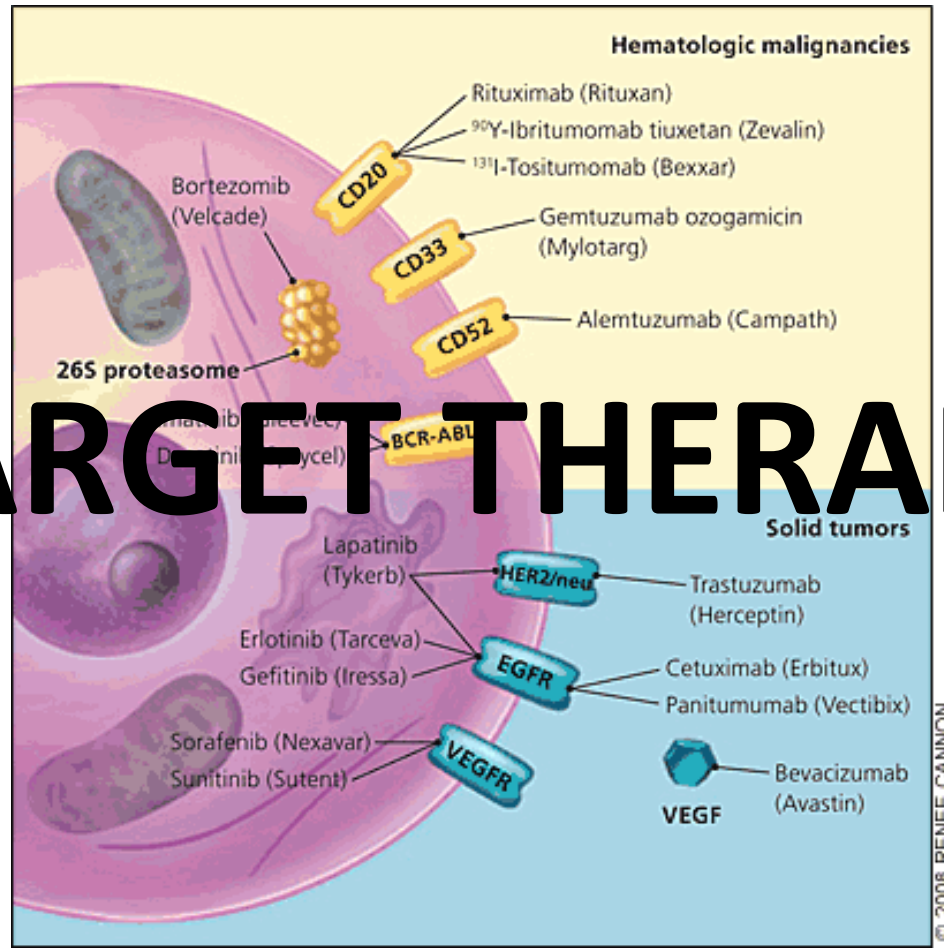
# The bases of clinical oncology: from bench to bedside

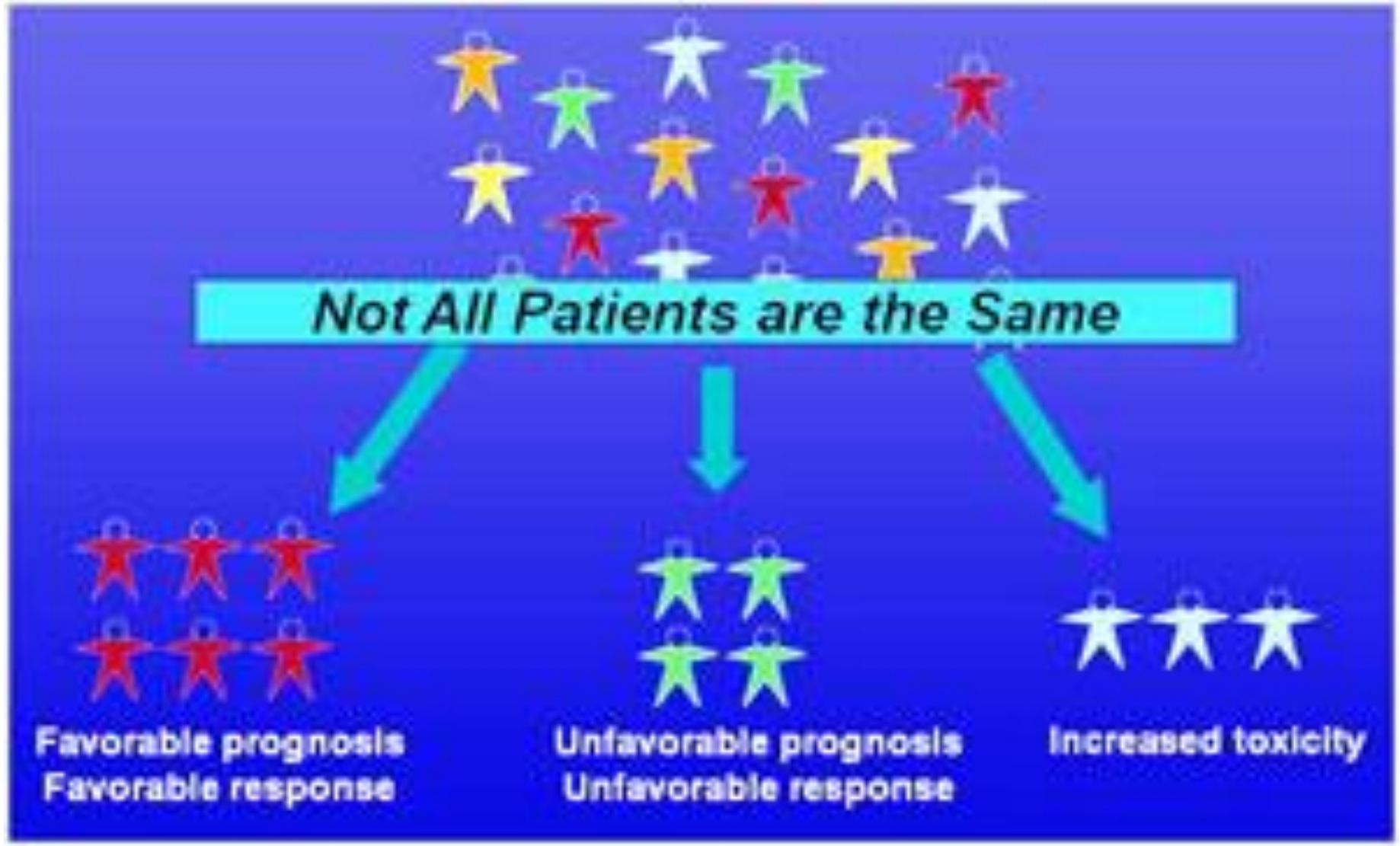
**Prof. Annarosa Arcangeli**
**Department of Experimental and Clinical Medicine**

**Erasmus course Basic & Translational Oncology**
**In collaboration with Université de Paris**
**Firenze, 20-24 January 2020**

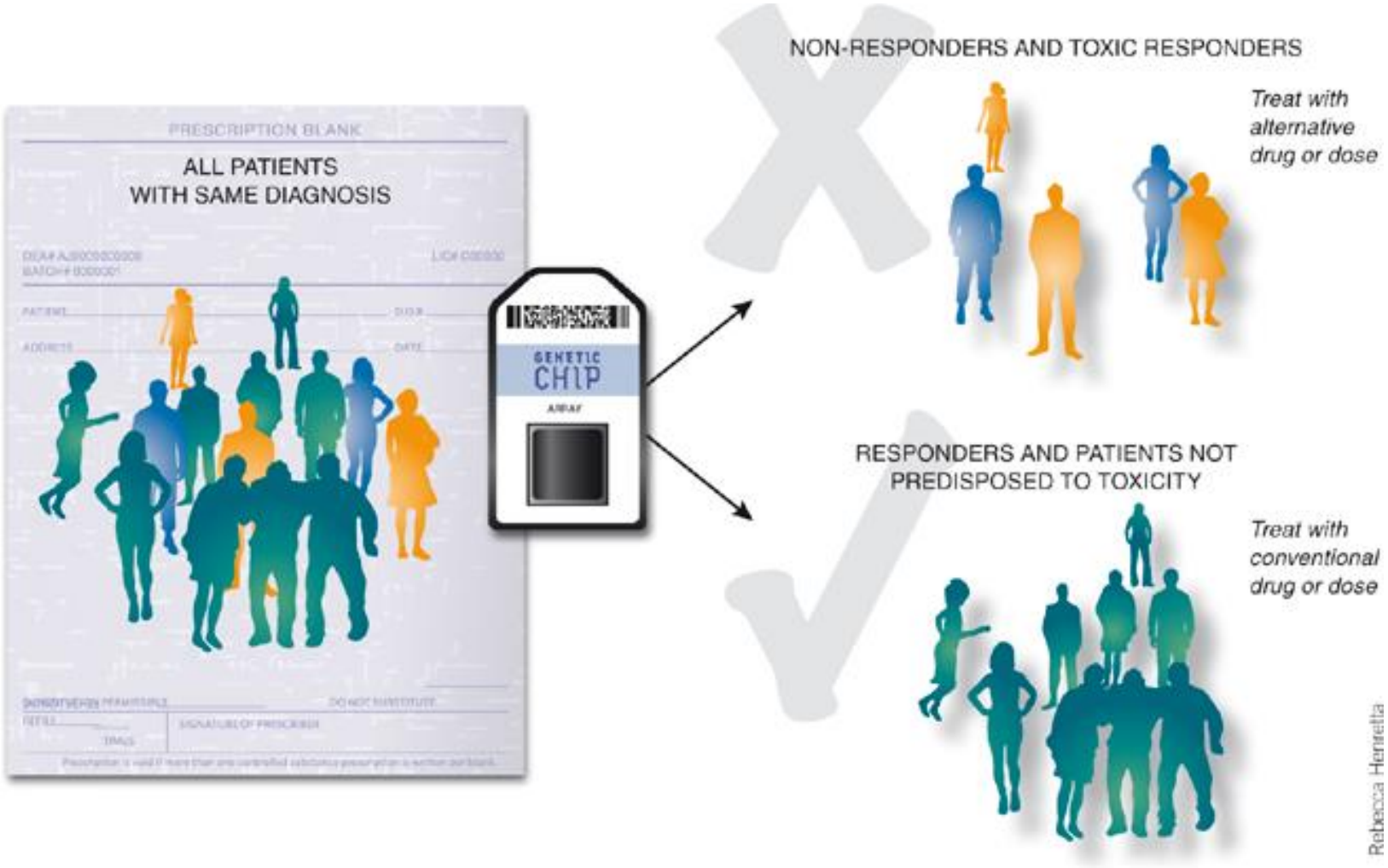
# How can we translate basic science to the medical practice in oncology?




# TARGET THERAPY


Not All Patients are the Same
Favorable prognosis
Favorable response
Unfavorable prognosis
Unfavorable response
Increased toxicity

# Personalized medicine
# Biomarkers
# Predictors of benefits



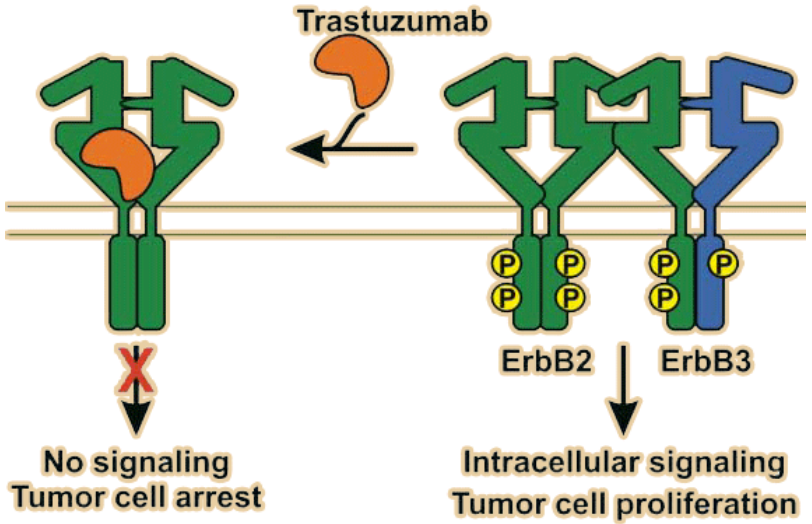
# Some examples

- **HER2**
- **K-Ras**
- **BCR-ABL (9:22 translocation)**

# HER2/neu

- The *HER2/neu* gene encodes one of a family of human epidermal growth-factor receptors.
- This gene is frequently amplified in breast cancer cells, resulting in increased amounts of HER2 cell surface protein.
- HER2-expressing tumors are sensitive to Transtuzumab (herceptin), a monoclonal antibody therapy.
- HER2 protein is detected by immunohistochemistry (IHC).
- *HER2/neu* gene amplification is detected by fluorescence in situ hybridization (FISH).
- **Patients carrying a HER2/neu positive cancer (e.g.Breast Cancer) can be treated with Transtuzumab (Herceptin)**

# Herceptin




*Badache and Hynes, Cancer Cell 2004*

Trastuzumab is a humanized monoclonal antibody anti-ErbB-2

Efficacy on primary tumors with ErbB-2 amplification:
- Inhibits angiogenesis
- induces cytotoxicity
- increase response to chemioterapy
- Inhibits the activation of ErbB-2

Metastatic tumors develop resistance to Herceptin within 12 months

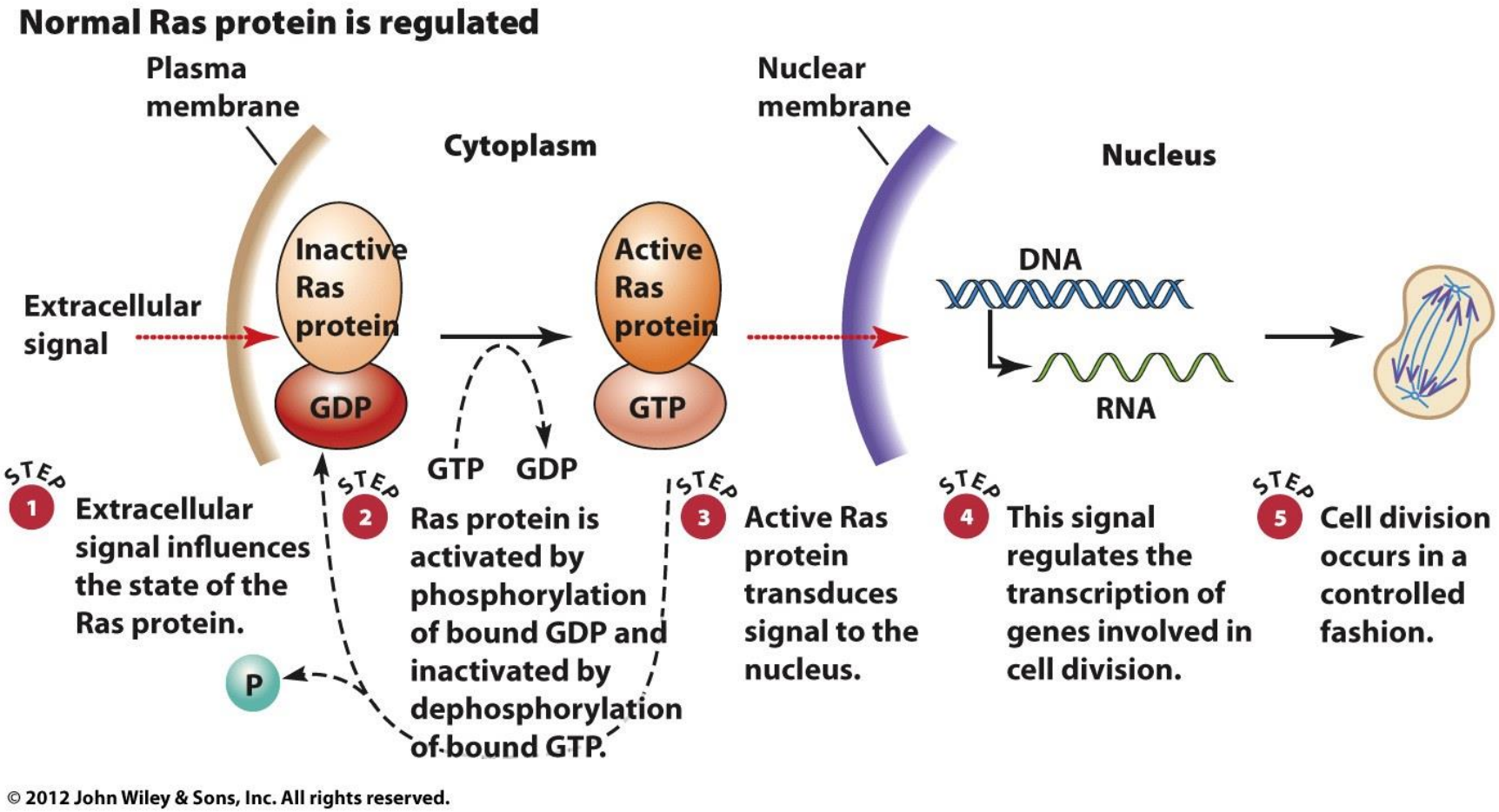
↓

Increase of PI3K activation

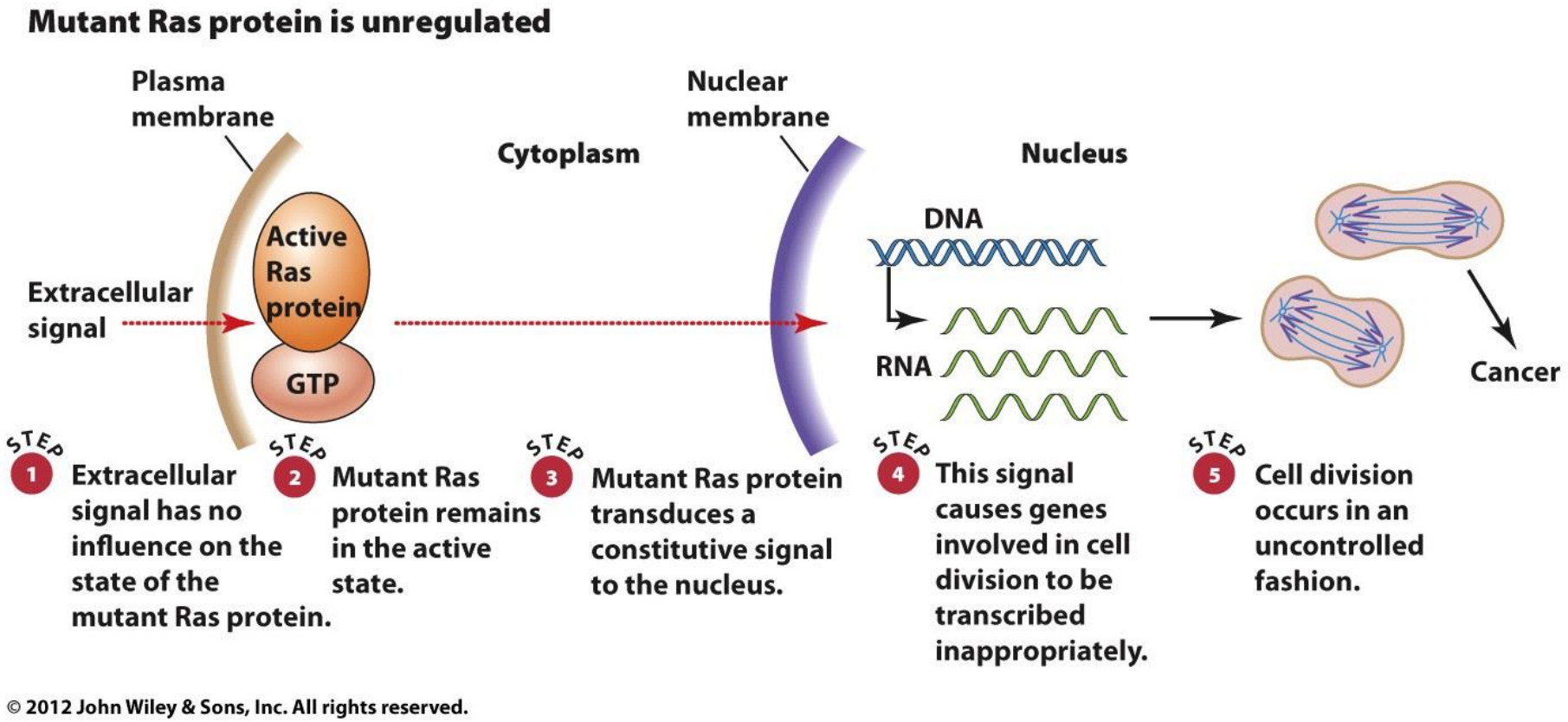
# *K-ras*

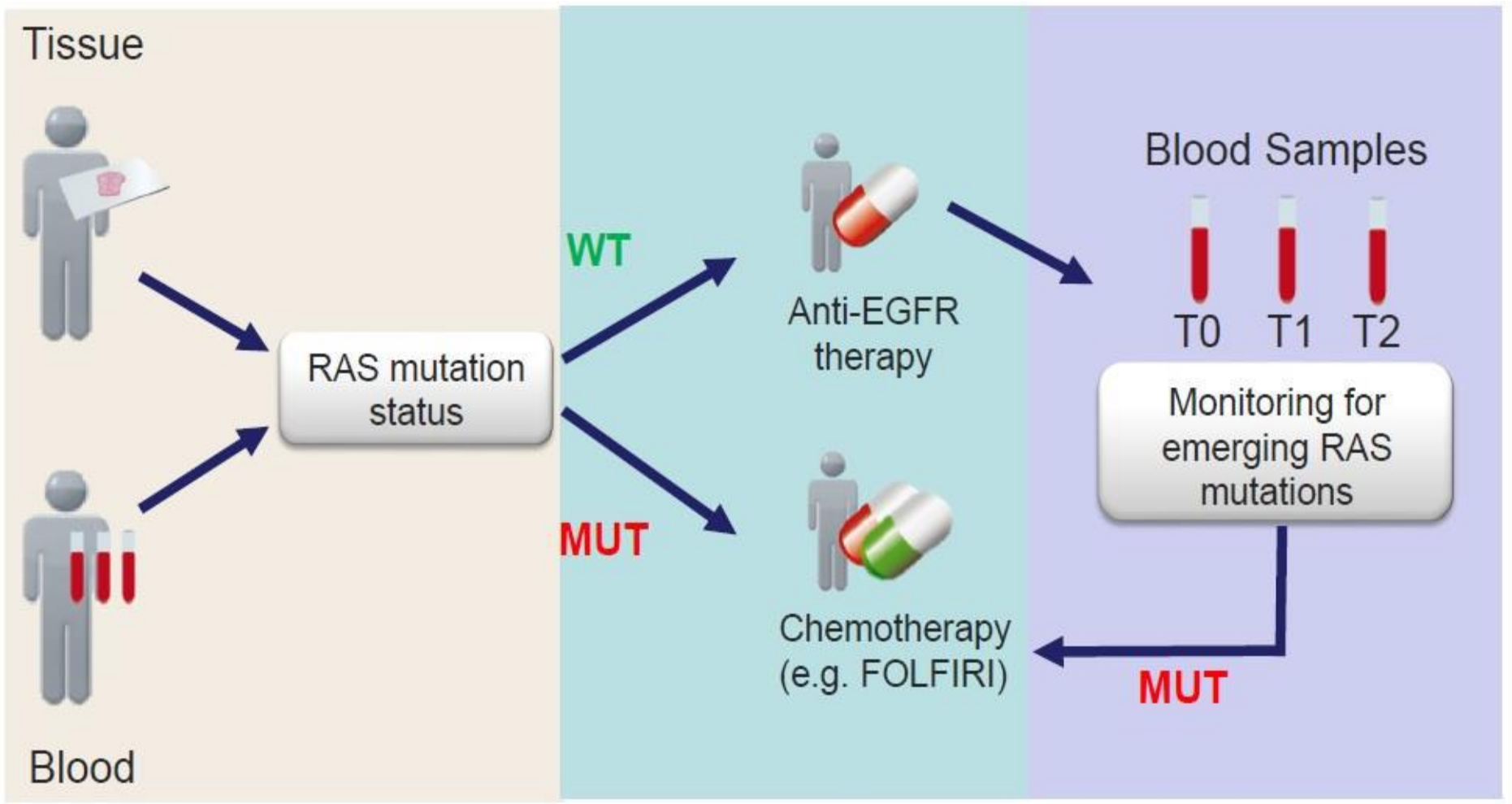
- The Kirsten rat sarcoma viral oncogene (*K-ras*) encodes a key component of cell signaling.
- Mutations in *K-ras* are the most common oncogene mutations in cancer.
- *K-ras* mutations are associated with tumor malignancy and may affect response to some therapies.
- *K-ras* gene mutations are detected by SSCP or direct sequencing.

