effect *in vivo* on B-ALL (697 cells): increase in survival



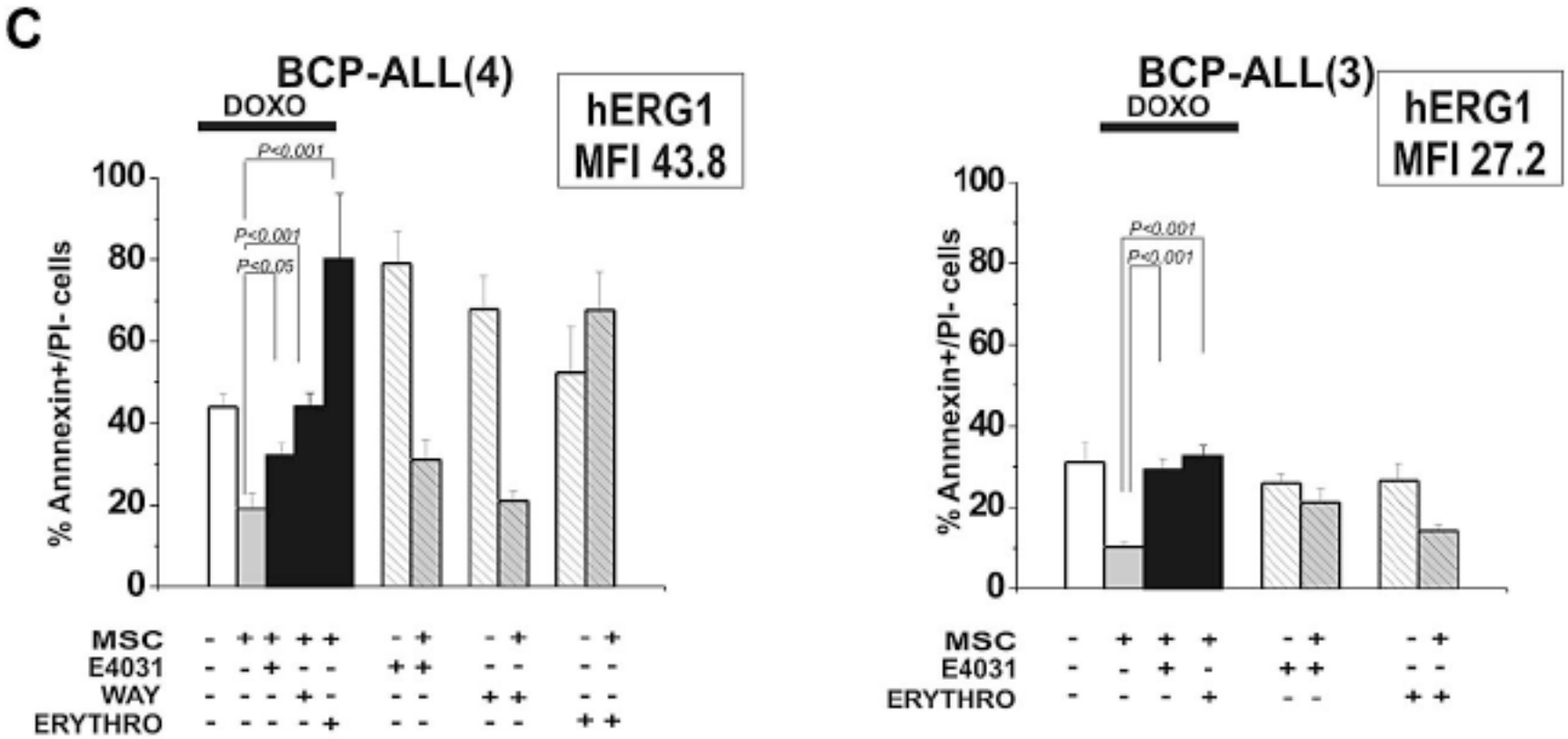
E4031 has a therapeutic effect *in vivo* on B-ALL (corticosteroid-resistant REH cells)



Non torsadogenic hERG1 blockers (Erythromycin and Sertindole) and R-Roscovitine have the same effects of E4031



Effects of Erythromycin on primary B-ALL



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- Development of compounds (antibodies, peptides) which disassemble the ion channel/ integrin complex.