# Normal Ras Protein Signaling



# Mutant Ras Protein (**V12 or G12V**) is Unregulated



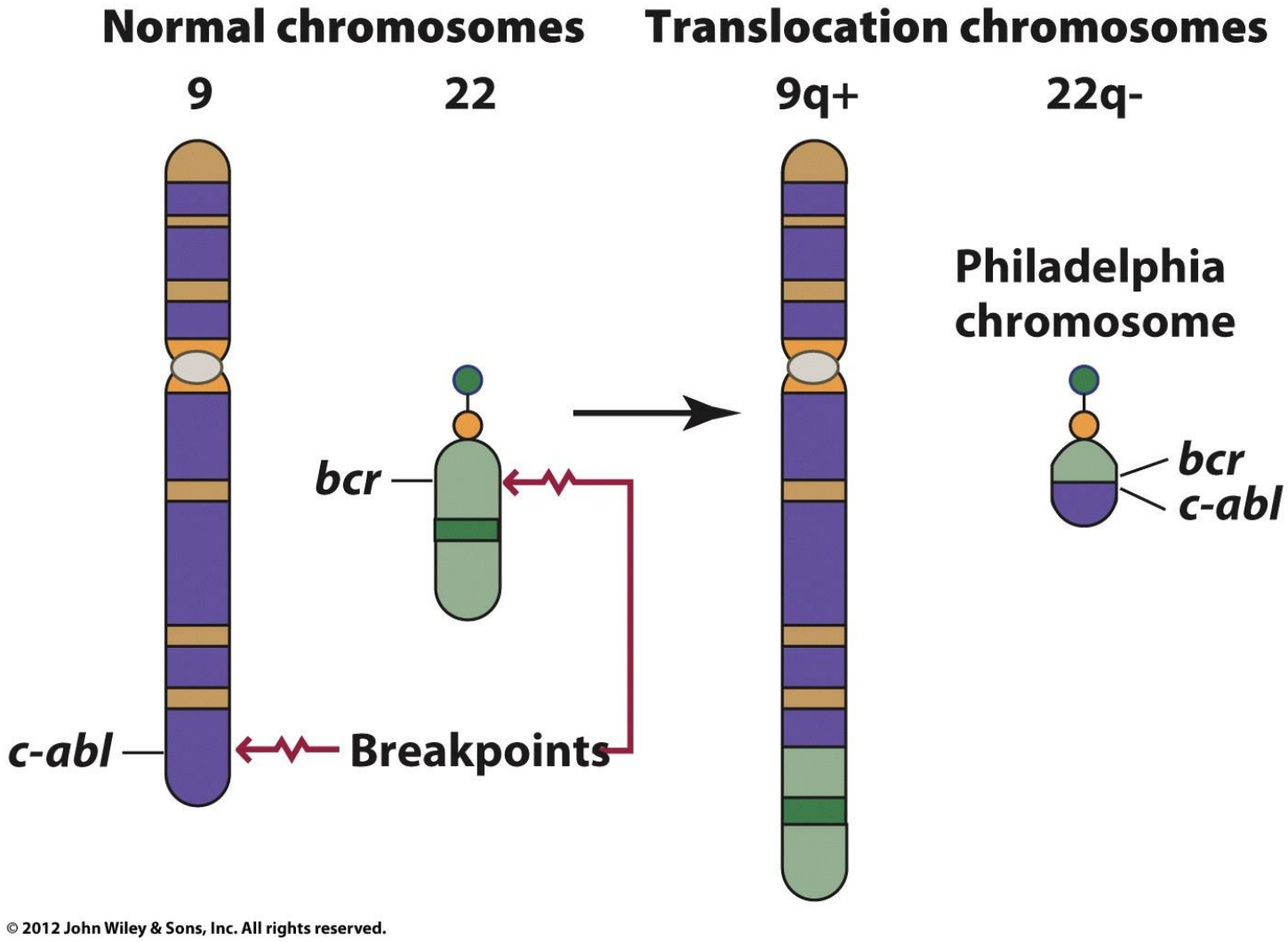



*Example: Blood-based RAS testing for colorectal cancer*

# Philadelphia Chromosome/t(9; 22)/ Bcr/abl

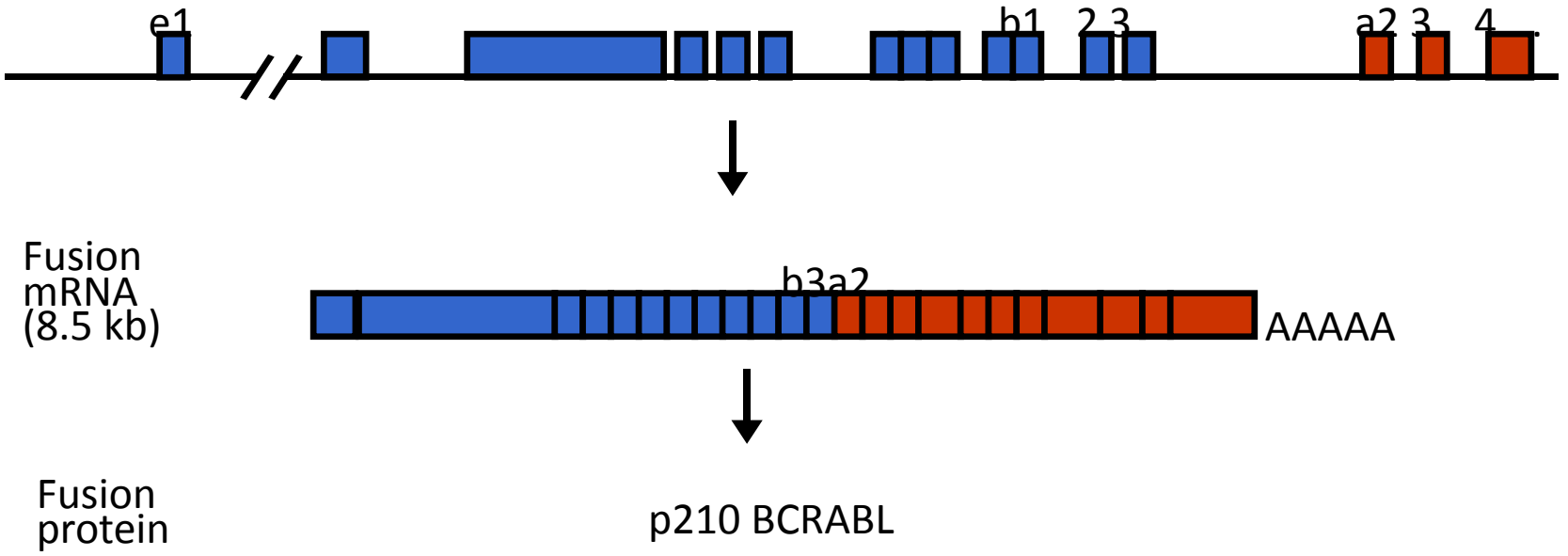
- t(9;22) is a reciprocal translocation between the long arms of chromosomes 9; 22 is found in chronic myelogenous leukemia and acute lymphoblastic leukemia.
- This translocation forms a chimeric gene between the breakpoint cluster region (BCR) gene on chromosome 22 and the Abelson leukemia virus (ABL) gene on chromosome 9.
- The translocated chromosome is the Philadelphia chromosome.

# The Philadelphia Chromosome

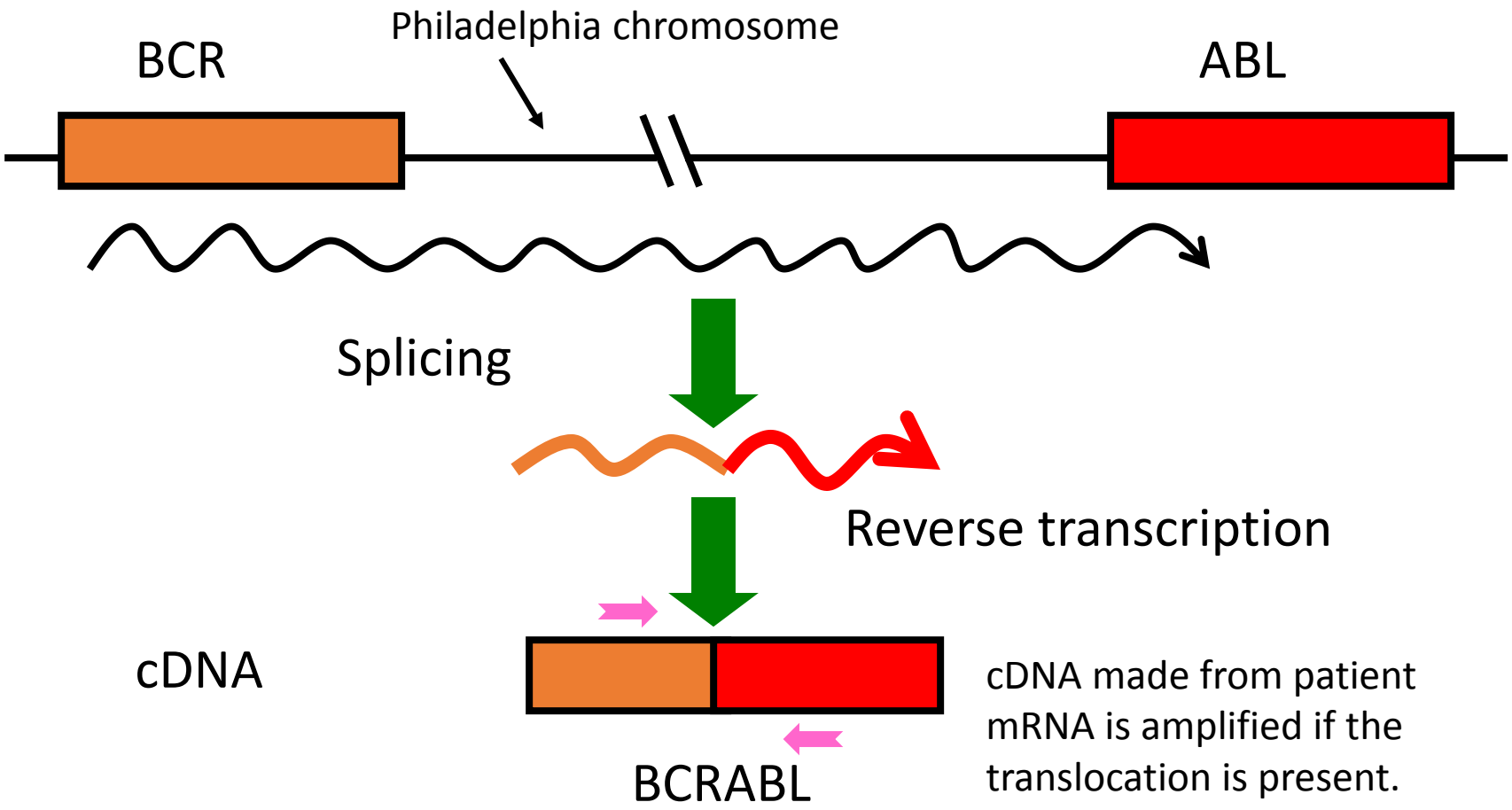


# Translocations Used in Diagnosis and Monitoring of Hematological Tumors: t(9; 22)

The chimeric gene, BCRABL, produces an abnormal protein that drives the tumor cell phenotype.



# Detection of  t(9; 22) by RT-PCR



# Detection of  t(9; 22) by RT-PCR

1 = molecular weight standard
2-5 = positive for translocation
6 = negative
7-11 = amplification controls
12 = blank

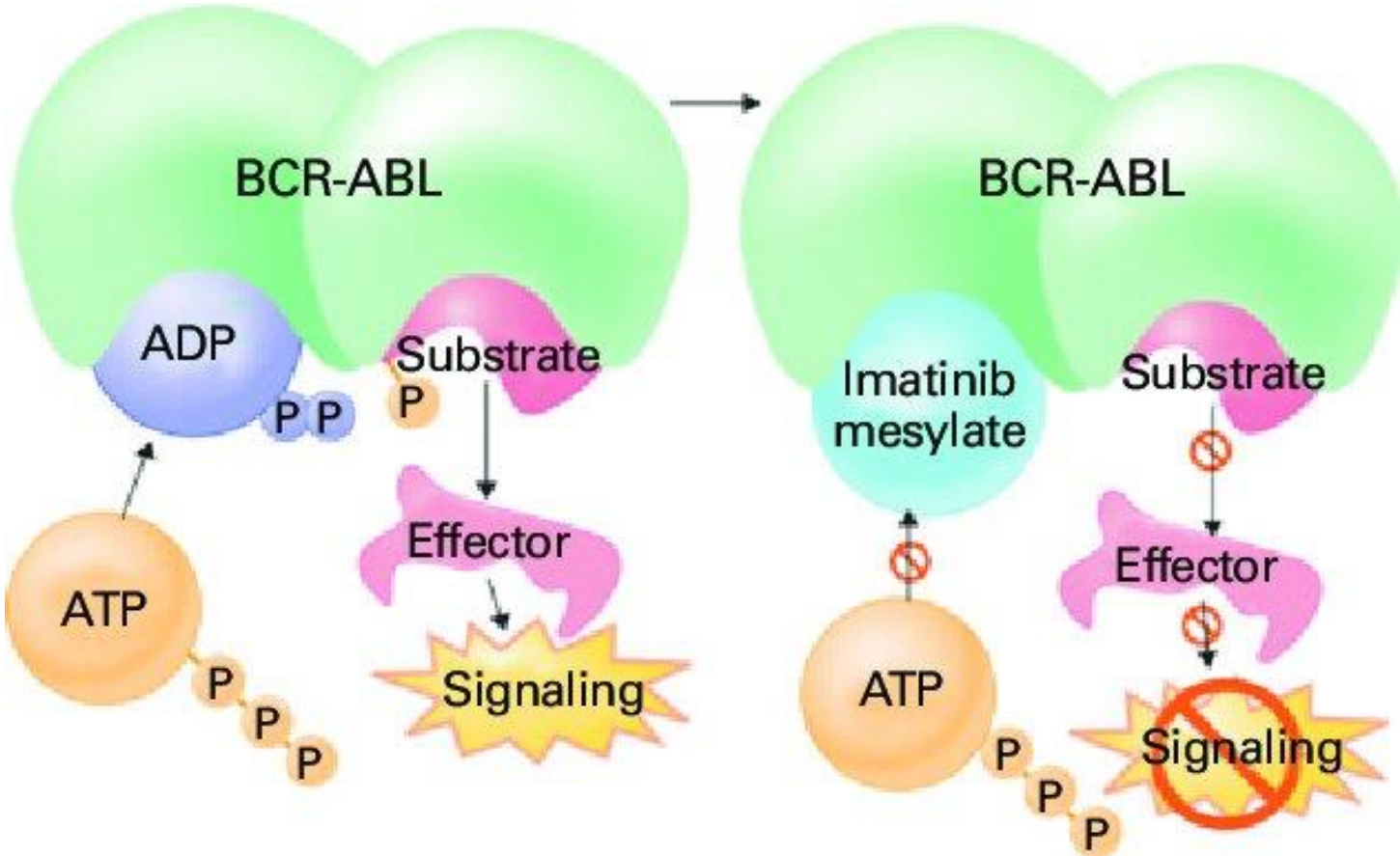
Translocation products (BCRABL)

The band size is determined by different chromosome 22 breakpoints.


Agarose gel

Translocation products (ABL)

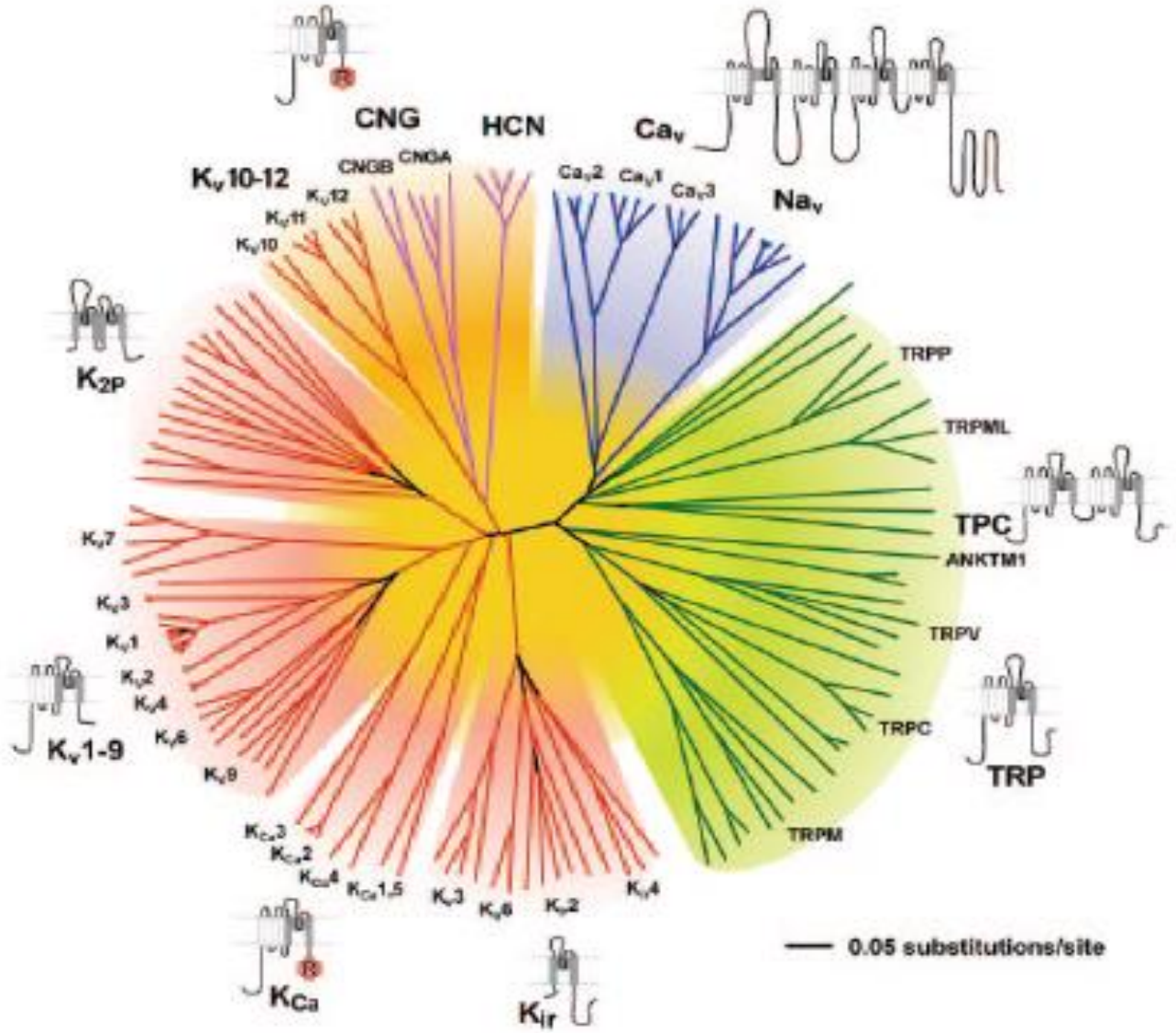
# IMATINIB

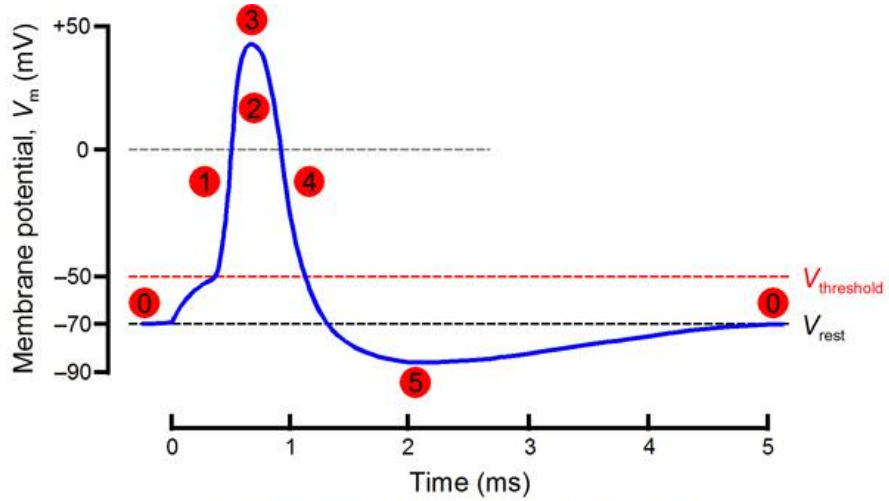

BCR-ABL
ADP
P P
Substrate
P
Effector
ATP
P P P
Signaling
BCR-ABL
Imatinib
mesylate
Substrate
Effector
ATP
P P P
Signaling

# How can we translate basic science in ion channels to the medical practice in oncology?

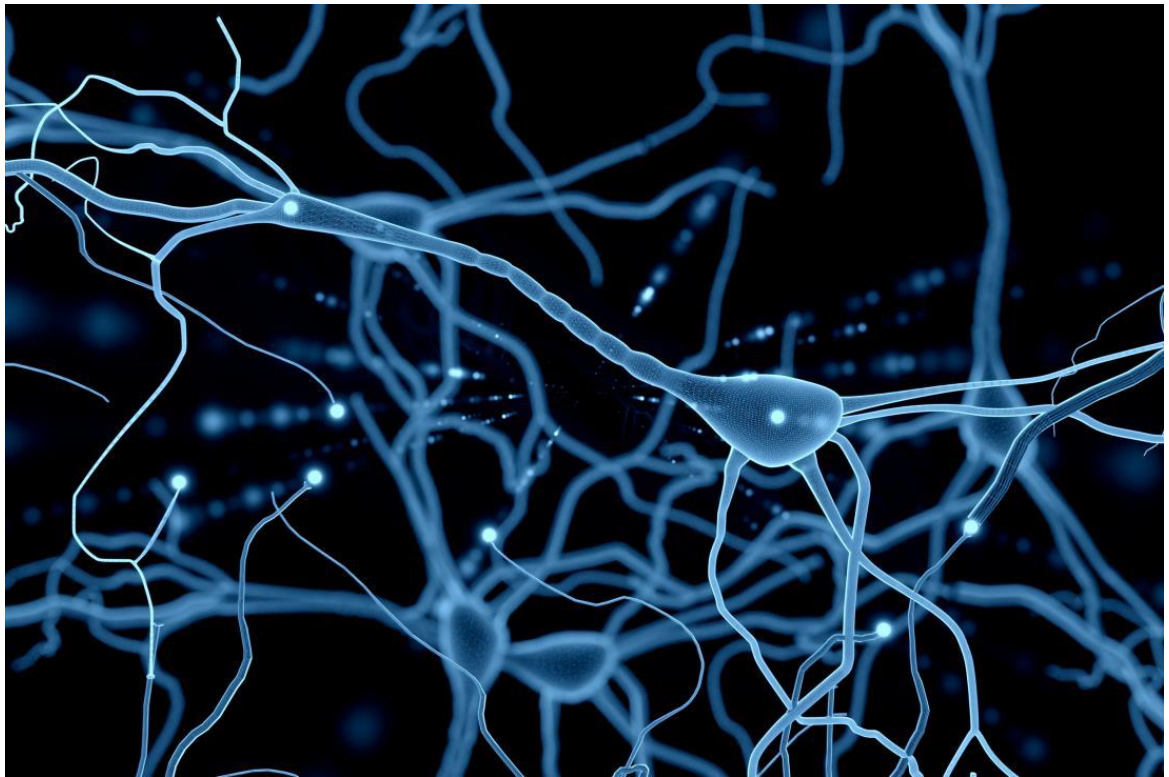
# ION CHANNELS IN CANCER

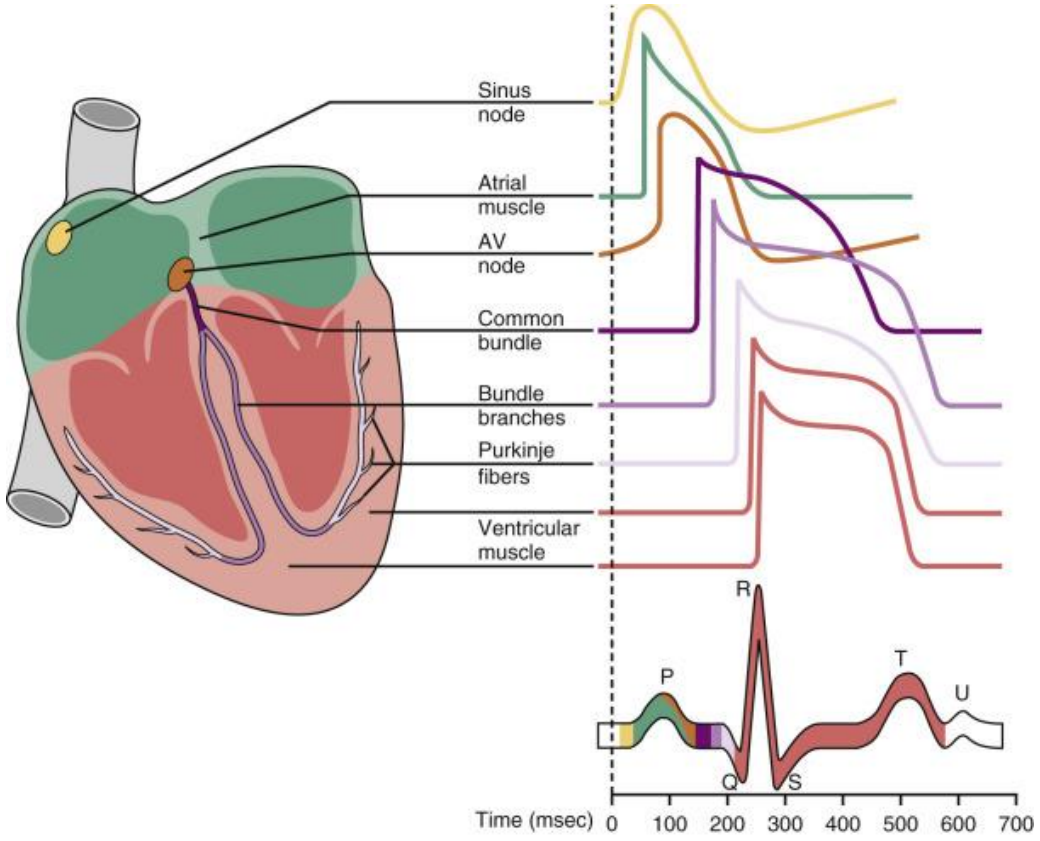
# ION CHANNELS



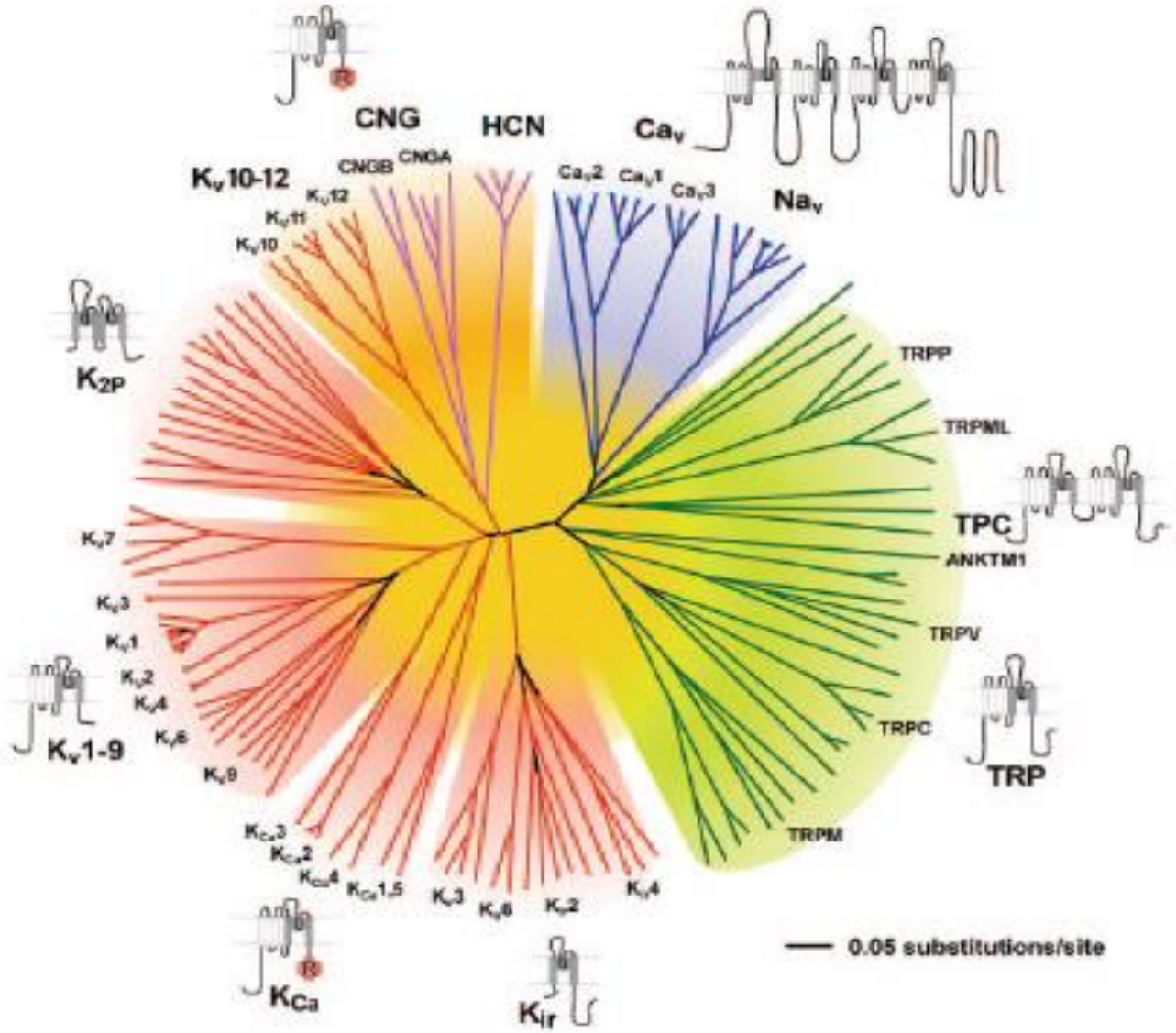




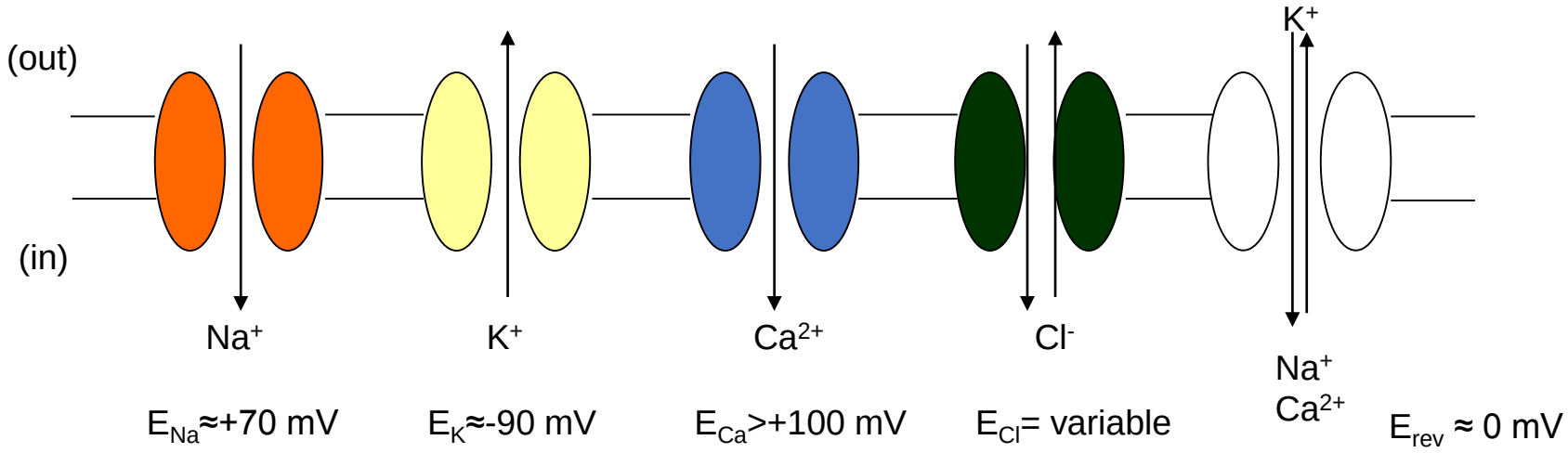





# ION CHANNELS



# Ion channels and membrane potential ($V_m$)




An ion channel highly selective for a given ion type i tends to bring $V_m$ close to the equilibrium (Nernst) potential ($E_i$) for that ion, at which the net flux of i is null.

$E_K$ is around -90 mV, in physiological $[K]_o/[K]_i$. When $V_m = E_K$, the $K^+$ outflow driven by the concentration gradient is balanced by the influx driven by the negative $V_m$.

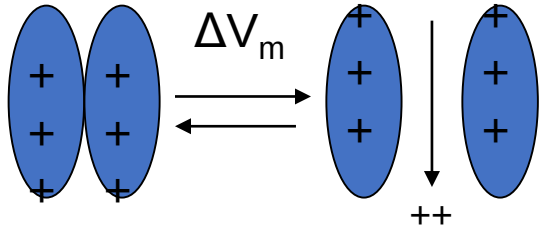
At $V_m$'s negative of $E_K$, $K^+$ flows into the cell.

At $V_m$'s  positive of $E_K$, $K^+$ flows out of the cell

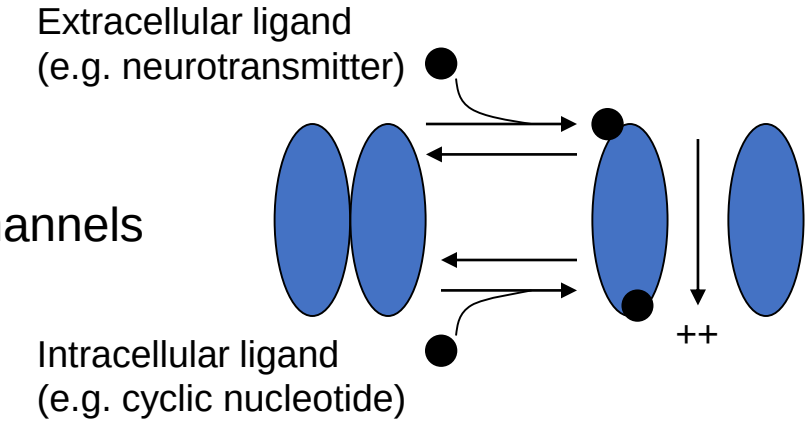
# Gating of ion channels

Closed ⇄ Open

Voltage-gated channels



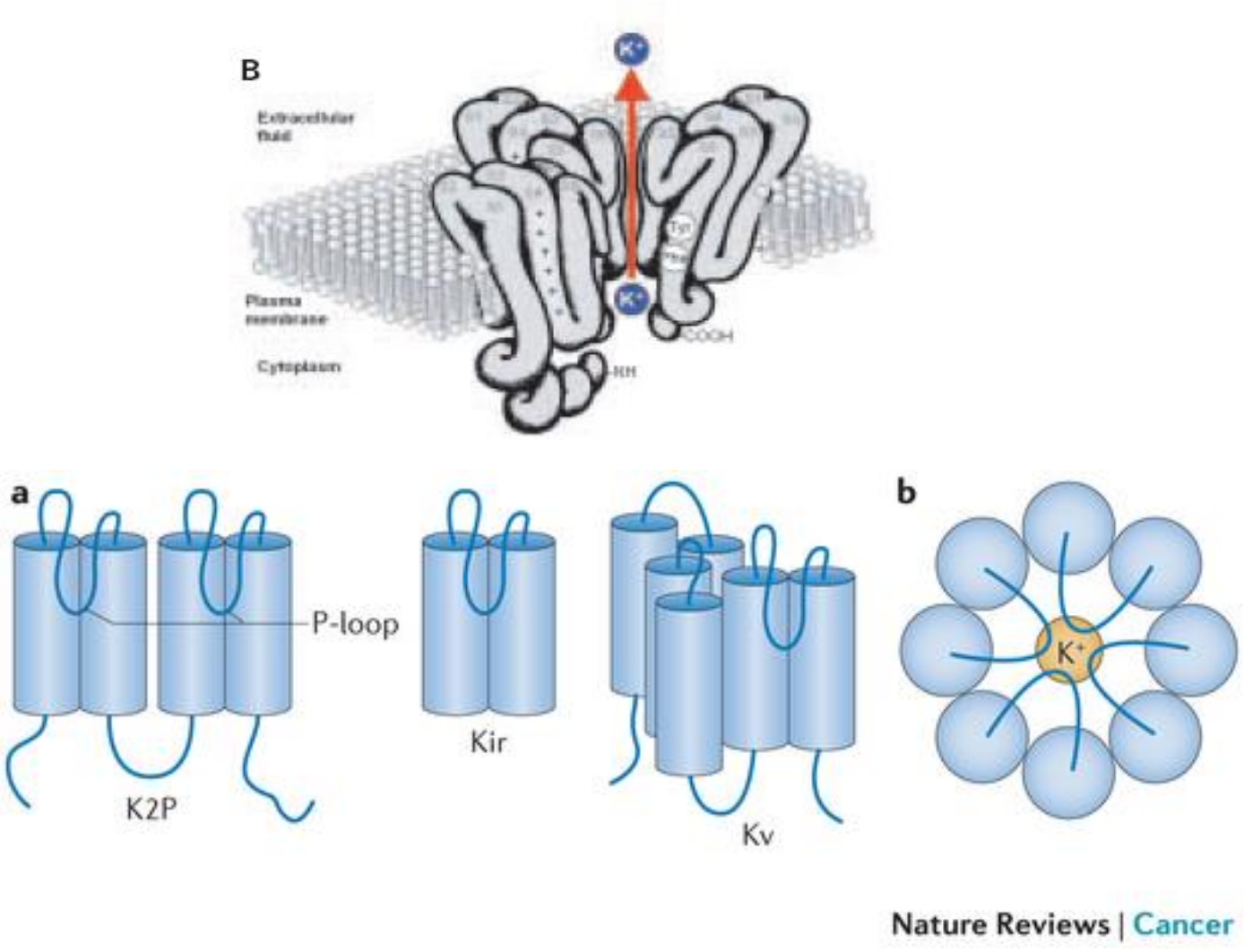

Ligand-gated channels



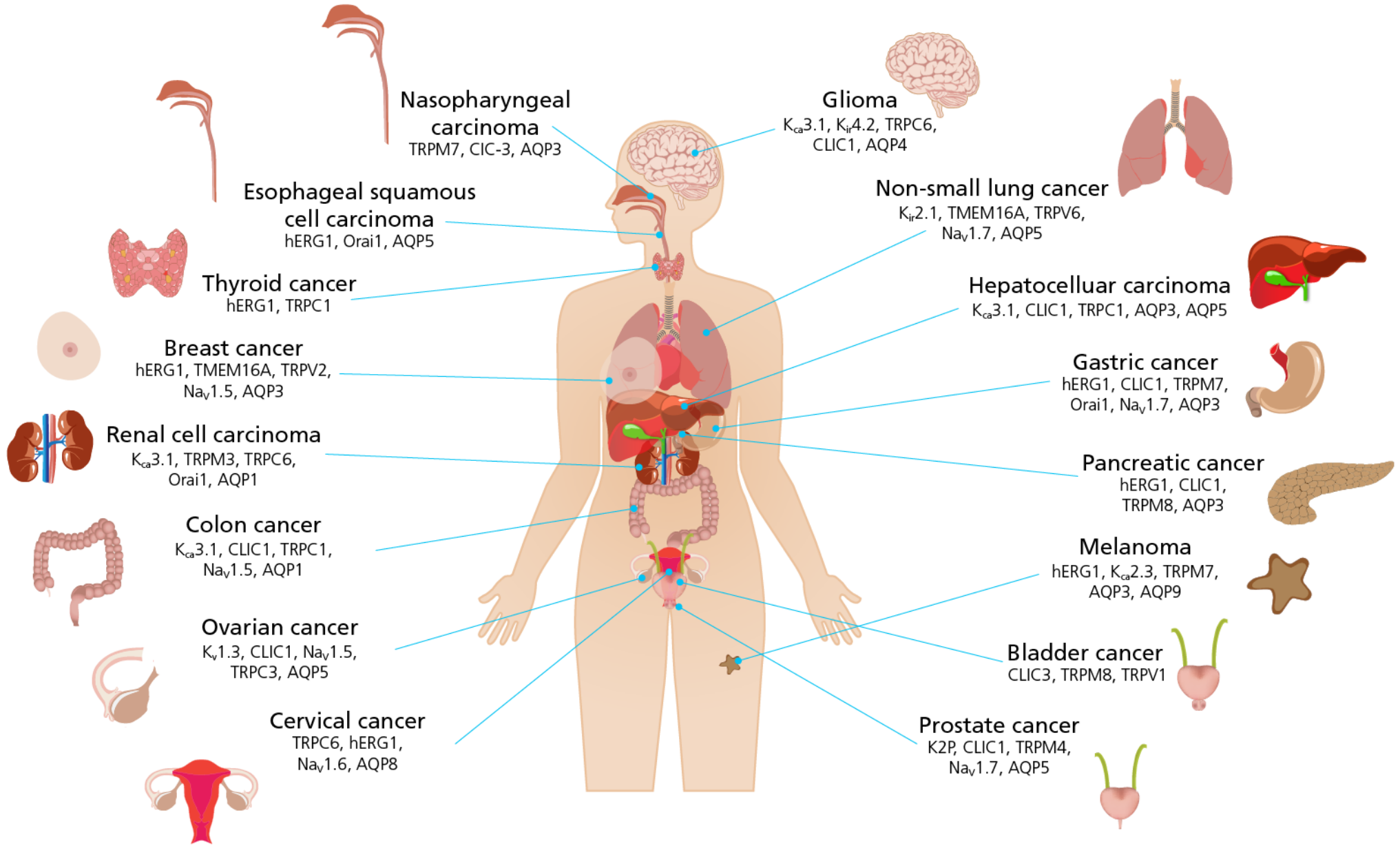

Other gating mechanisms:

- mechanically-gated channels (cytoskeleton)
- conformational coupling

# $K^+$ channels: structure



# ION CHANNELS IN CANCER



Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

# Ion channel expression as promising cancer biomarker☆

Elena Lastraioli, Jessica Iorio, Annarosa Arcangeli *

*Department of Experimental and Clinical Medicine, Section of Internal Medicine University of Florence, Florence, Italy*

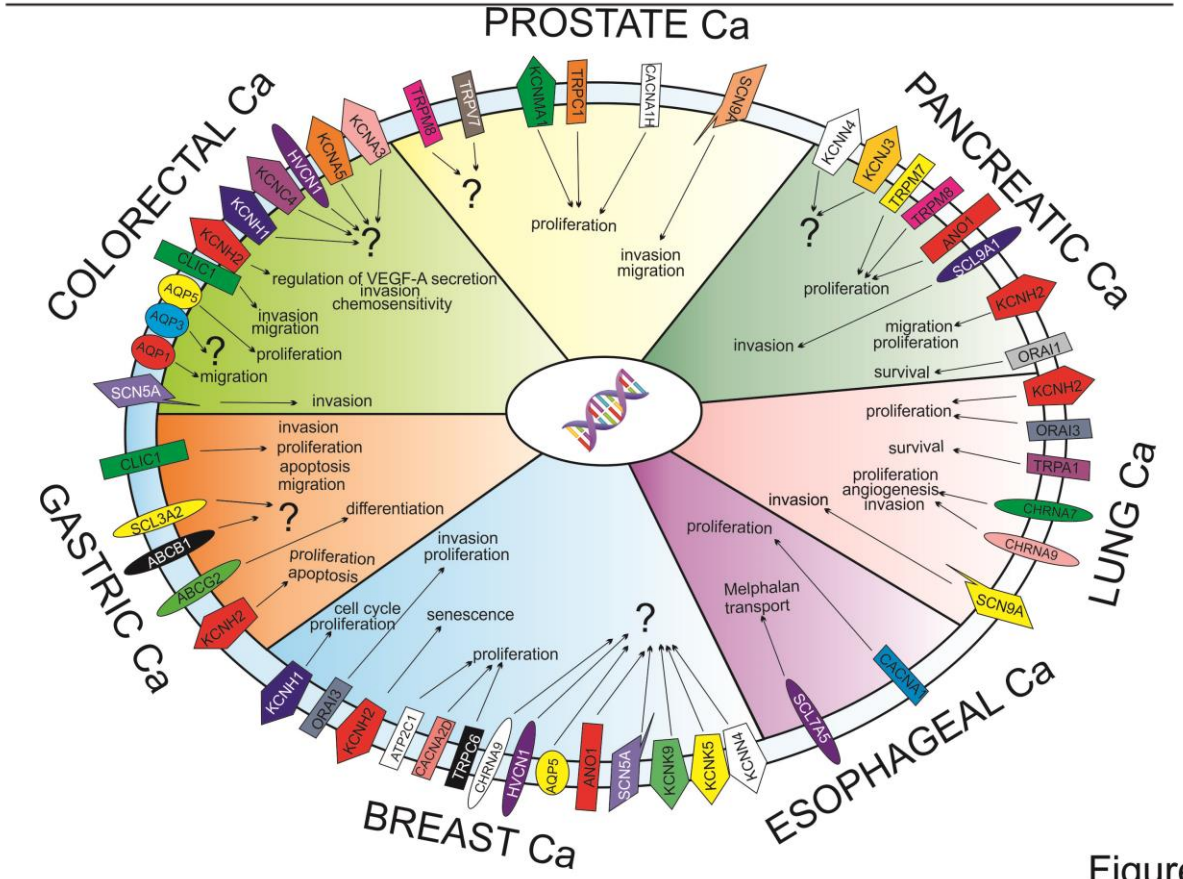
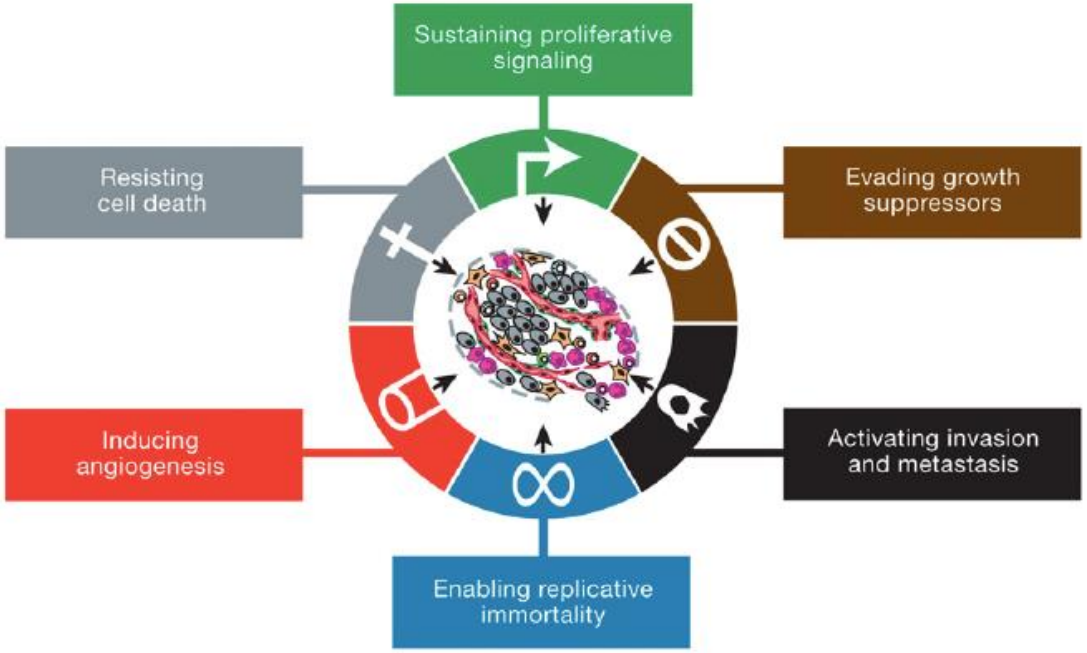