BlackSwan Pharma

W. Tiedke

Leipzig, Germany

CD-160130: A Novel Small Molecule Apoptosis Promoter For The Treatment Of CLL

Mugridge, K., Letschert, S., Schulze, A., Daghish, M., Schwind, S. and Tiedke, W.
BlackSwan Pharma GmbH, Deutscher Platz 5e, 04103, Leipzig, Germany

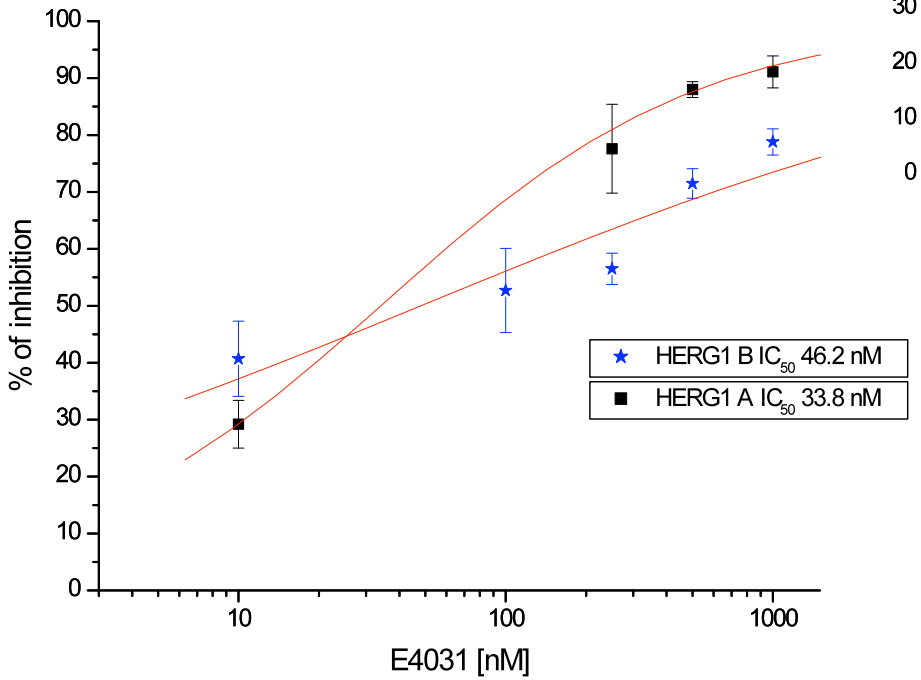
Effects of CD-160130 on proliferation of leukemia cell lines and hERG1A/hERG1B-expressing cells :LD50 values (comparison with E 4031)

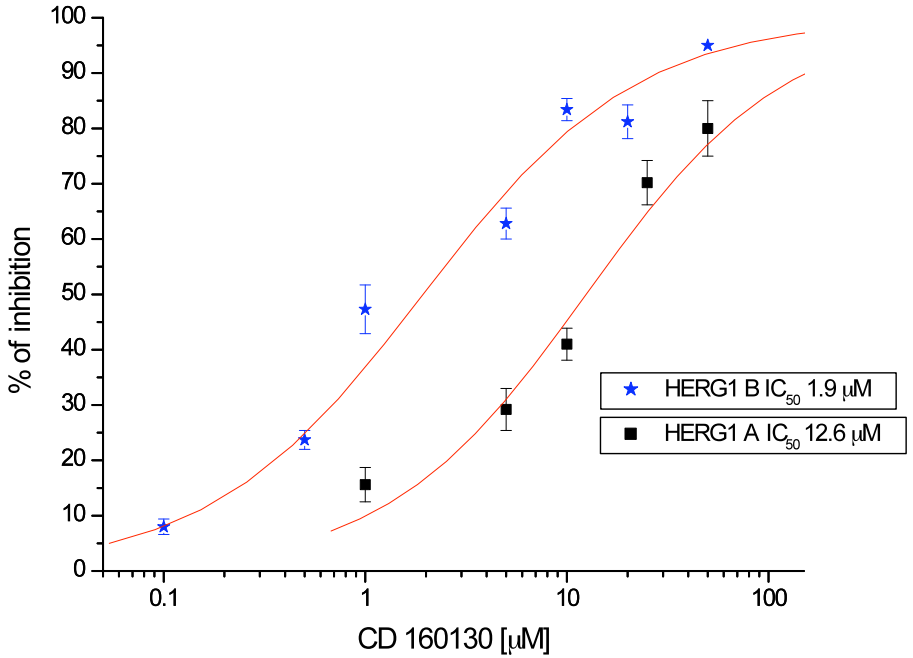
	CD 160130	*E 4031*
MEC 1	5.36±0.94 µM	58.11±16.27 µM
REH	4.12±0.80 µM	
697	9.11±1.50 µM	22.01±2.34 µM
FLG 29.1	3.87±0.30 µM	
HEK293-HERG1A	9.74±1.39 µM	27.03±4.46 µM
HEK293-HERG1B	5.71±0.72 µM	33.25±4.18 µM

LD50 values for CD16030 in different cell lines. LDC50 were evaluated by non-linear regression analysis using the Origin 6 (Microcal Software) softwares .