Figure 1

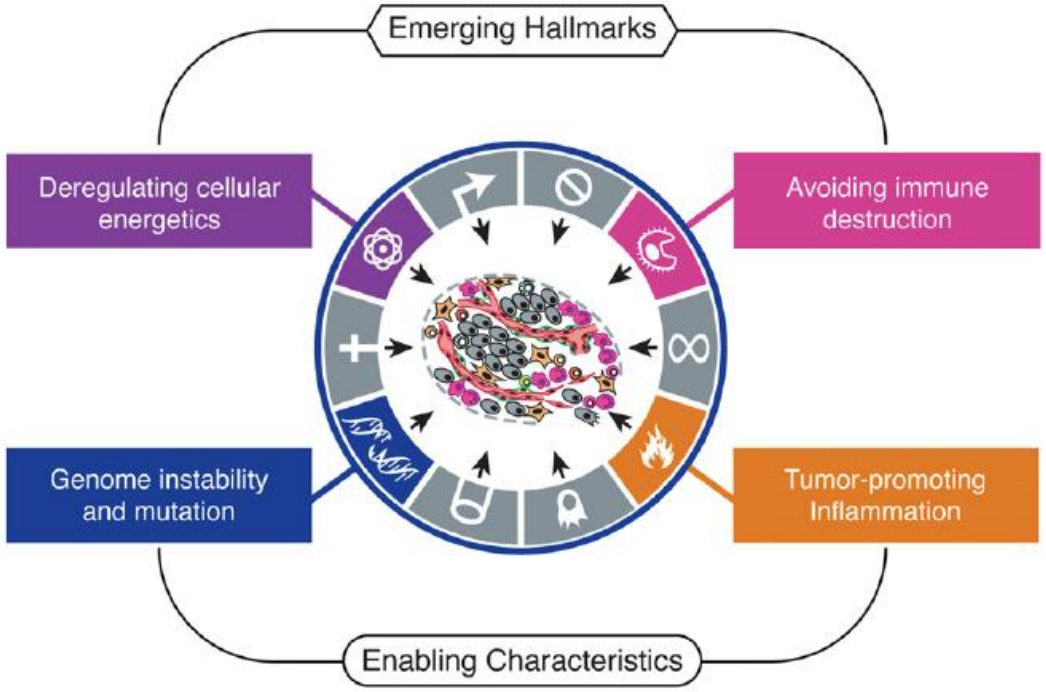
# Hallmarks of Cancer

Hanahan and Weimberg, Cell, 2000

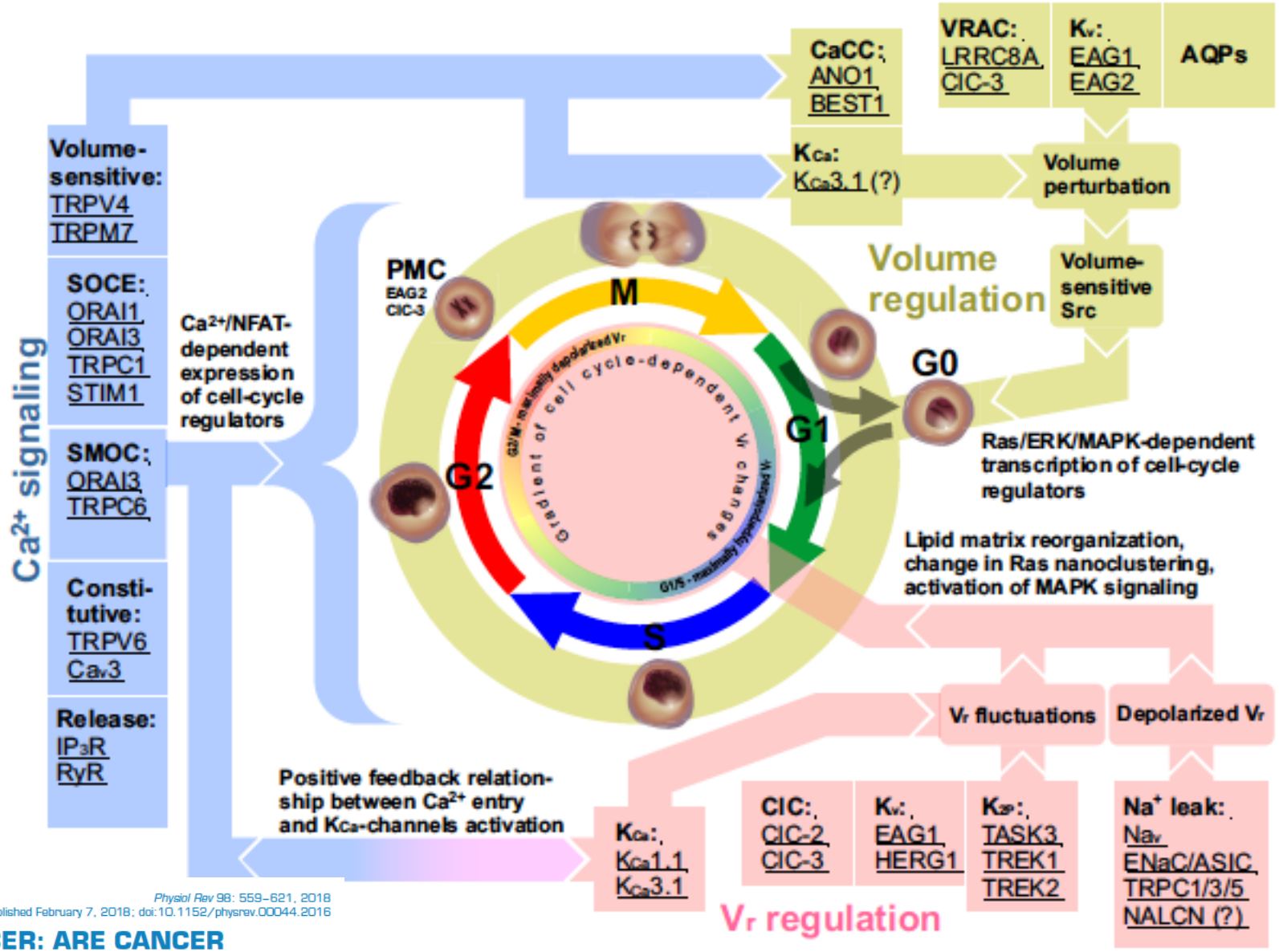



# Hallmarks of Cancer: The Next Generation

Hanahan and Weimberg, Cell, 2011



# Contribution of ion channels to proliferation and cell cycle progression of cancer cells




*Physiol Rev* 98: 559–621, 2018
Published February 7, 2018; doi:10.1152/physrev.00044.2016

**ION CHANNELS IN CANCER: ARE CANCER HALLMARKS ONCOCHANNELOPATHIES?**

Natalia Prevarskaya, Roman Skryma, and Yaroslav Shuba

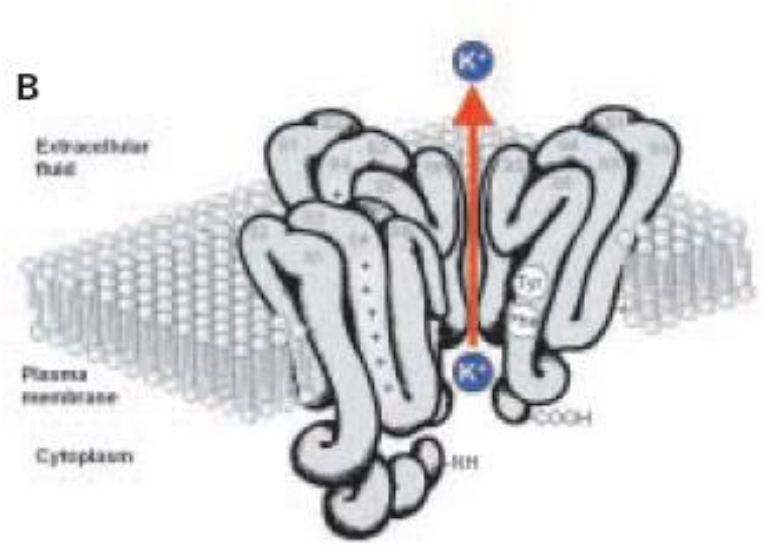
# Some channel-mediated mechanisms in cell proliferation

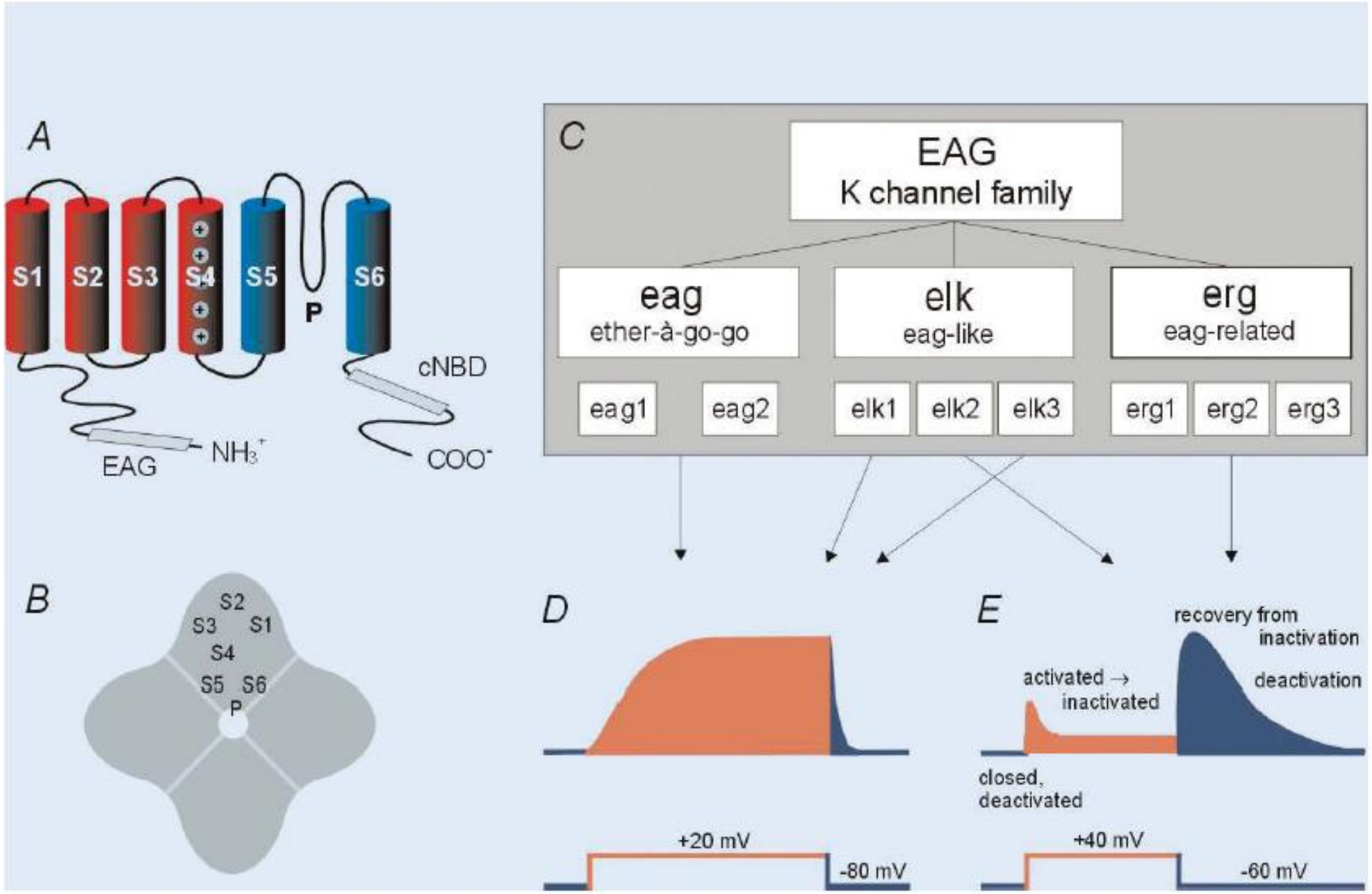
- Direct modulation of the cell cycle machinery (by e.g., $Ca^{2+}$, pH).
- Depolarization + $Ca^{2+}$ regulate exocytosis of hormones and paracrine factors (GFs, cytokines, etc.).
- Regulation of cell volume ($K^+$ plus $Cl^-$ channels, and KCC transporters for solute extrusion; mainly $Na^+$ plus $Cl^-$ channels, and NKCC transporters, for solute absorption).
- **Regulation of cell adhesion and GF release by non-conductive mechanisms (stimulation of intracellular cascades and conformational coupling with other membrane proteins)**

# Non conductive roles of ion channels

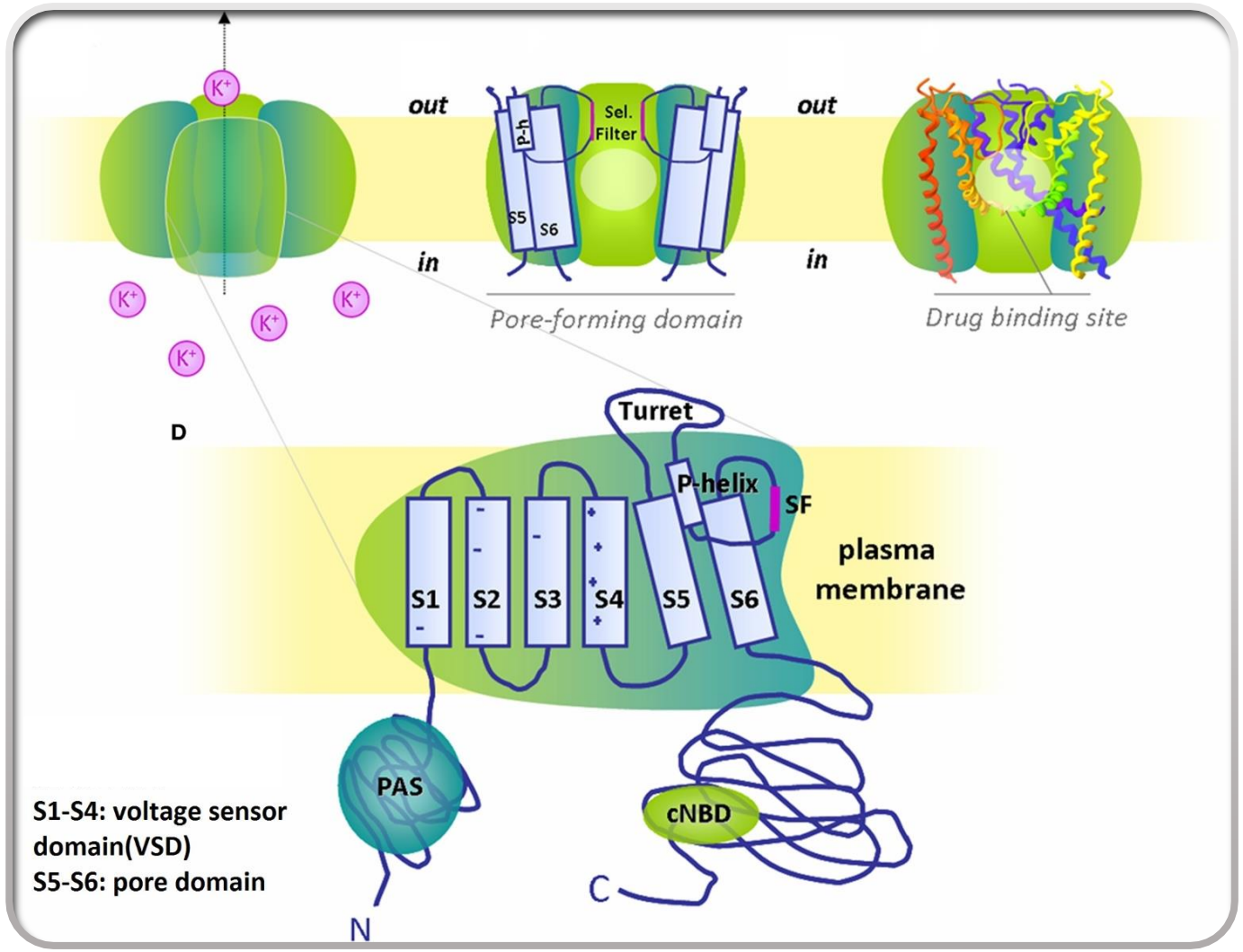
- Regulatory domains (e.g. kinase domains).
- **Formation and modulation of membrane multiprotein complexes (e.g. hERG or AMPA glutamate receptors with integrins).**
- Conformational coupling with cytosolic proteins (e.g. cytoskeleton).

# Kv 11.1 (hERG1) CHANNELS IN CANCER




A
S1
S2
S3
S4
S5
P
S6
cNBD
EAG
NH3+
COO-
B
S2
S3
S1
S4
S5
S6
P
C
EAG
K channel family
eag
ether-à-go-go
elk
eag-like
erg
eag-related
eag1
eag2
elk1
elk2
elk3
erg1
erg2
erg3
D
+20 mV
-80 mV
E
recovery from
inactivation
activated →
inactivated
deactivation
closed,
deactivated
+40 mV
-60 mV

# hERG1


out
in
Sel. Filter
P-h
S5
S6
Pore-forming domain
out
in
Drug binding site
K+
D
Turret
P-helix
SF
plasma membrane
S1
S2
S3
S4
S5
S6
PAS
cNBD
C
N
S1-S4: voltage sensor domain(VSD)
S5-S6: pore domain

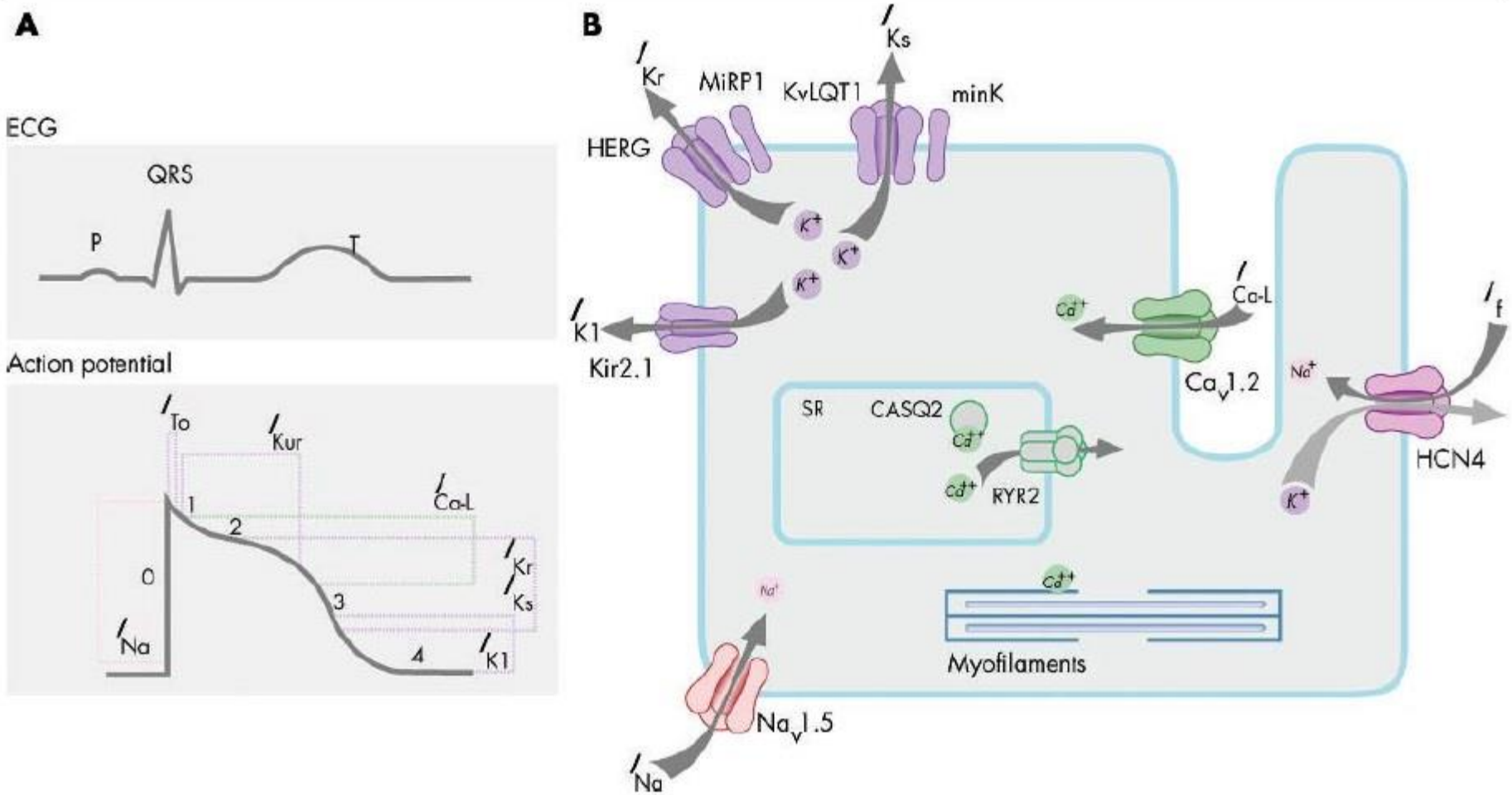
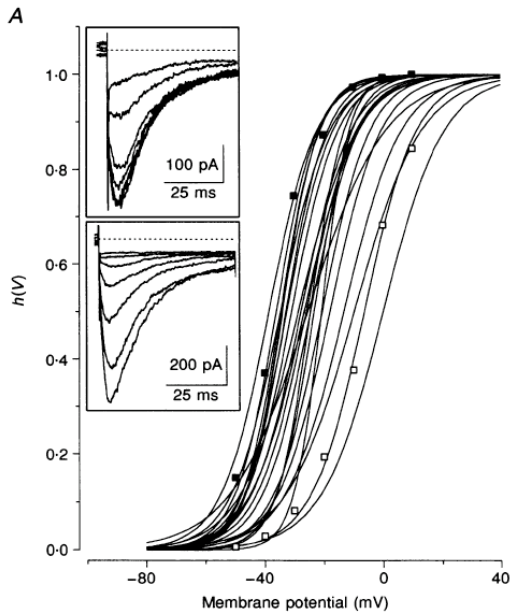


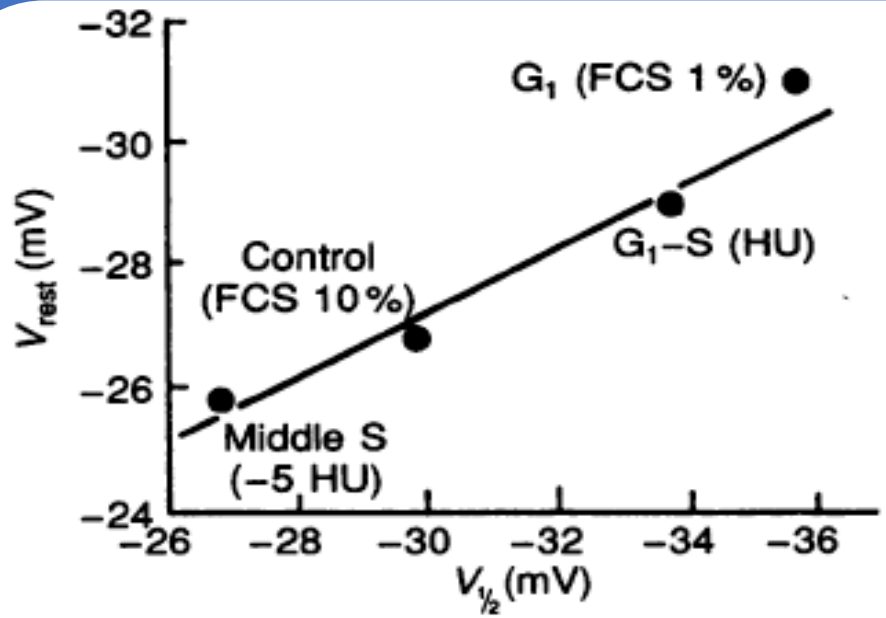

**Figure 1** Ionic currents contributing to the ventricular action potential (A) and schematic representation of a cardiomyocyte displaying (only) those proteins involved in the pathogenesis of inherited arrhythmia syndromes (B). In panel A, the action potential is aligned with its approximate time of action during the ECG. In panel B, ankyrin-B, an adapter protein involved in the long QT syndrome type 4, is not depicted.

*Journal of Physiology* (1995), **489.2**, *pp.*455–471

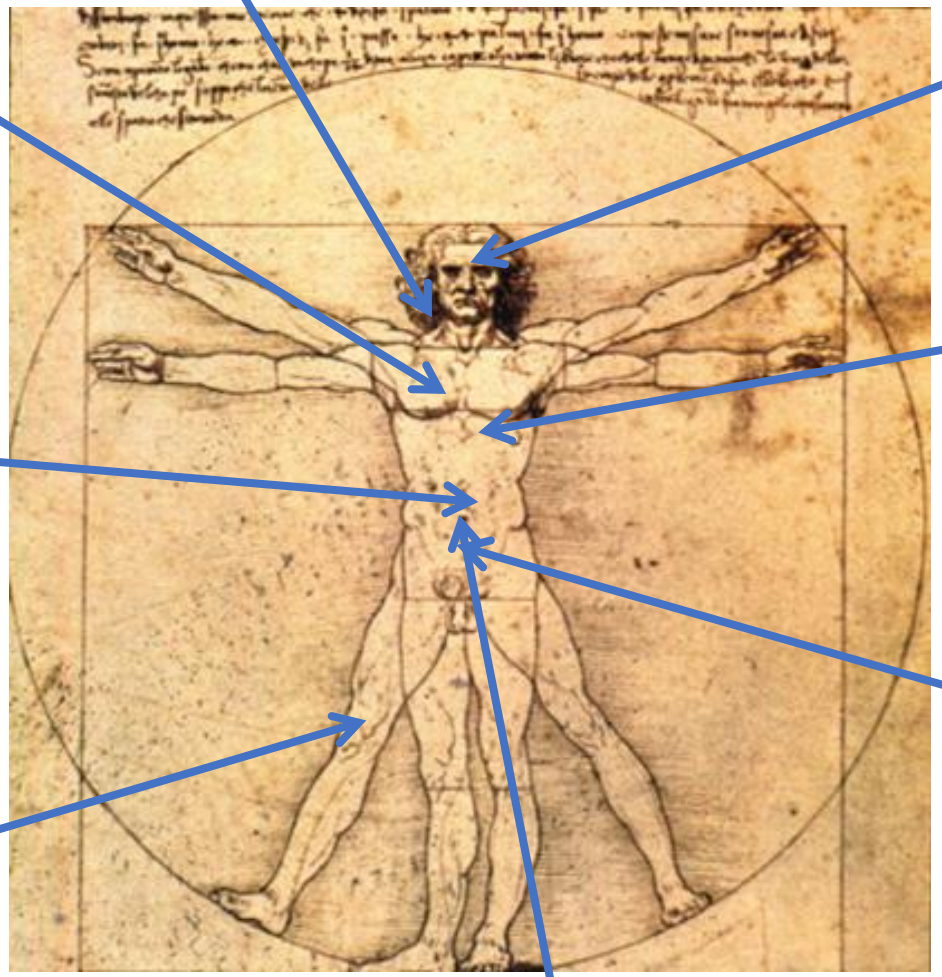
# A novel inward-rectifying $K^+$ current with a cell-cycle dependence governs the resting potential of mammalian neuroblastoma cells

Annarosa Arcangeli*, Laura Bianchi, Andrea Becchetti, Laura Faravelli, Marcella Coronnello†, Enrico Mini†, Massimo Olivotto* and Enzo Wanke‡



# hERG1 expression and role in human cancers



***HEAD & NECK CANCER:***
***Reduced survival, tumor recurrence***

***BREAST:***
***-metastasis***
***-correlation with molecular subtype, G and Ki67***

***•BRAIN: GBM***
***•VEGF secretion***
***•Prognosis***
***•Target for therapy***

***PANCREAS:***
***-PDAC :Proliferation, Migration***
***•Negative PF in Stages I-III***
***•pNET:***
***•Positive PF***

***STOMACH:***
***Intestinal type ADK proliferation***
***•VEGF secretion***
***•Negative PF in TNM Stage I***

***BONE MARROW-LEUKEMIAS:***
***Proliferation (AML)***
***Migration (AML)***
***•VEGF secretion (AML)***
***•Apoptosis/Chemoresistance (ALL)***

***COLORECTAL:***
***-Migration/invasiveness***
***•VEGF secretion***
***•Negative PF in TNM Stages I-II***
***•Positive factor for response to Bevacizumab in TNM Stage IV***

***ENDOMETRIAL CANCER***
***OVARIAN CANCER:***
***Overexpression***

# hERG1 in Colorectal Cancer (CRC)

[CANCER RESEARCH 64, 606–611, January 15, 2004]

# *herg1* Gene and HERG1 Protein Are Overexpressed in Colorectal Cancers and Regulate Cell Invasion of Tumor Cells

**Elena Lastraioli,[1] Leonardo Guasti,[1] Olivia Crociani,[1] Simone Polvani,[1] Giovanna Hofmann,[1] Harry Witchel,[4] Lapo Bencini,[3] Massimo Calistri,[3] Luca Messerini,[2] Marco Scatizzi,[3] Renato Moretti,[3] Enzo Wanke,[5] Massimo Olivotto,[1] Gabriele Mugnai,[1] and Annarosa Arcangeli[1]**

*Departments of [1]Experimental Pathology and Oncology, and [2]Human Pathology and Oncology, University of Firenze, Firenze, Italy; [3]First Division of General Surgery and Transplantation, Careggi Hospital, Firenze, Italy; [4]Department of Physiology and Cardiovascular Research Laboratories, School of Medical Sciences, University of Bristol, Bristol, United Kingdom; and [5]Department of Biotechnology and Biosciences, University of Milano Bicocca, Milan, Italy*

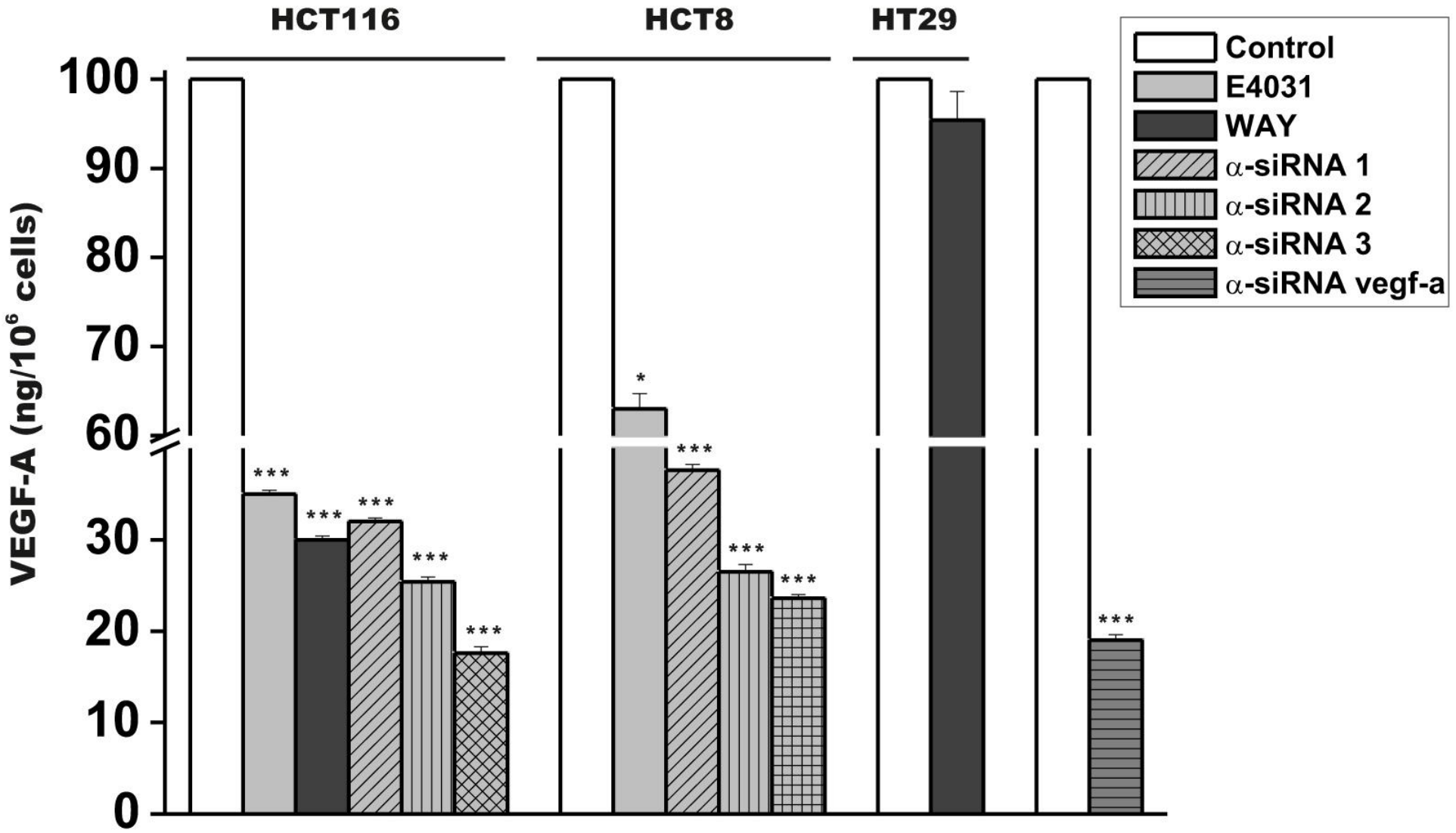

OPEN

SUBJECT AREAS:
COLORECTAL CANCER
INTEGRIN SIGNALLING
TUMOUR ANGIOGENESIS
ION CHANNEL SIGNALLING

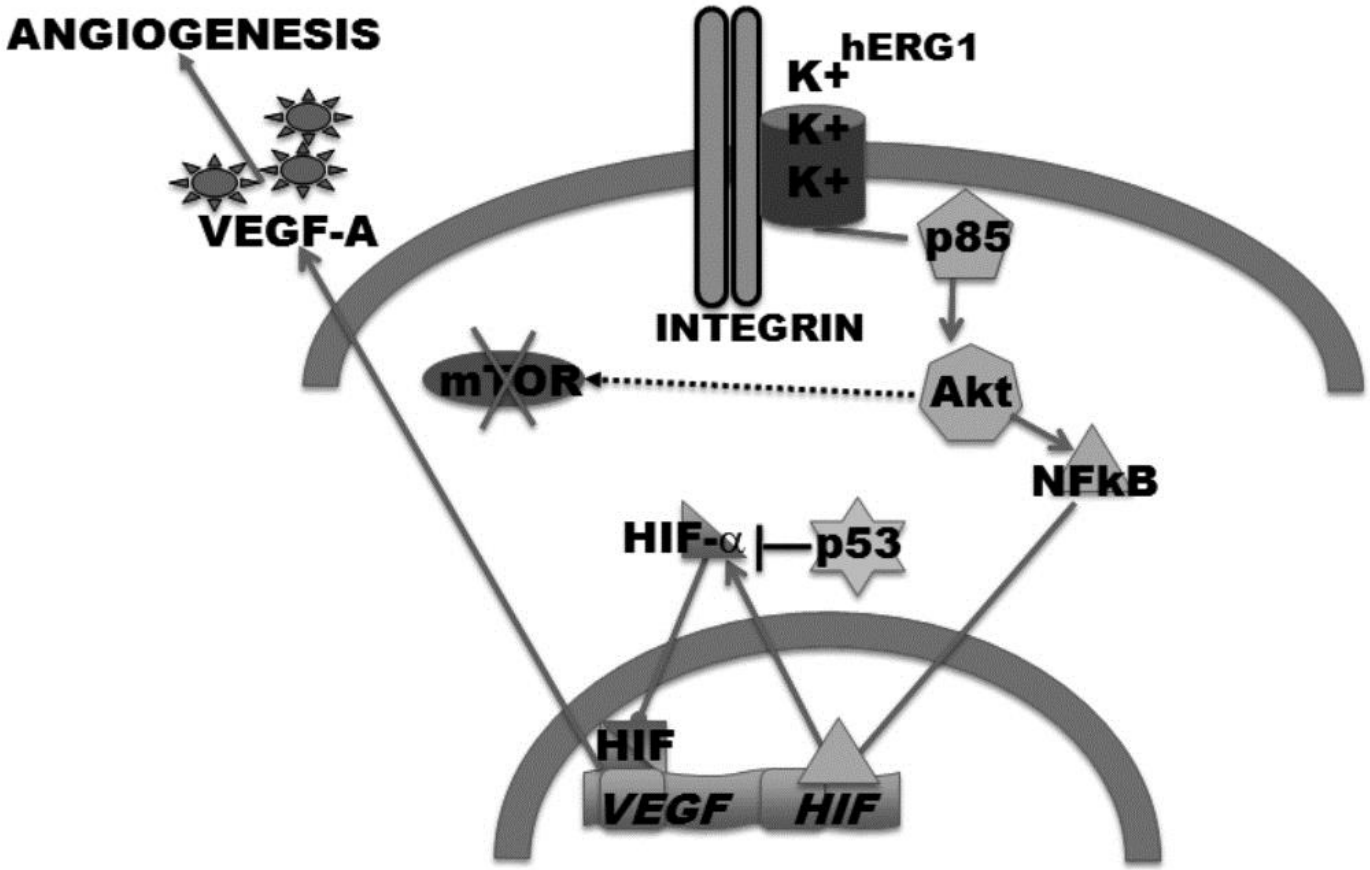
# hERG1 channels modulate integrin signaling to trigger angiogenesis and tumor progression in colorectal cancer

Olivia Crociani[1], Francesca Zanieri[1], Serena Pillozzi[1], Elena Lastraioli[1], Matteo Stefanini[1], Antonella Fiore[1], Angelo Fortunato[1], Massimo D'Amico[1], Marika Masselli[1], Emanuele De Lorenzo[1], Luca Gasparoli[1], Martina Chiu[2], Ovidio Bussolati[2], Andrea Becchetti[3] & Annarosa Arcangeli[1]

# hERG1 regulates VEGF-A expression and secretion



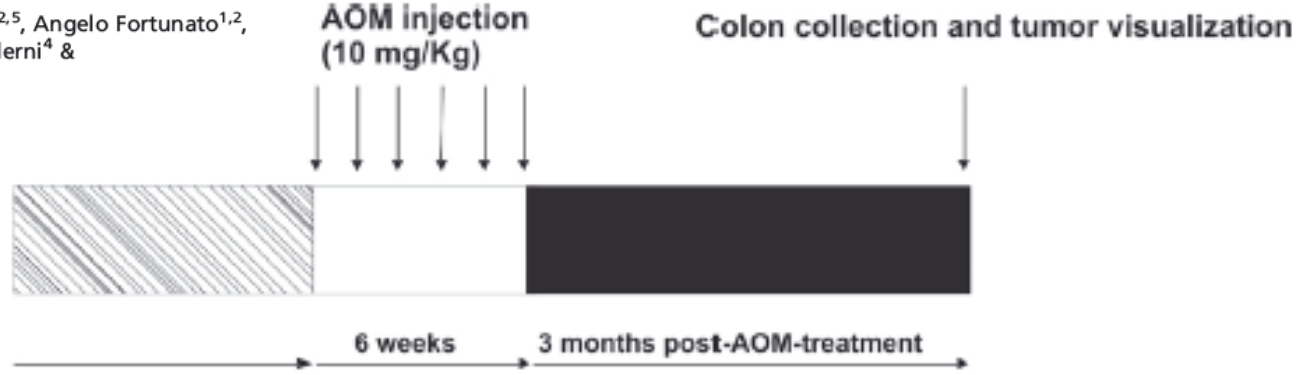
# The hERG1-centered pro-angiogenic signaling pathway in CRC



Cancer Medicine
Open Access
ORIGINAL RESEARCH

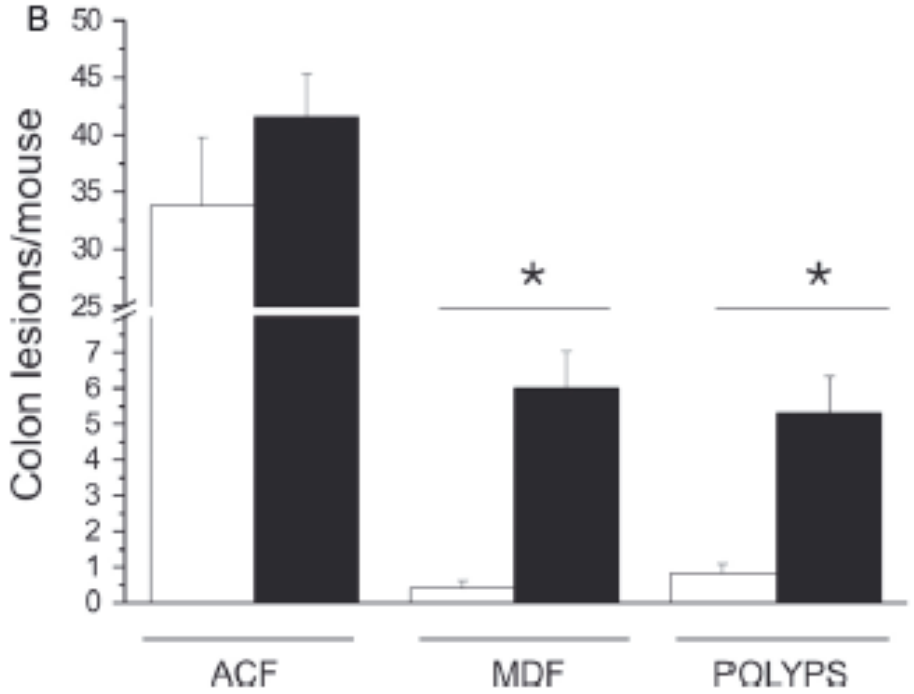
# Characterization of hERG1 channel role in mouse colorectal carcinogenesis

Antonella Fiore[1,2,a], Laura Carraresi[3,a], Angela Morabito[1,2], Simone Polvani[1,2,5], Angelo Fortunato[1,2], Elena Lastraioli[1,2], Angelo P. Femia[4], Emanuele De Lorenzo[1,6], Giovanna Caderni[4] & Annarosa Arcangeli[1,2]




**White bars= WT mice**

**Black bars = hERG1 TG (over expressing) mice**



# hERG1 positivity and Glut-1 negativity identifies high-risk TNM stage I/II Colorectal Cancer patients

Translational Oncology
www.transonc.com

Volume 5 Number 2 April 2012 pp. 105–112 105

## hERG1 Channels and Glut-1 as Independent Prognostic Indicators of Worse Outcome in Stage I and II Colorectal Cancer: A Pilot Study[1]

Elena Lastraioli*, Lapo Bencini†, Elisa Bianchini‡, Maria Raffaella Romoli*, Olivia Crociani*, Elisa Giommoni‡, Luca Messerini†, Silvia Gasperoni‡, Renato Moretti‡, Francesco Di Costanzo‡, Luca Boni‡,§ and Annarosa Arcangeli*,§

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Universitaria, Careggi,
Azienda Ospedaliero-U
§Department of Huma
of Florence, Florence,

OncoTargets and Therapy

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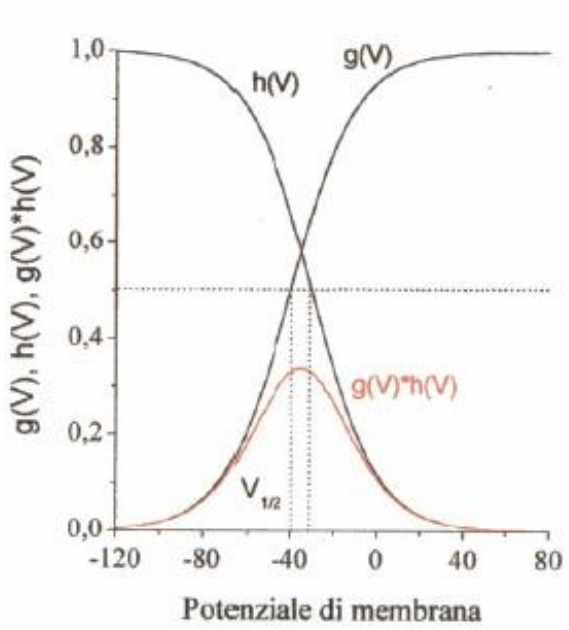
ORIGINAL RESEARCH

## hERG1 positivity and Glut-1 negativity identifies high-risk TNM stage I and II colorectal cancer patients, regardless of adjuvant chemotherapy

# The dual mode of action of hERG1: ion flux vs non-conductive mechanisms

# *hERG1 role in human cancers*

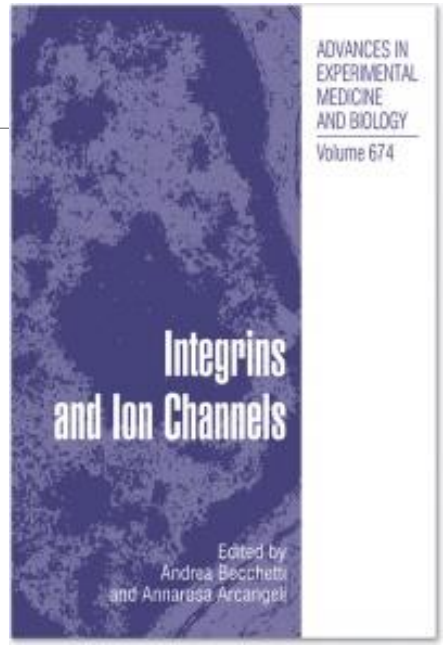
- ✓ How can hERG1 exert different roles?




Membrane potential

- ✓ Regulation of $V_{rest}$
- ✓ Regulation of intracellular signaling

# *hERG1 and integrins*




Review TRENDS in Cell Biology Vol.16 No.12



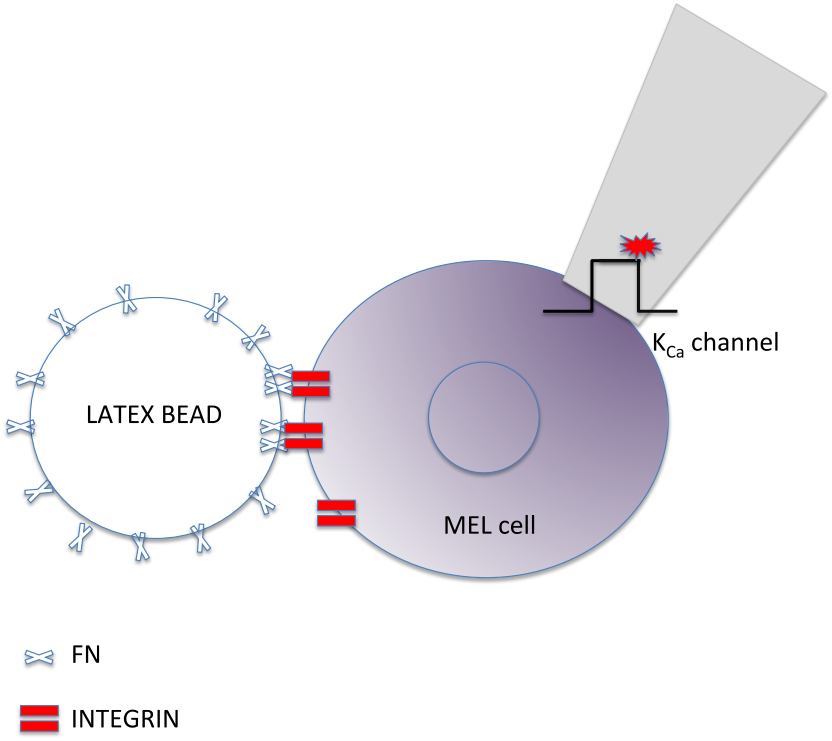
ELSEVIER

## Complex functional interaction between integrin receptors and ion channels

**Annarosa Arcangeli[1] and Andrea Becchetti[2]**

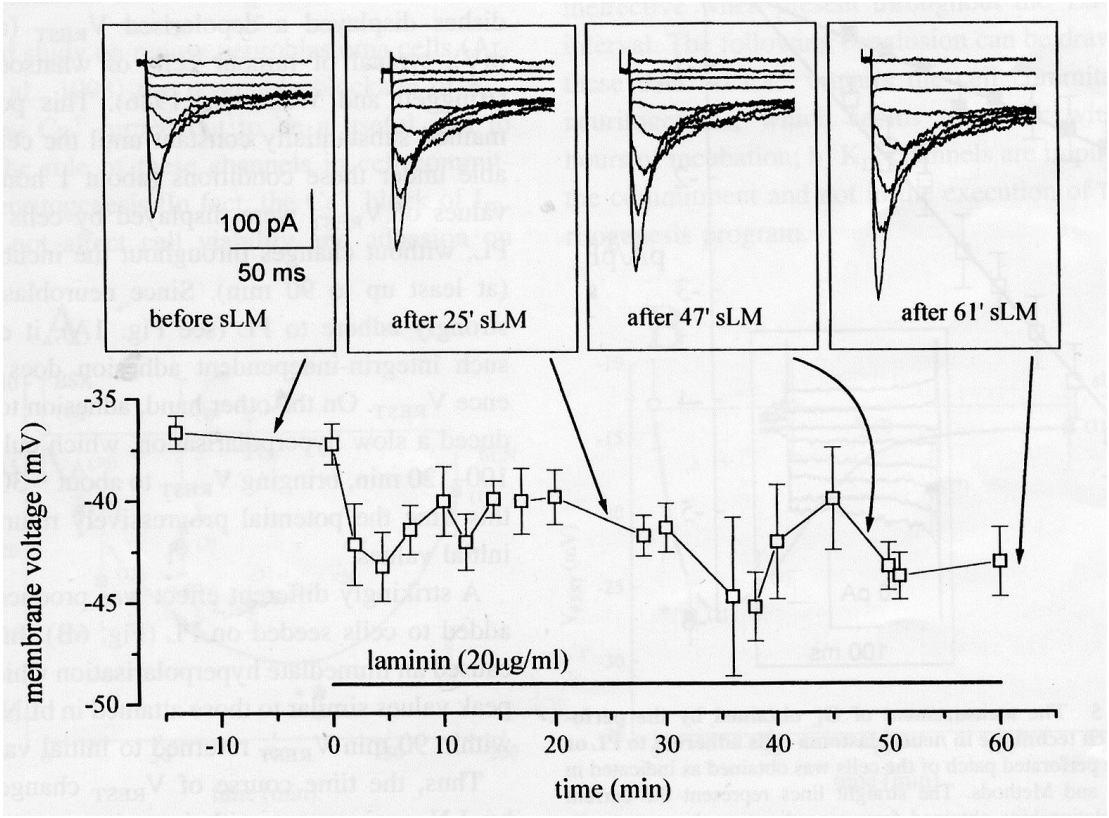
[1] Department of Experimental Pathology and Oncology, University of Firenze, Viale G.B. Morgagni 50, 50134 Firenze, Italy
[2] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy

# Engagement of the $\beta_1$ integrin activates $Ca^{2+}$-dependent $K^+$ channels in murine erythroleukemia (MEL) cells



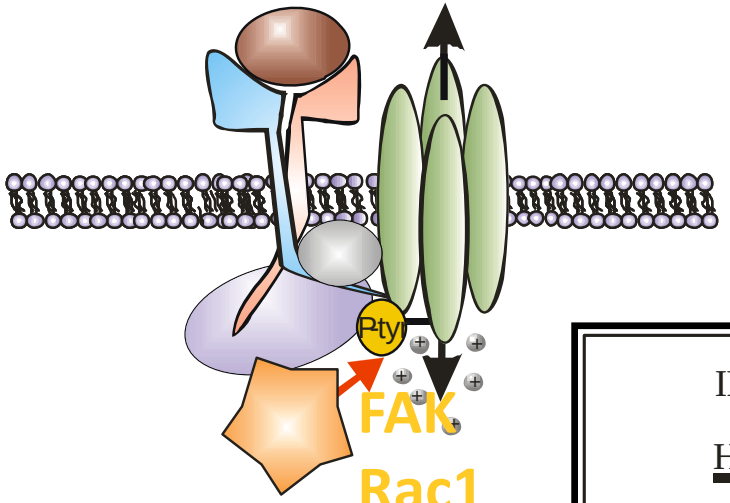

**Arcangeli, A.**,et al.. **Biochem. Biophys. Res. Commun.**, 146, 1450-1457, **1987**.
**Arcangeli A. et al., Biochem.Biophys.Res.Commun., 177, 1266-1272, 1991.**
**Becchetti A. et al., Proc. Royal Soc. Ser.B, 248, 235-240, 1992**

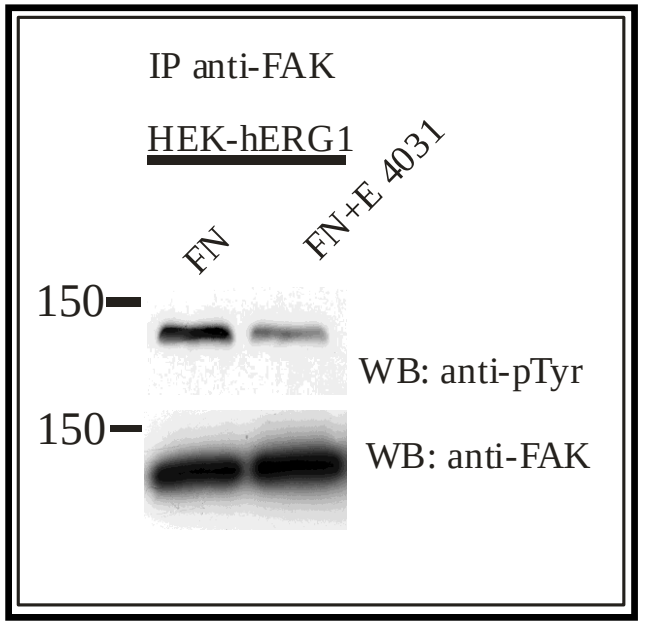
# Engagement of the $\beta_1$ integrin activates hERG1 $K^+$ channels in neuroblastoma cells.