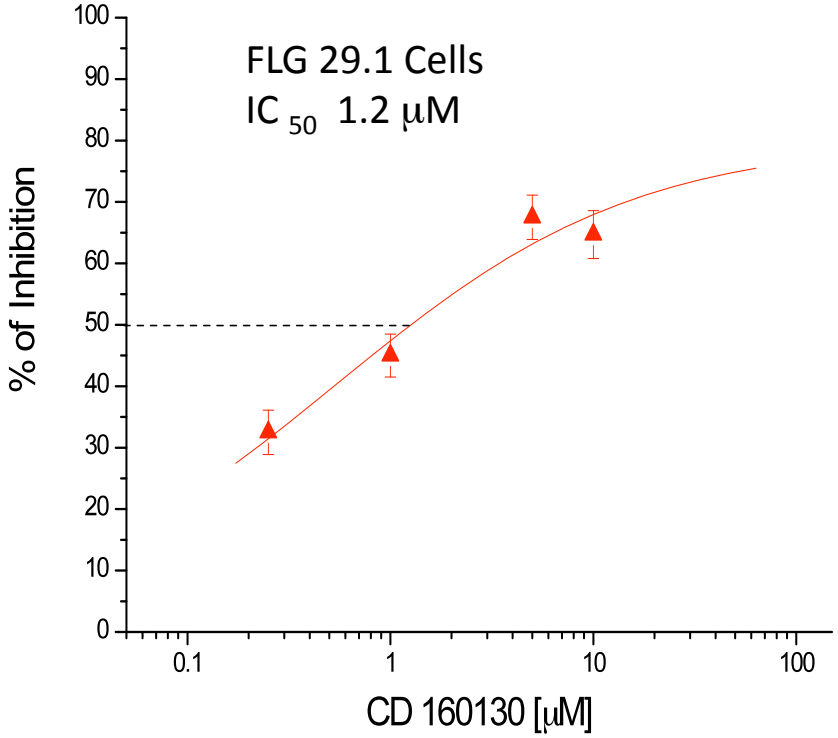
Massimo D'Amico
Luca Gasparoli

CD-160130 preferentially inhibits the hERG1B isoform (comparison with E 4031)

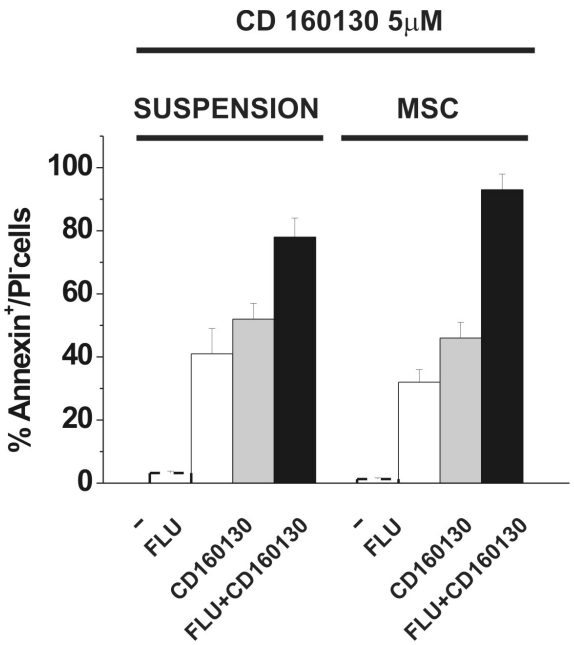


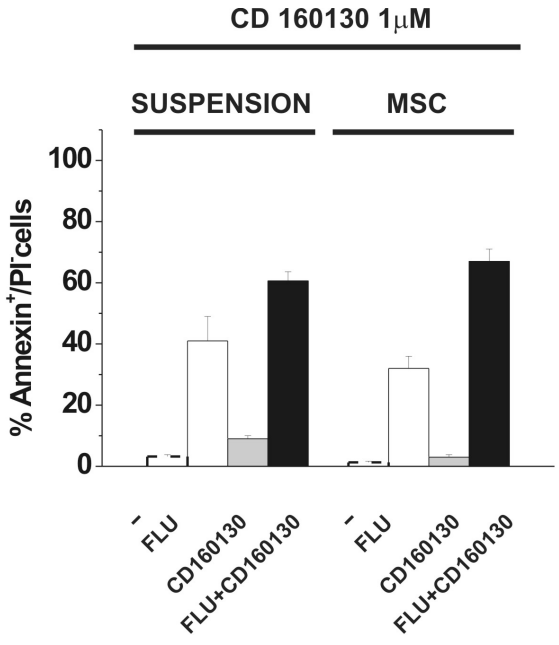


CD-160130 preferentially blocks hERG1B isoform … also in leukemia cells



CD-160130 is synergic with Fludarabine on apoptosis of MEC1 cells (CLL) on MSC





	SUSPENSION	*P value (SUSPENSION vs MSC)*	MSC	*P value (CD160130 5 μM vs CD160130 1 μM)*
MEC1 cell line				
FLUDARABINE+CD160130 5μM	>1	*0.037*	0.568±0.123	*0.035*
FLUDARABINE+CD160130 1μM	0.786±0.052	*0.002*	0.276±0.020	

- Less harmful compounds can be developed which block hERG1 channels in cancer cells.

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ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO

AIRC Con la ricerca, contro il cancro

ITT Istituto Toscano Tumori

Supported By:

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University of Milano Bicocca

Prof.Enzo Wanke