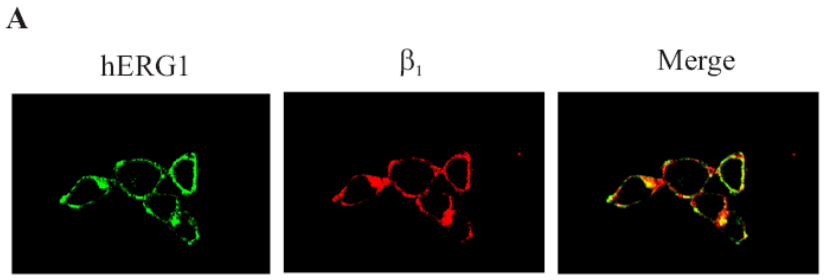


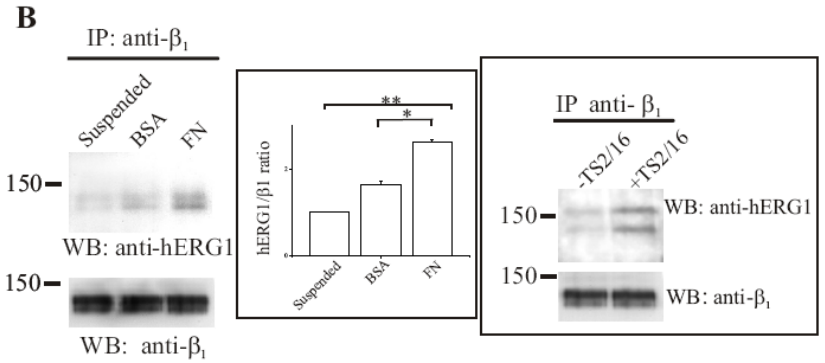

**Arcangeli A. et al., J. Cell. Biol.,122: 1131-1143, 1993**
**Arcangeli, A**. et al., **J.Physiol**., **489, 455-471,1995**
**Arcangeli A., et al., Cell Adhesion Commun., 4: 369-384, 1996**

# Integrin-channel complex: hERG1/β1




**Cherubini A. et al., Mol.Biol.Cell, 2005**

# The hERG1/β1 integrin complex in CRC

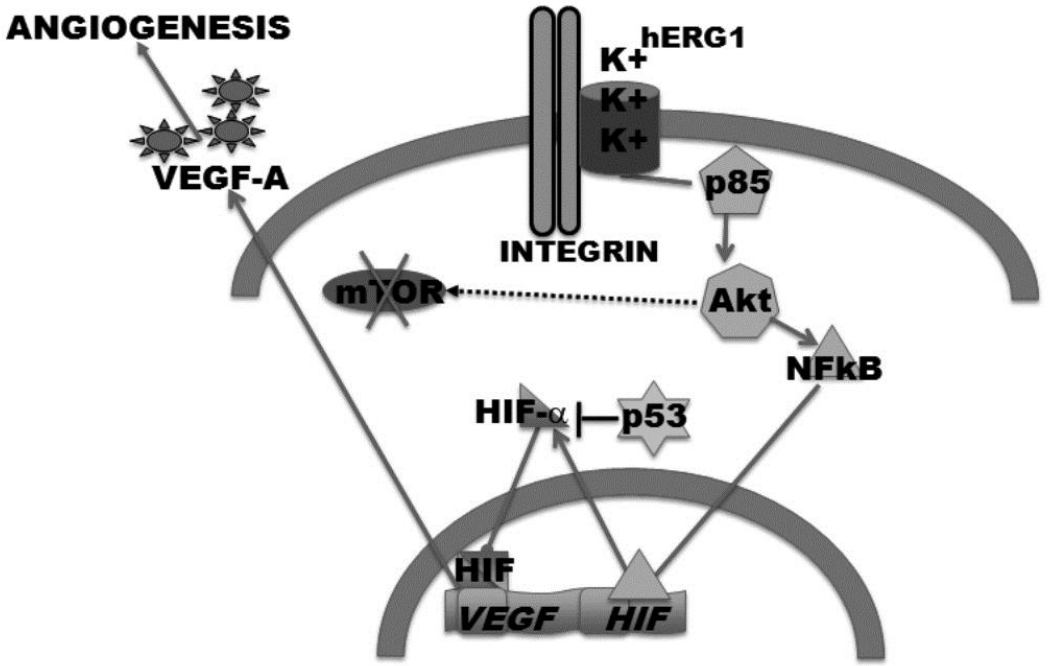
SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
COLORECTAL CANCER
INTEGRIN SIGNALLING
TUMOUR ANGIOGENESIS
ION CHANNEL SIGNALLING

## hERG1 channels modulate integrin signaling to trigger angiogenesis and tumor progression in colorectal cancer

Olivia Crociani[1], Francesca Zanieri[1], Serena Pillozzi[1], Elena Lastraioli[1], Matteo Stefanini[1], Antonella Fiore[1], Angelo Fortunato[1], Massimo D'Amico[1], Marika Masselli[1], Emanuele De Lorenzo[1], Luca Gasparoli[1], Martina Chiu[2], Ovidio Bussolati[2], Andrea Becchetti[3] & Annarosa Arcangeli[1]



SCIENCE SIGNALING | RESEARCH ARTICLE

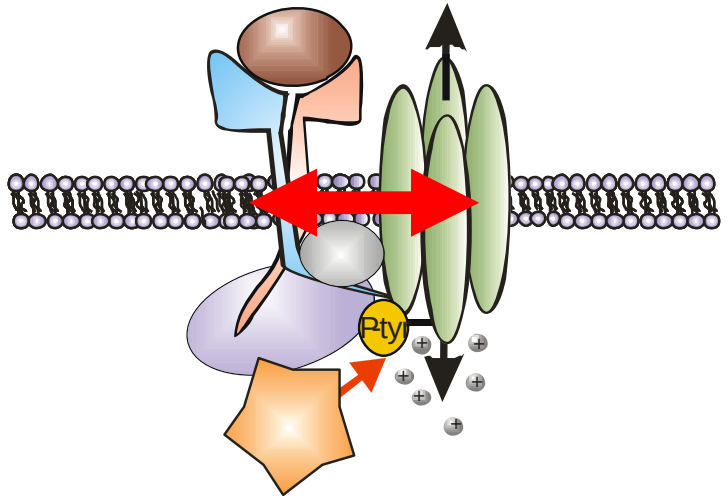
CANCER

# The conformational state of hERG1 channels determines integrin association, downstream signaling, and cancer progression



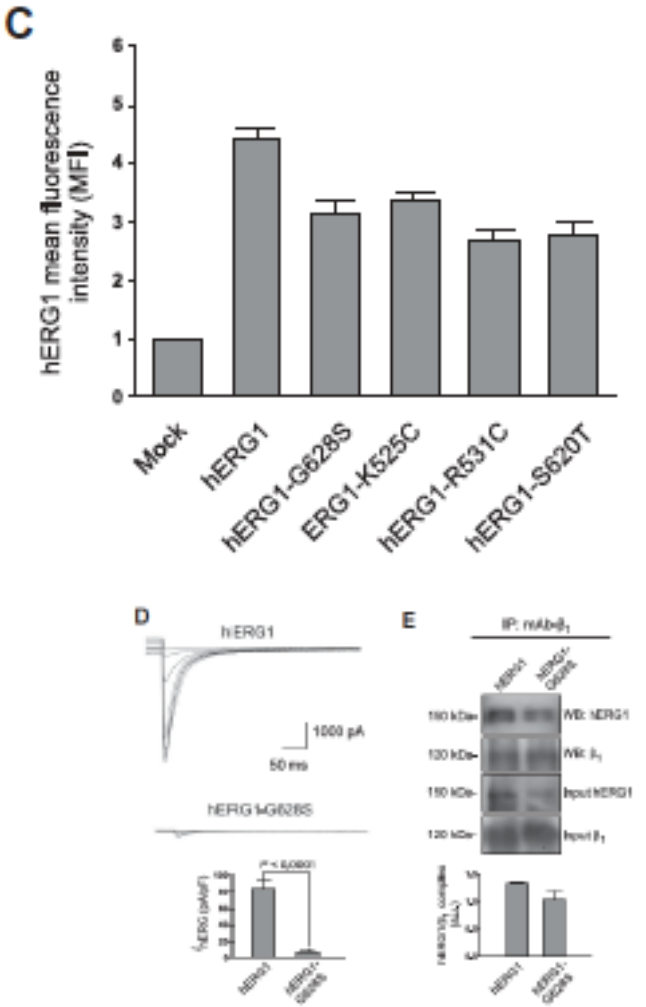
Andrea Becchetti,[1] Silvia Crescioli,[2] Francesca Zanieri,[2] Giulia Petroni,[2] Raffaella Mercatelli,[3] Stefano Coppola,[4] Luca Gasparoli,[2] Massimo D'Amico,[5] Serena Pillozzi,[2] Olivia Crociani,[2] Matteo Stefanini,[5] Antonella Fiore,[2] Laura Carraresi,[5] Virginia Morello,[6]* Sagar Manoli,[2] Maria Felice Brizzi,[7] Davide Ricci,[8] Mauro Rinaldi,[8] Alessio Masi,[2]† Thomas Schmidt,[4] Franco Quercioli,[3] Paola Defilippi,[4] Annarosa Arcangeli[2]‡

**The association between the channel and the integrin occurs through the TM domains of either proteins**



# *The hERG1 conformational state determines (the closed state favours) integrin association*

***hERG1 mutants:***
***G628S: non conductive***
***S620T: non inactivating***
***K525C: S4 (voltage sensor) mutant****
***preferentially in the open state***
***R531C: S4 (voltage sensor) mutant****
***preferentially in the closed state***
****=alterations of gating***



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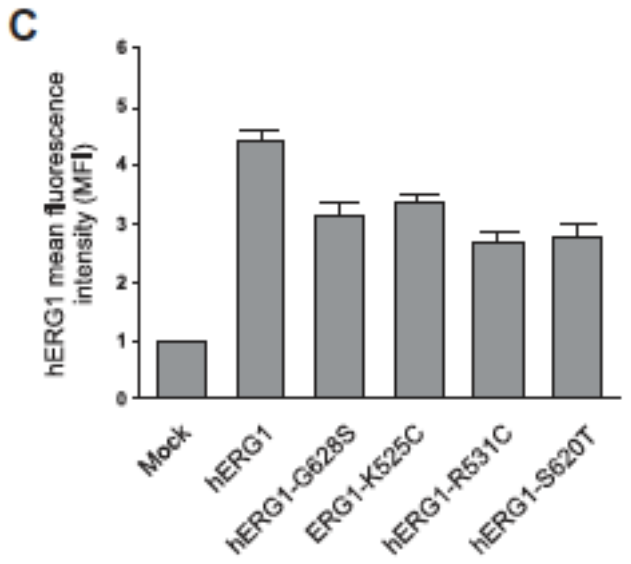


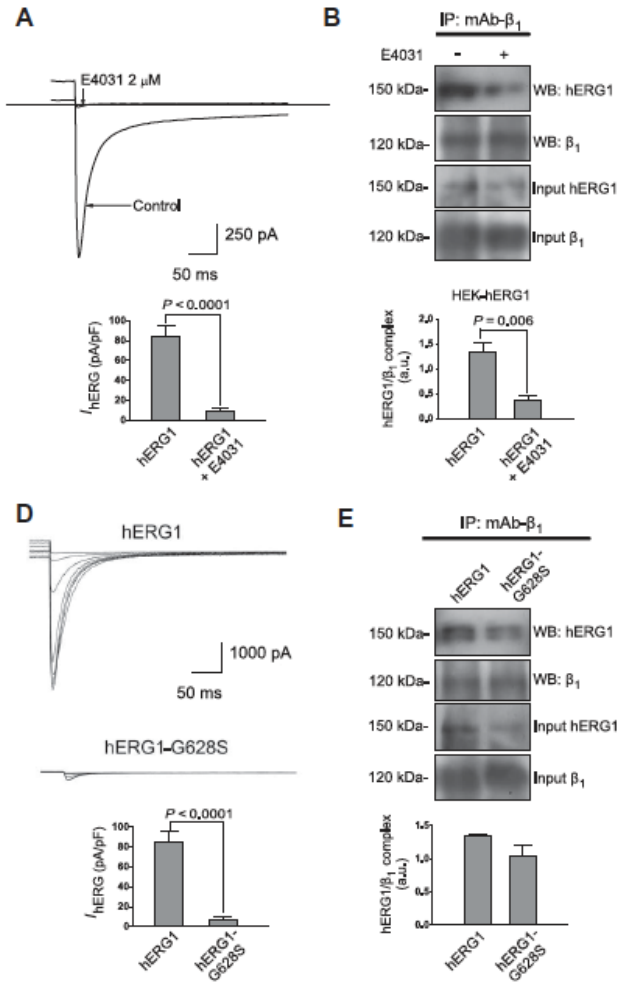


*hERG1 mutants:*
*G628S: non conductive*
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*K525C: S4 (voltage sensor) mutant**
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**=alterations of gating*

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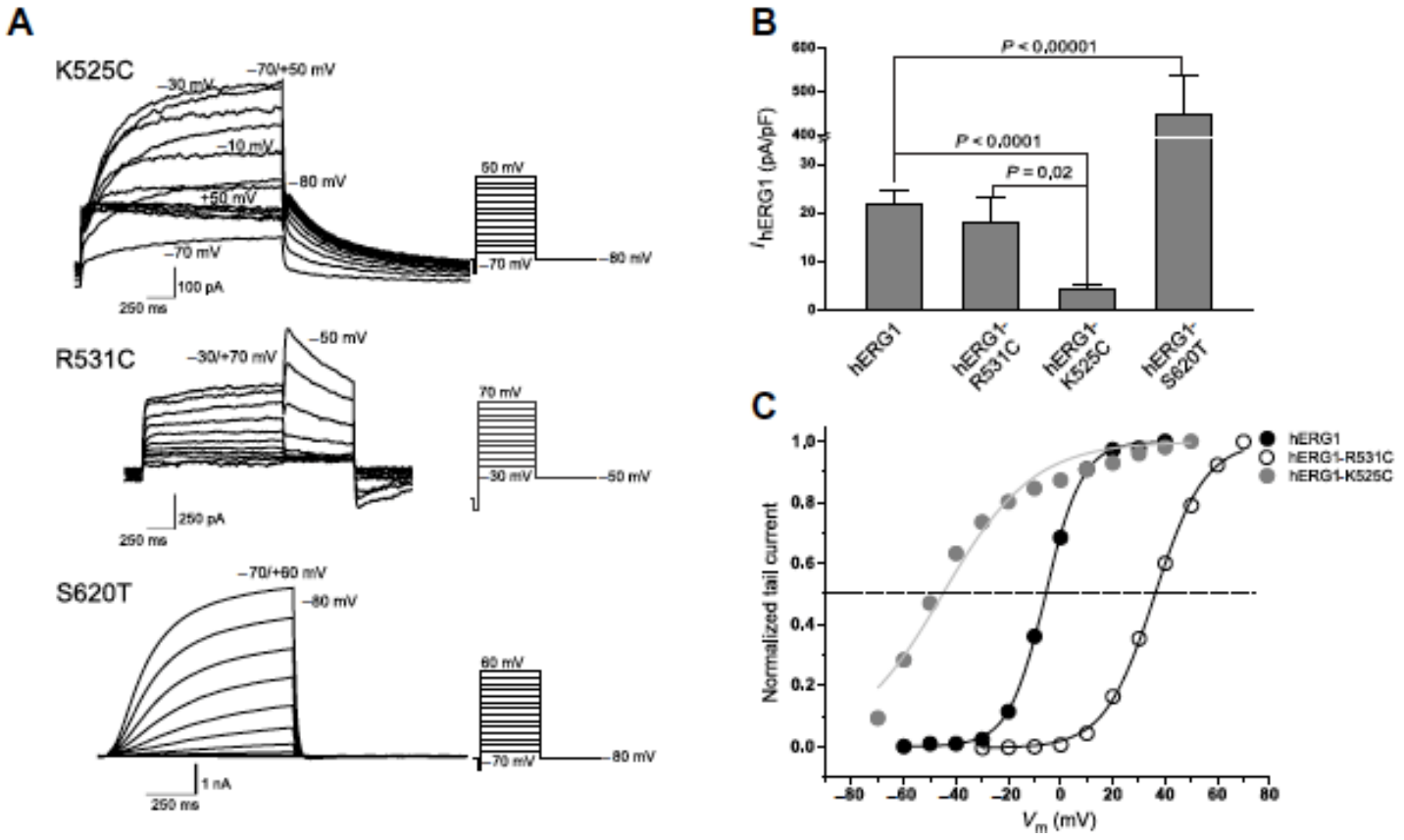

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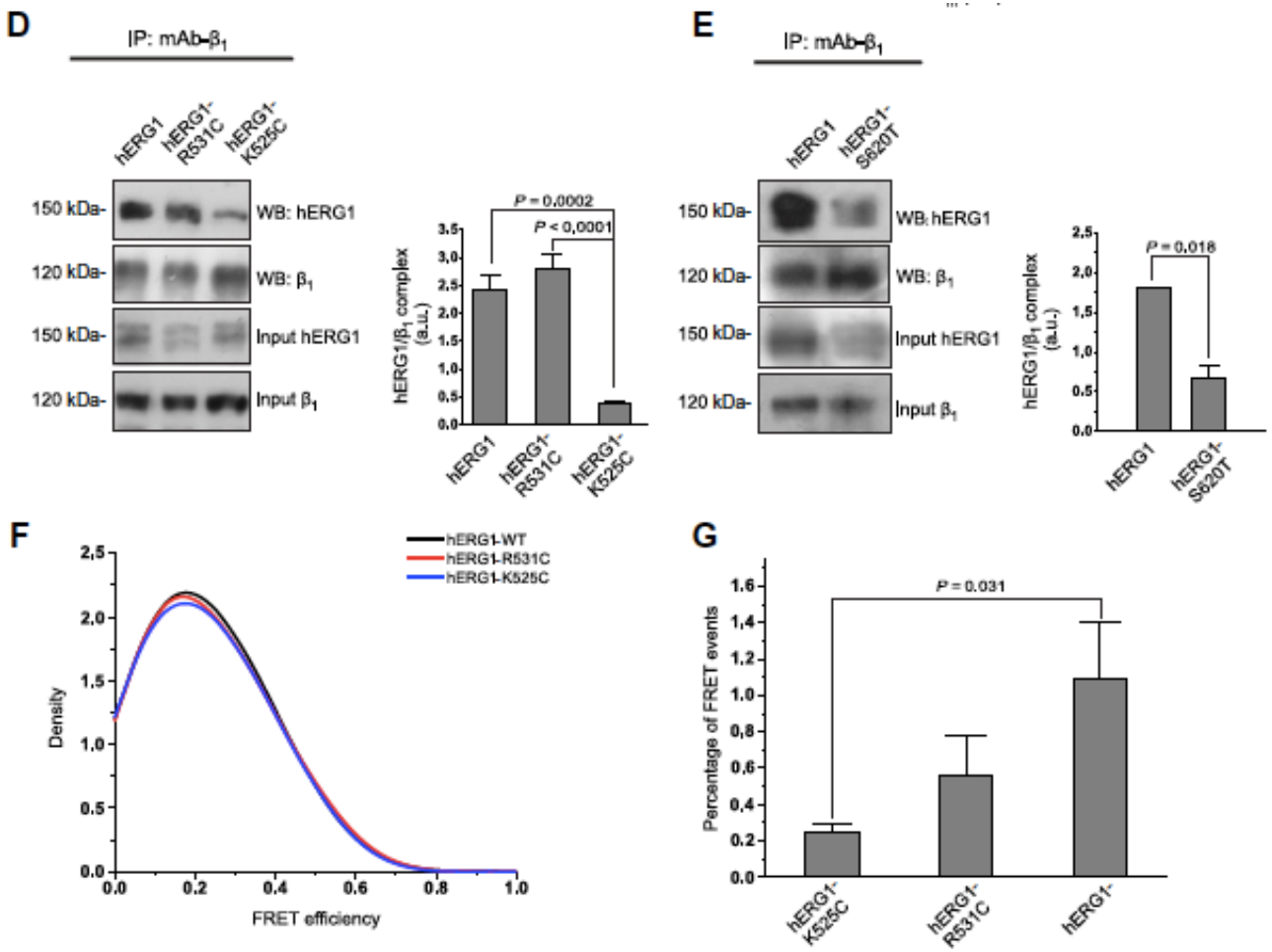

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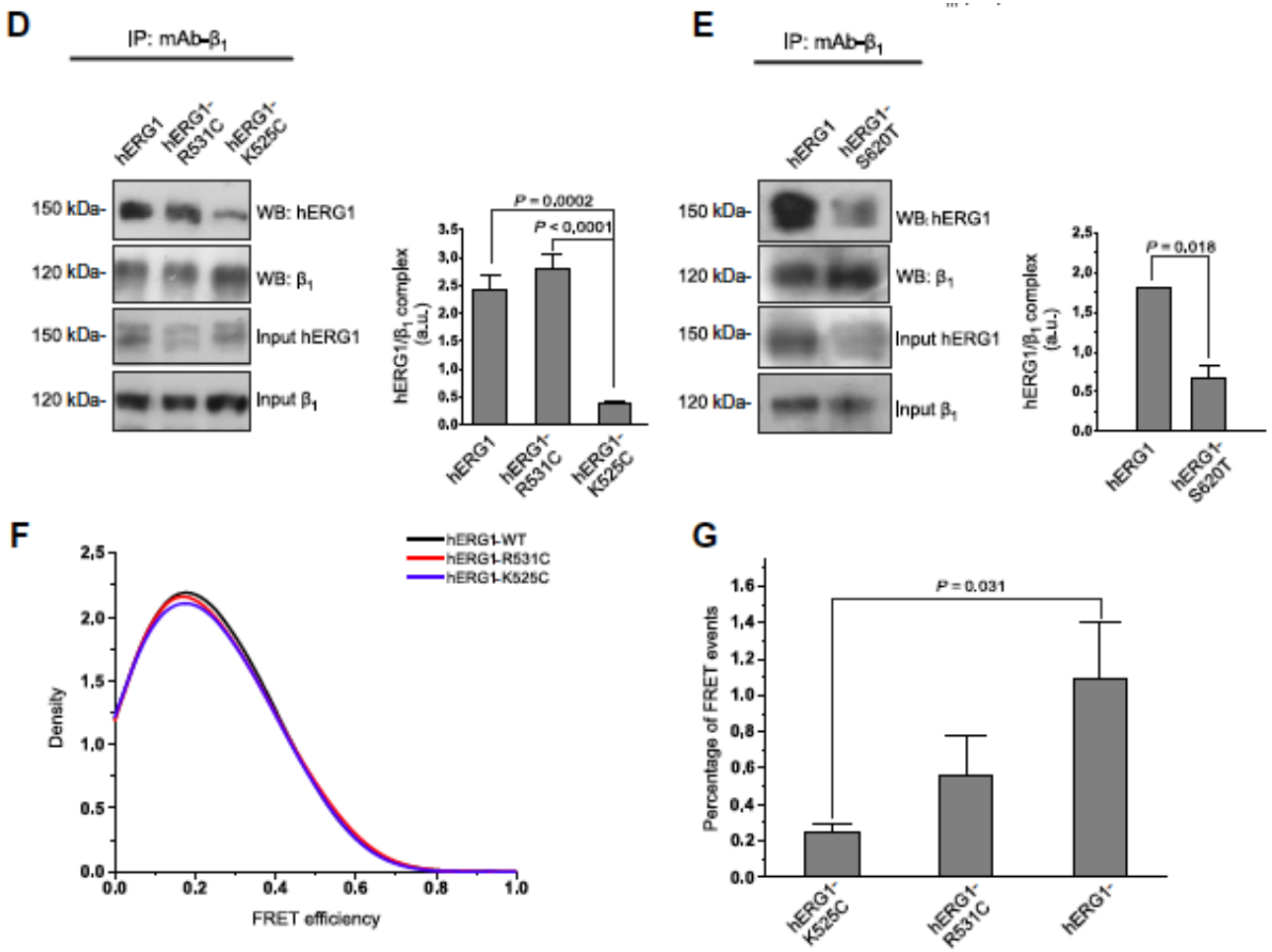
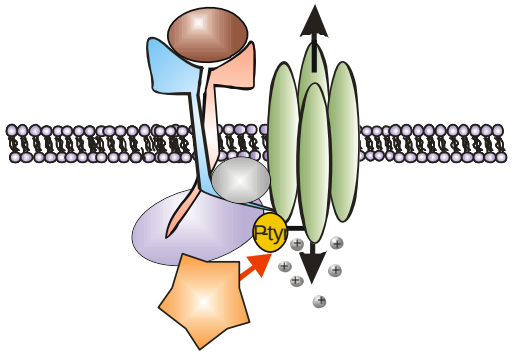
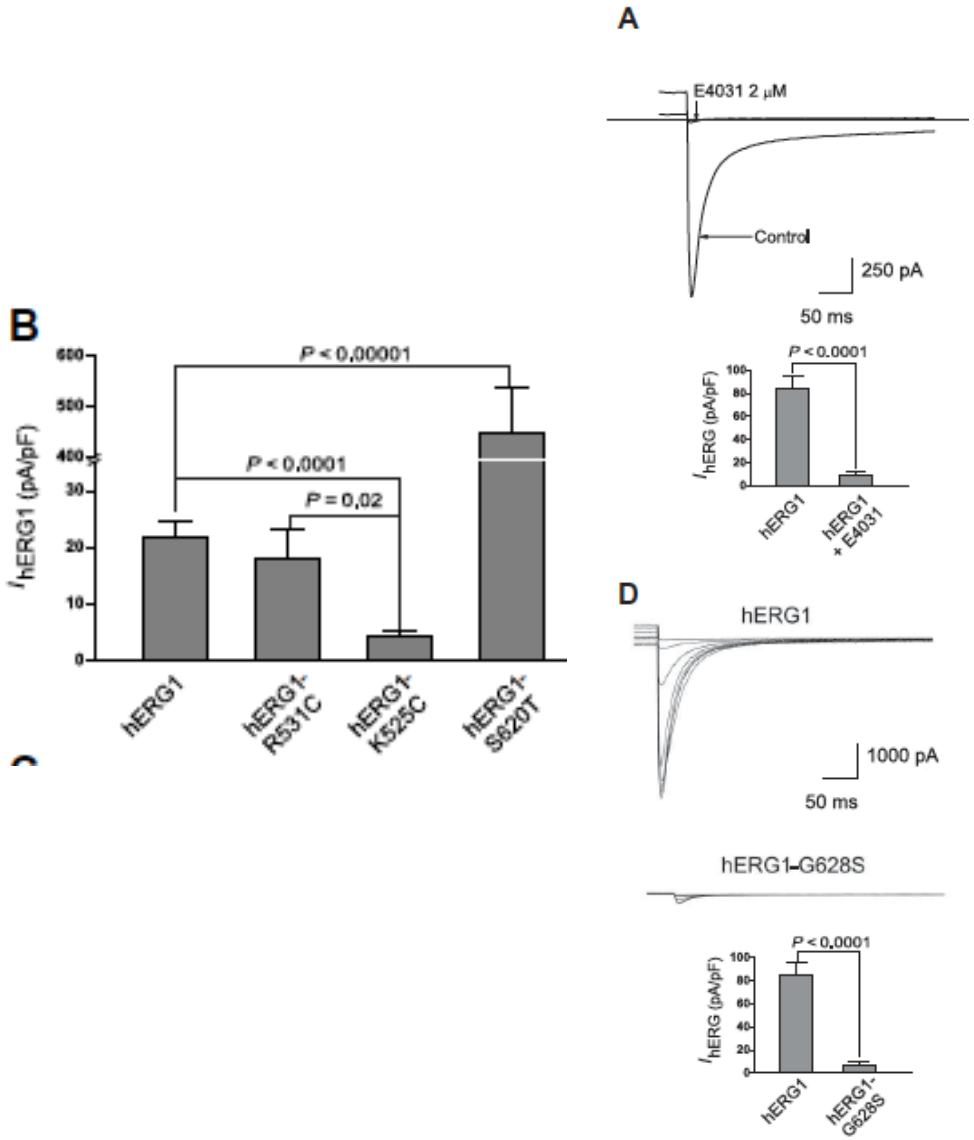
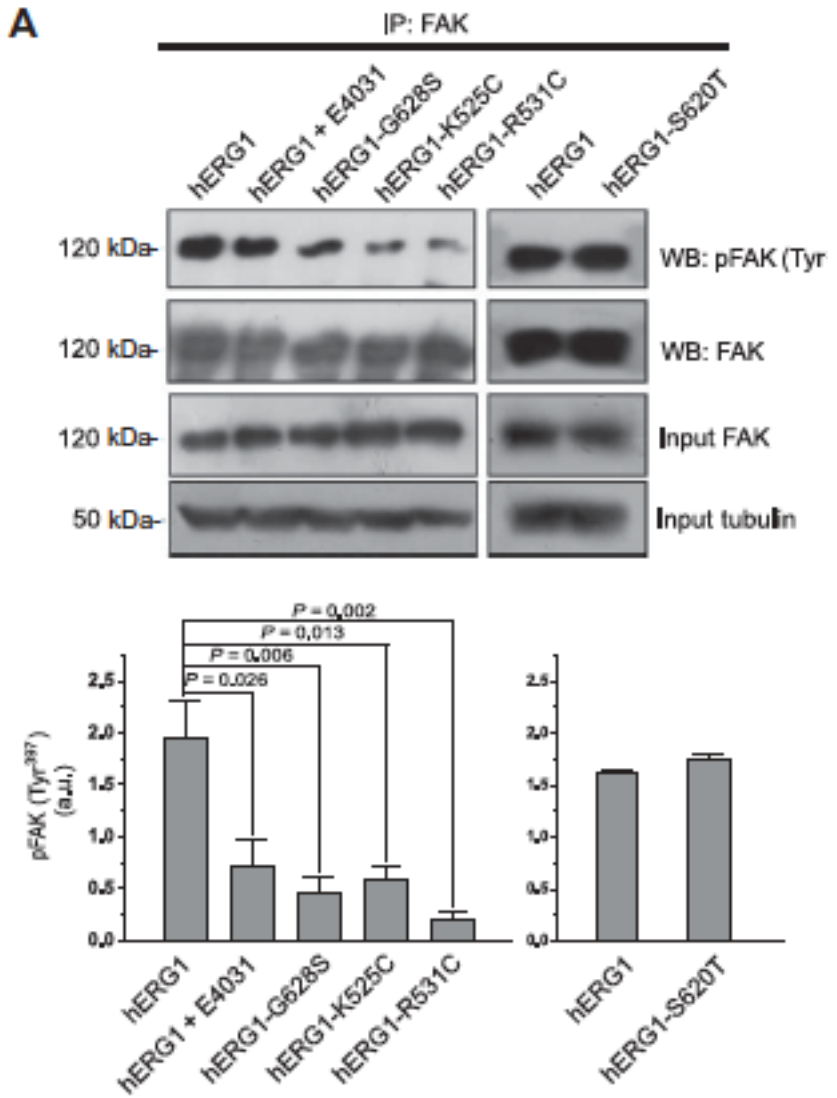




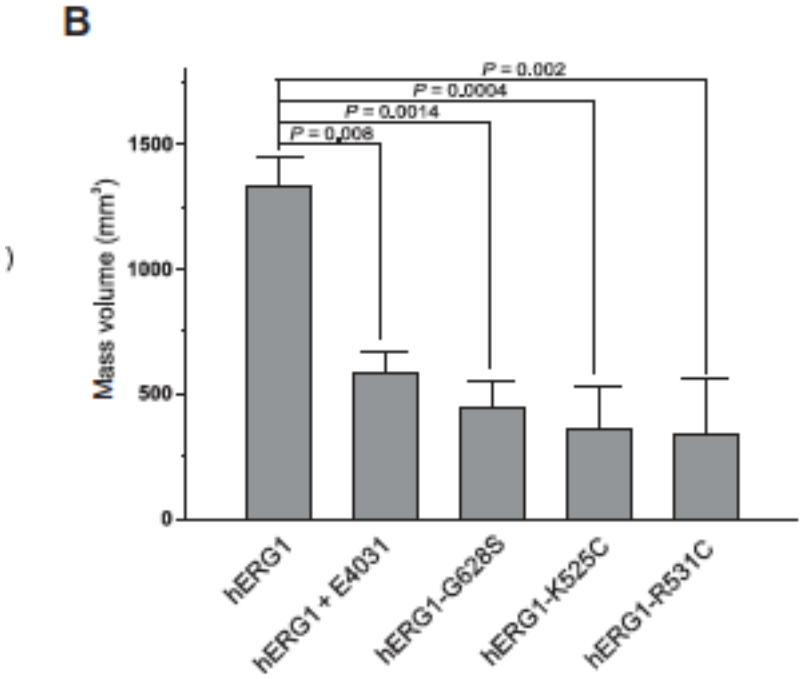

| hERG1 mutant | hERG1 conformational state | hERG1 currents | hERG1/b1 integrin complex |
|---|---|---|---|
| WT | Open/Closed | +++ | +++ |
| G628S | Non Conductive | 0 | ++/+++ |
| R531C | Closed | + | +++/++++ |
| K525C | Open | + | + |
| S620T | No inactivation | ++++ | +/++ |
| E4031 | Blocked (binding in the open state) | 0 | +/++ |

# hERG1 currents (ion flux) regulate FAK phosphorylation

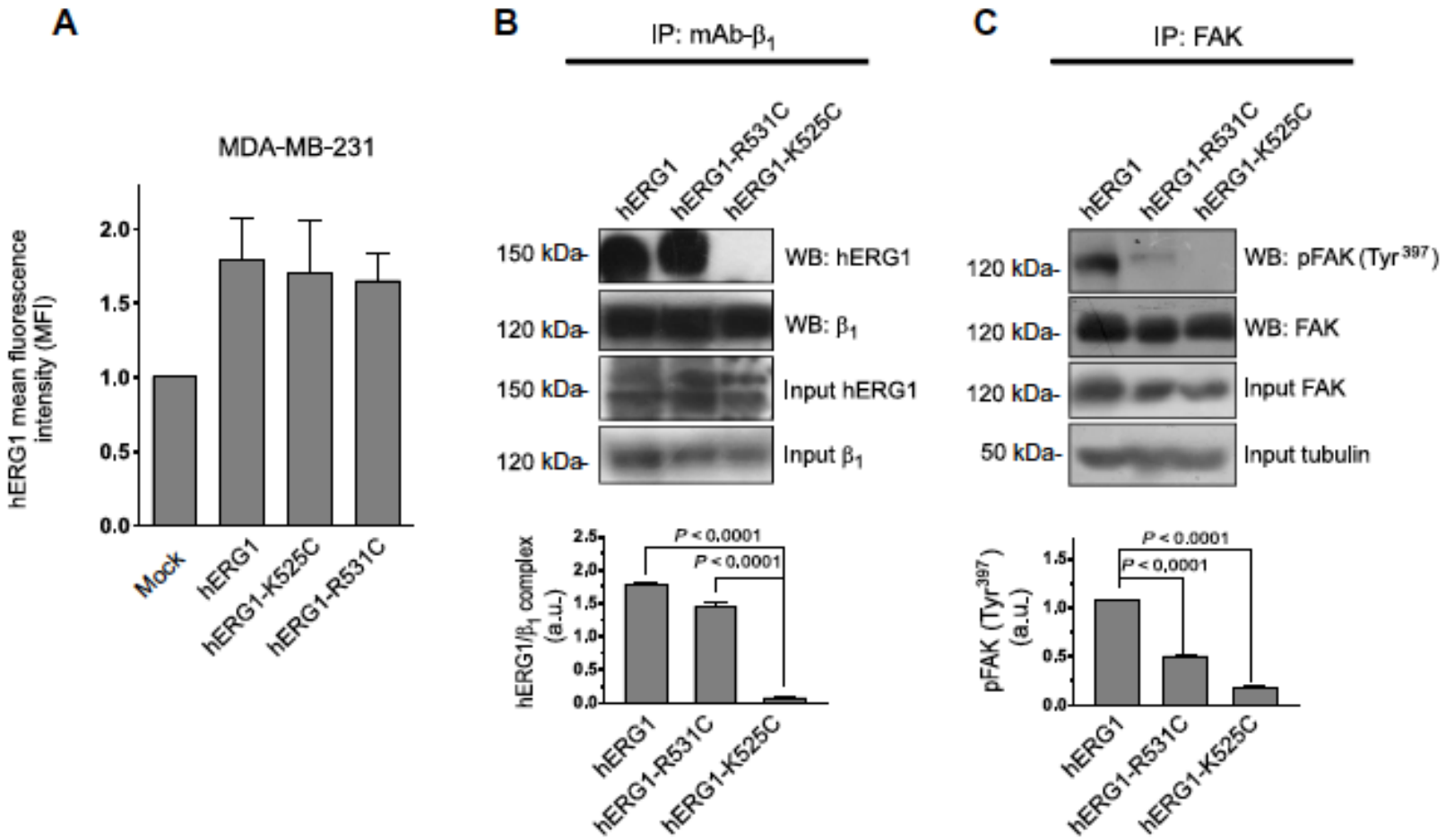


# hERG1 currents (ion flux) regulate FAK phosphorylation……and local tumor growth



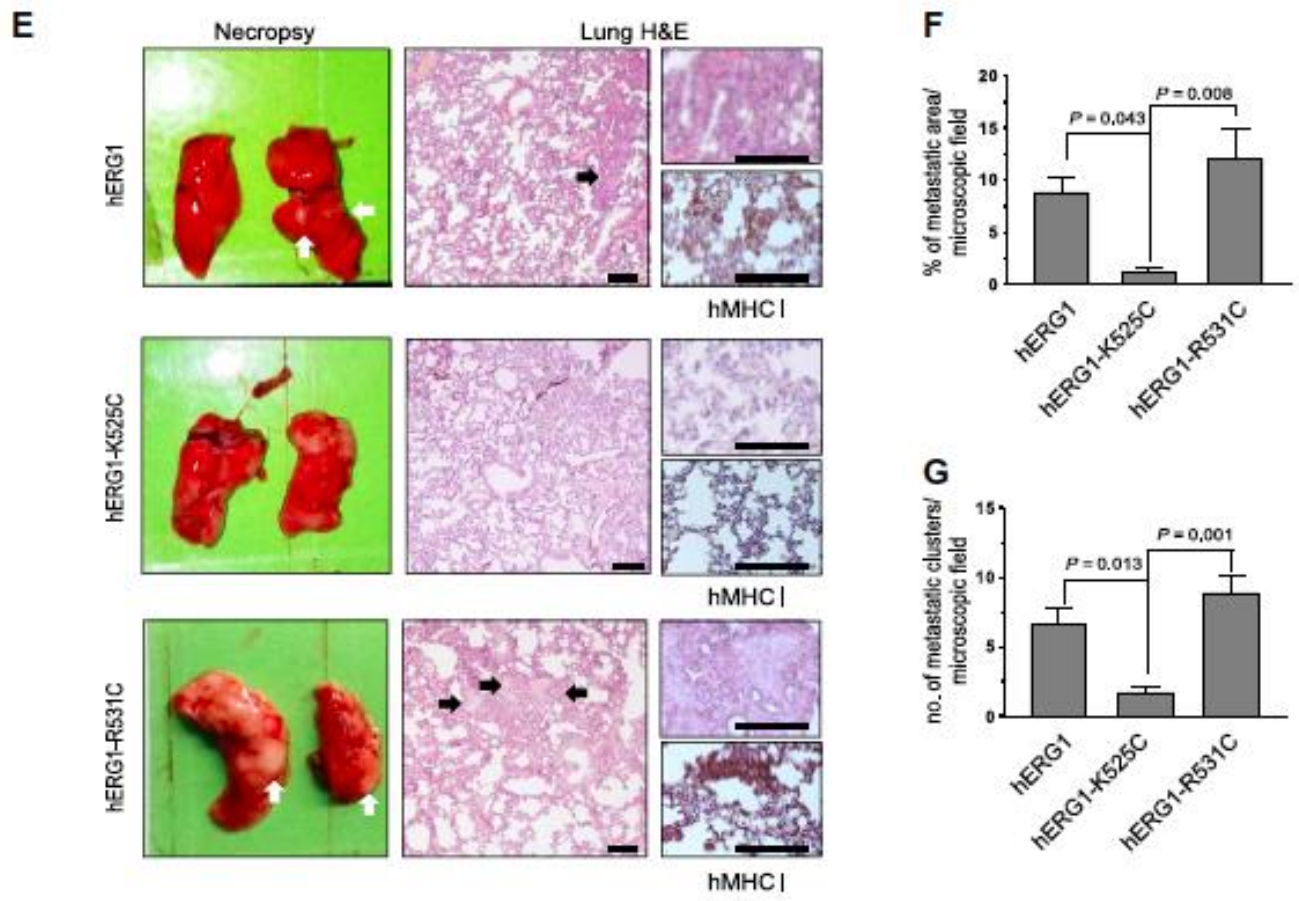
# *The hERG1 conformational state determines (the closed state favours) integrin association: MDA-MB-231 breast cancer cells*



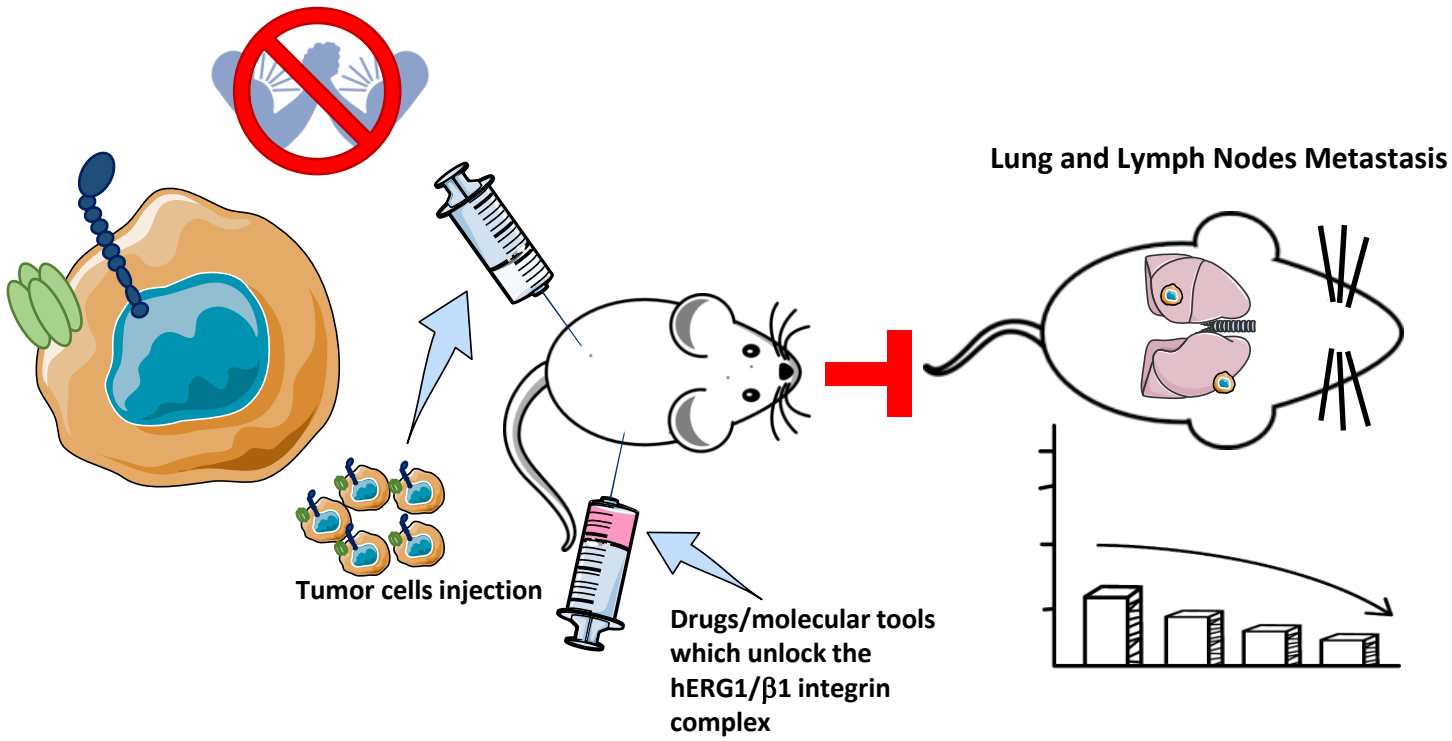
# The hERG1 conformational state determines (the closed state favours) integrin association…..and tumor metastasis (MDA-MB-231 breast cancer cells)

| MDA-MB-231 | hERG1 | hERG1-K525C | hERG1-R531C |
|---|---|---|---|
| Local tumor growth | | | |
| Number of tumor masses (%) | 9/10 (90%) | 10/10 (100%) | 9/10 (90%) |
| Median tumor volume ($mm^3$) | 150 (19–300) | 122 (33–300) | 212 (33–300) |
| Metastases | | | |
| Inguinal lymph nodes<br>Number of mice with macroscopic metastases (%) | 2/5 (40%) | 0/5 (0%) | 3/5 (60%) |
| Lung<br>Number of mice with macroscopic metastases (%) | 2/5 (40%) | 0/5 (0%) | 4/5 (80%) |

# The hERG1 conformational state determines (the closed state favours) integrin association……and tumor metastasis (MDA-MB-231 breast cancer cells)



# Disrupting the hERG1/β1 integrin complex inhibits tumor metastasis



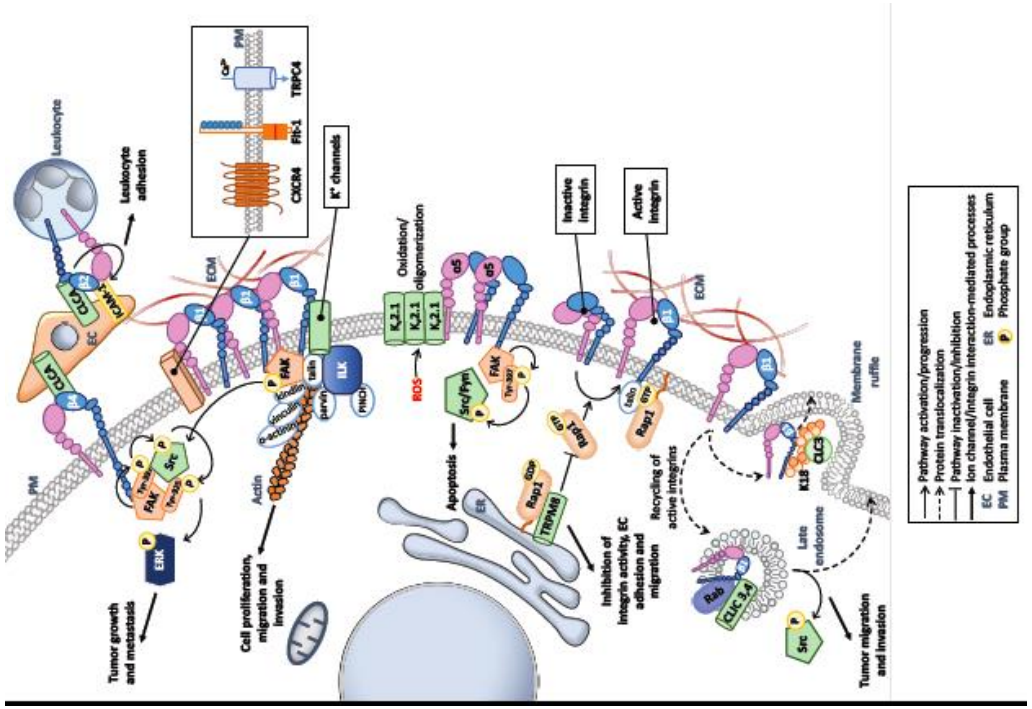
Trends in Cell Biology

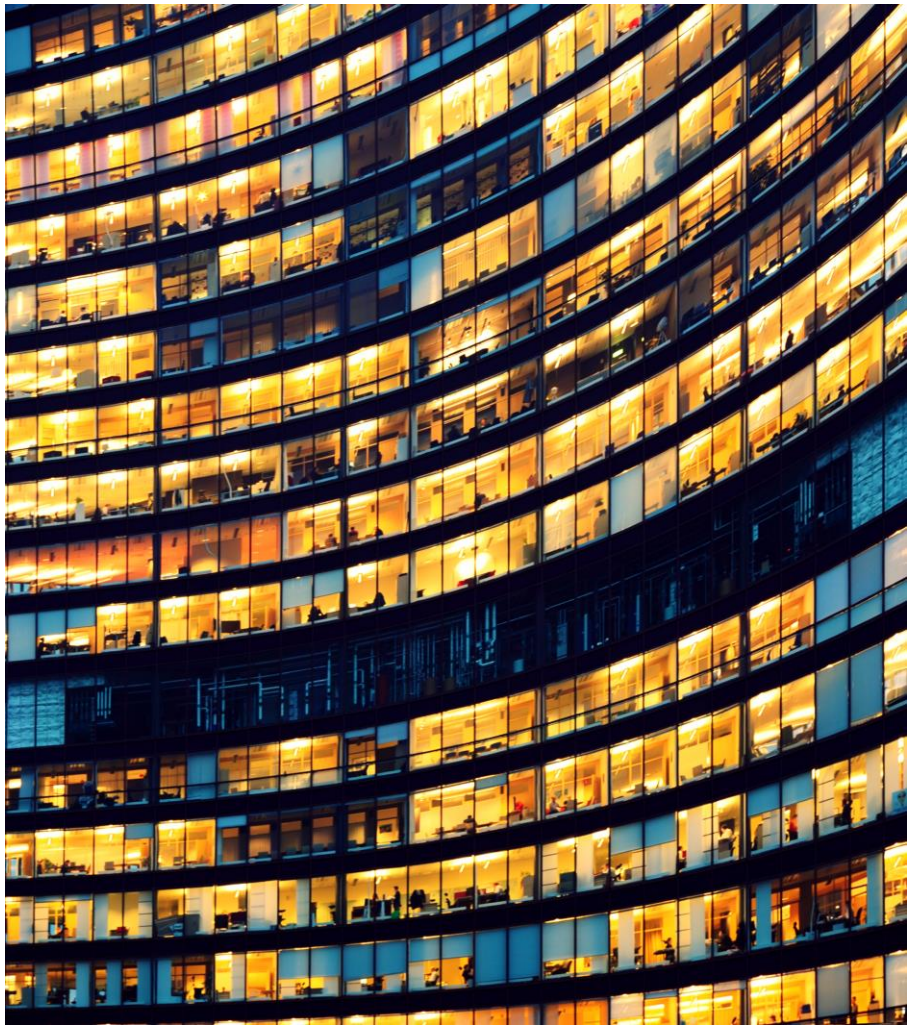
CellPress
REVIEWS

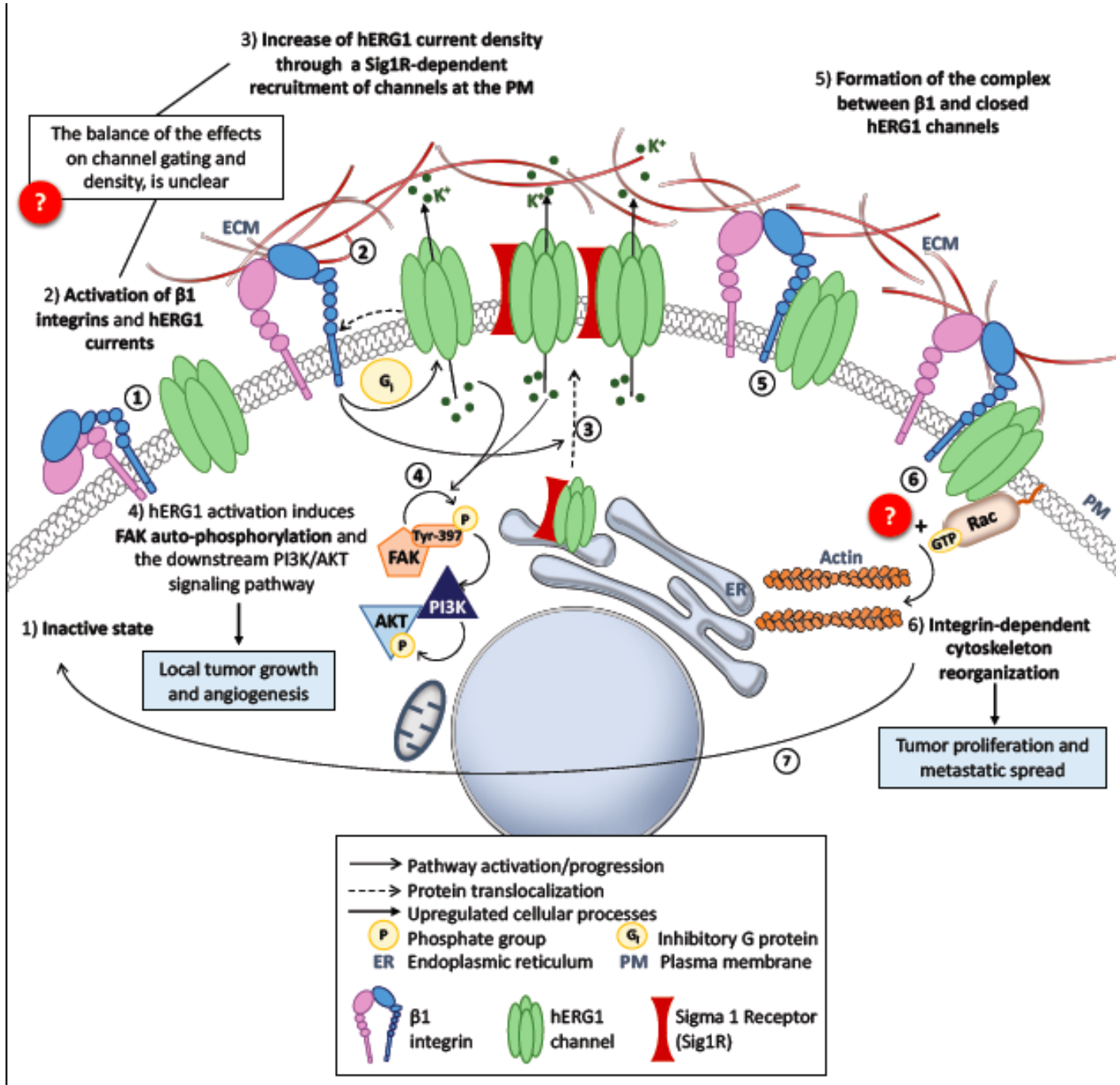
Opinion

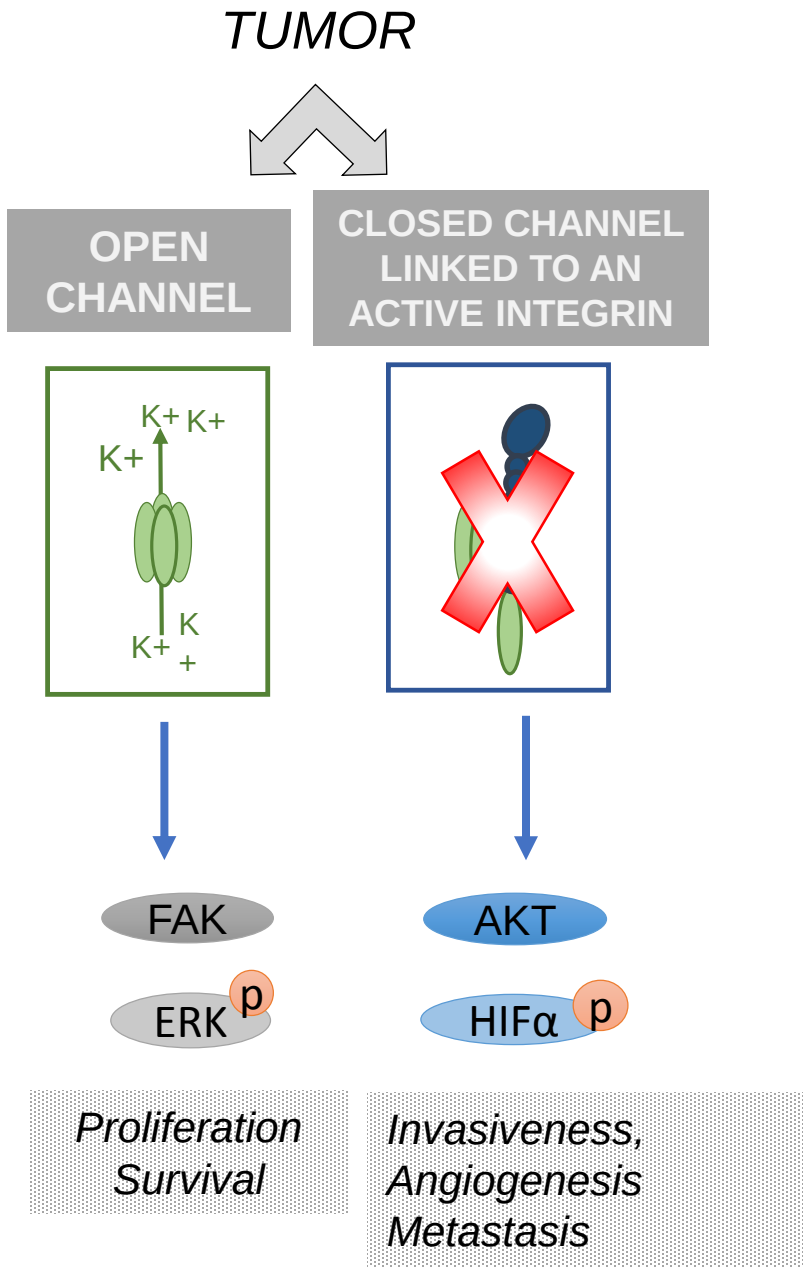
# Ion Channel Conformations Regulate Integrin-Dependent Signaling

Andrea Becchetti,[1,*] Giulia Petroni,[2] and Annarosa Arcangeli[2]

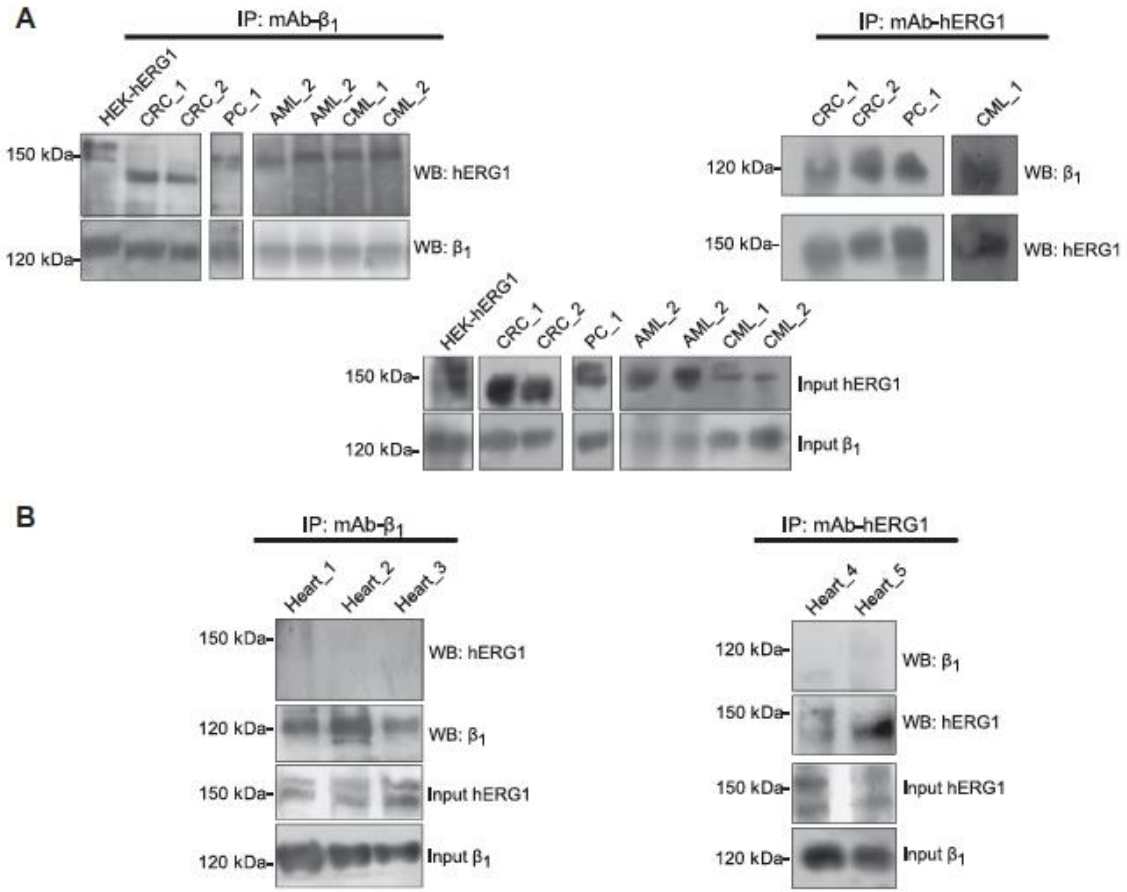


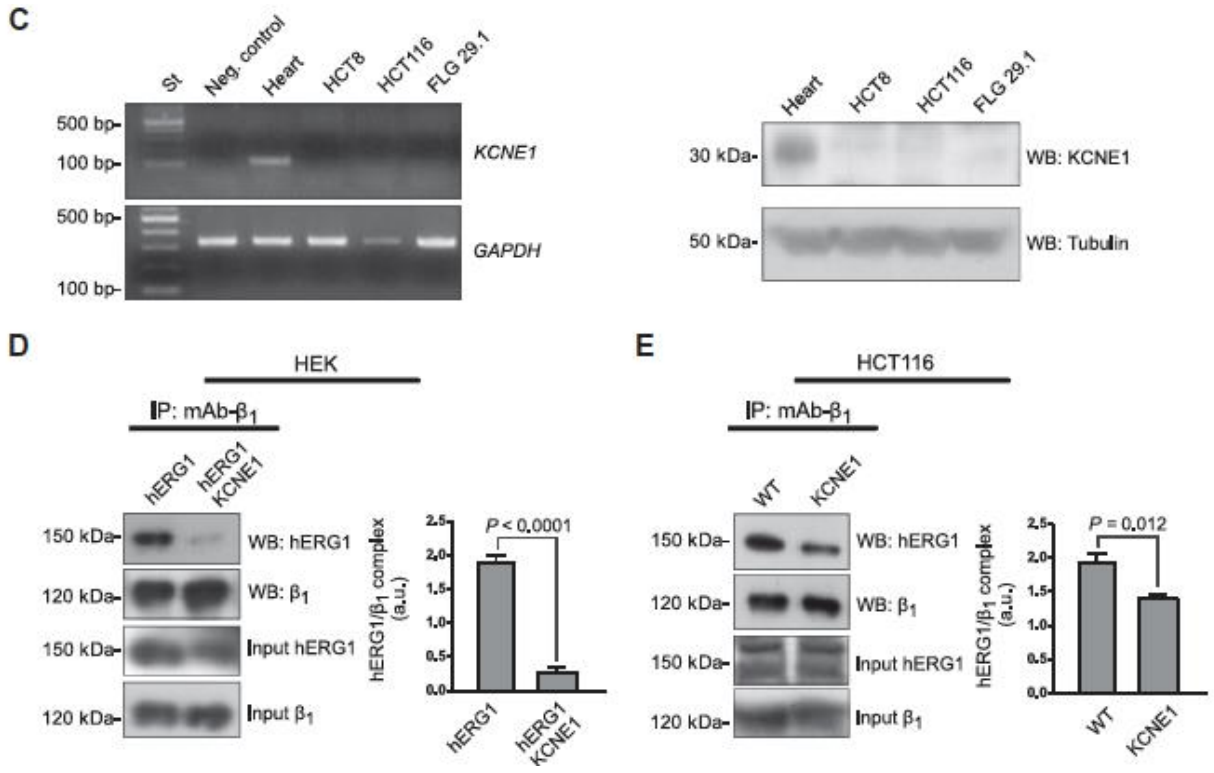

3) Increase of hERG1 current density through a Sig1R-dependent recruitment of channels at the PM
The balance of the effects on channel gating and density, is unclear
?
2) Activation of β1 integrins and hERG1 currents
5) Formation of the complex between β1 and closed hERG1 channels
ECM
ECM
K+
K+
K+
K+
1
2
3
4
5
6
7
Gi
4) hERG1 activation induces FAK auto-phosphorylation and the downstream PI3K/AKT signaling pathway
P
Tyr-397
FAK
PI3K
AKT
P
1) Inactive state
Local tumor growth and angiogenesis
ER
Actin
?
+
GTP
Rac
PM
6) Integrin-dependent cytoskeleton reorganization
Tumor proliferation and metastatic spread
Pathway activation/progression
Protein translocalization
Upregulated cellular processes
P Phosphate group
Gi Inhibitory G protein
ER Endoplasmic reticulum
PM Plasma membrane
β1 integrin
hERG1 channel
Sigma 1 Receptor (Sig1R)

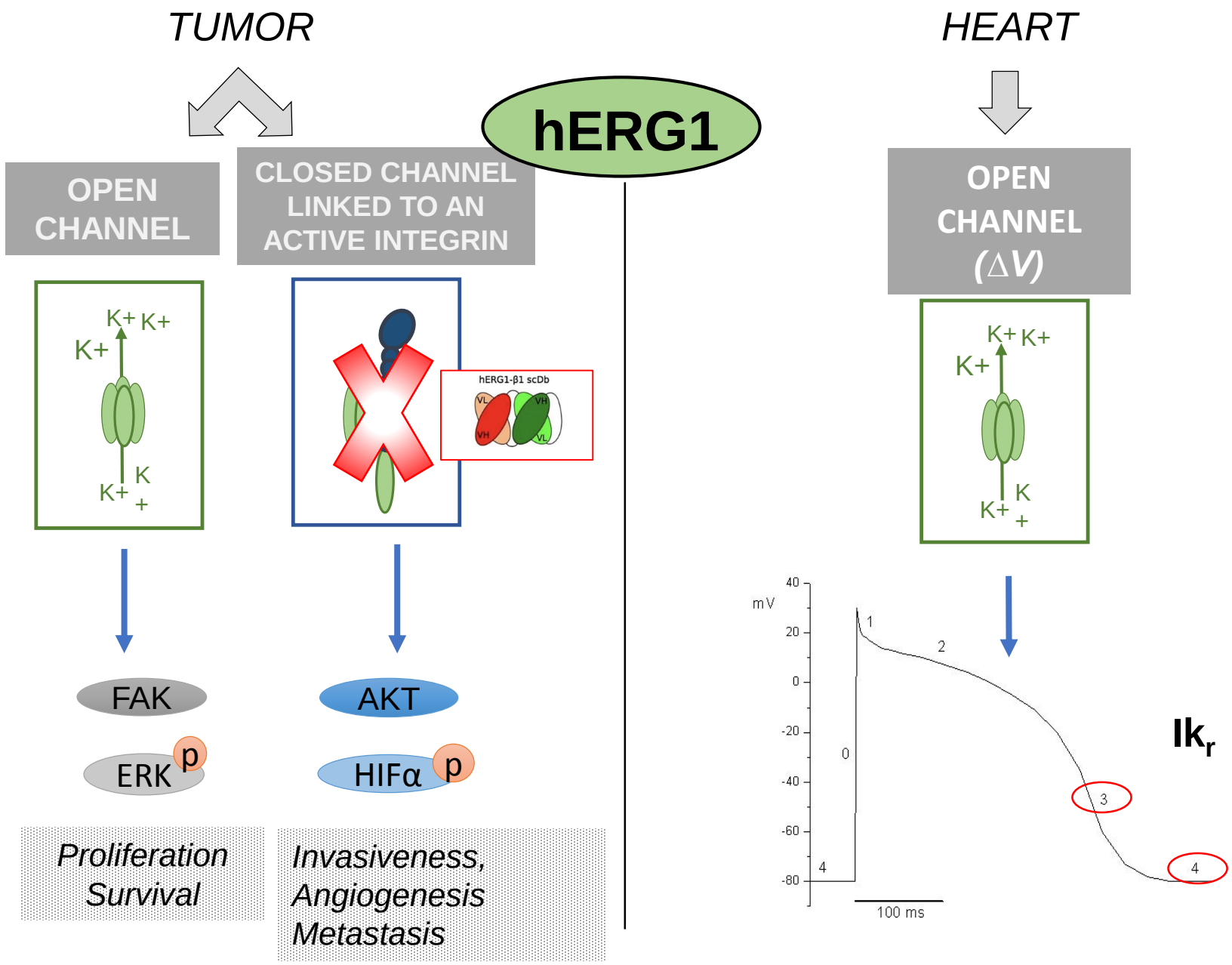

TUMOR
OPEN CHANNEL
CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN
K+ K+
K+
K+
K +
FAK
ERK p
AKT
HIFα p
Proliferation
Survival
Invasiveness,
Angiogenesis
Metastasis

# *The hERG1/β1 complex occurs in tumour cells, but not in the heart*



# … because tumour cells do not express “canonical” (KCNE1) beta subunits




TUMOR
HEART
hERG1
OPEN CHANNEL
CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN
OPEN CHANNEL (ΔV)
K+
hERG1-β1 scDb
VL
VH
FAK
ERK
p
AKT
HIFα
Proliferation
Survival
Invasiveness,
Angiogenesis
Metastasis
mV
40
20
0
-20
-40
-60
-80
100 ms
Ikr

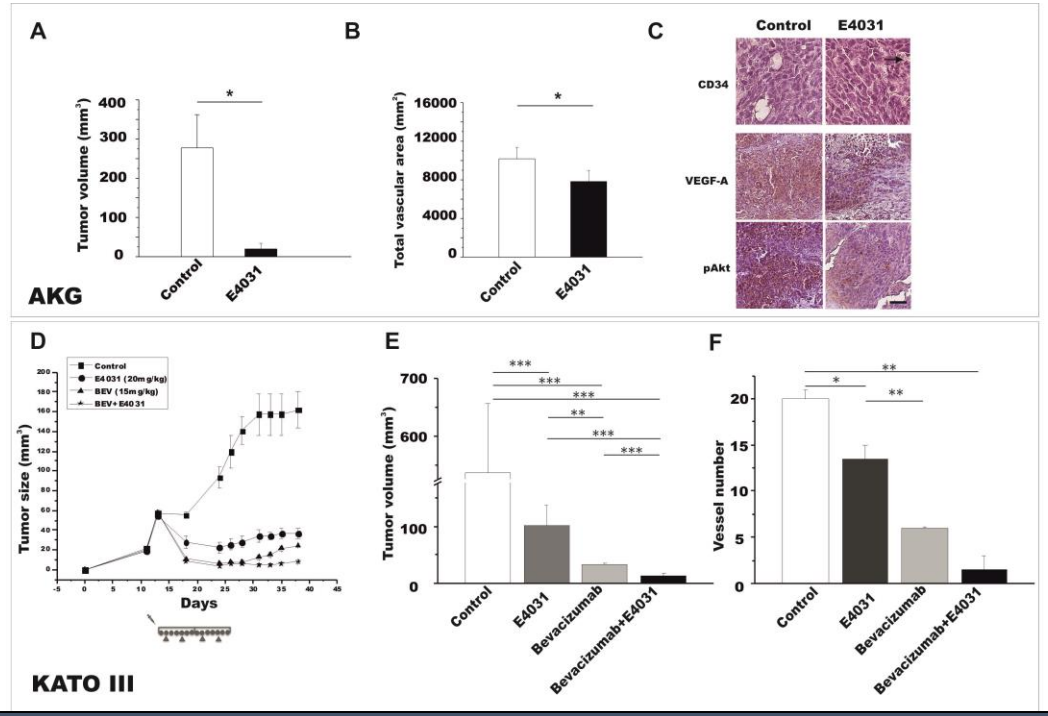
# Different physiological roles of hERG

- **Cardiac cells: regulates repolarization**
- **Other excitable cells: regulates $V_{rest}$ and firing**
- **Cancer cells: regulates slowly changing $V_{rest}$ and exerts non-conductive roles (e.g. molecular complexes with integrin receptors)**

# *Targeting hERG1 in cancer*

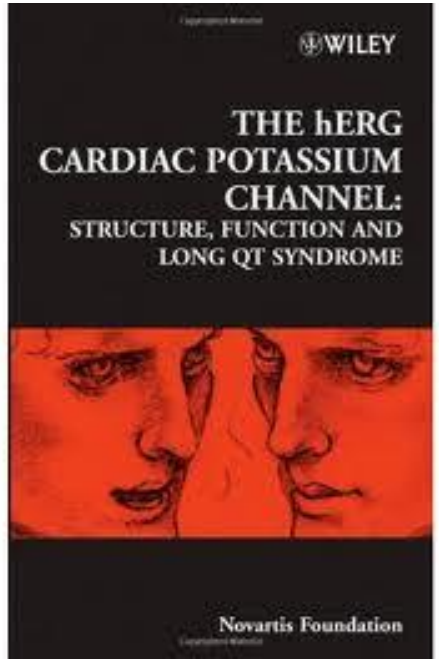
# *hERG1 in cancer*

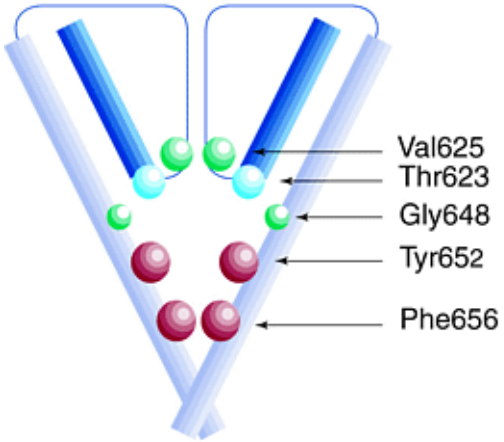
- Several preclinical data (mouse xenografts) indicate that blocking hERG1 inhibits tumour growth and metastatic spread




*Pillozzi et al., Blood, 2007;*
*Pillozzi et al., Blood ,2011;*
*Crociani et al., Sci.Rep.2013;*
*Crociani et al., Clin. Cancer Res., 2014;*
*Becchetti et al., Sci. Signal.,2017*

# hERG1 is considered an antitarget!
# hERG1 blockers can induce LQT syndrome and TdP




TRENDS in Pharmacological Sciences

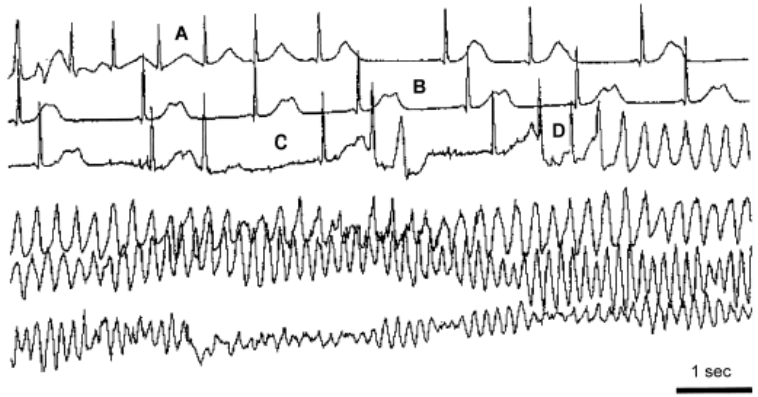



Fig. 2 Part of a continuous single-channel Holter recording from a patient with long QT syndrome. In this record, the characteristic prolonged QT interval (A), followed by giant late-repolarization 'T-wave humps' (B), led to premature beats with a bigeminal pattern with short-long-short sequences of R–R intervals (C) before onset of *torsade de pointes* (D). The episode progressed into ventricular fibrillation before spontaneously resolving into sinus rhythm; the patient was later treated successfully with pacing and beta-blockers (Reproduced with permission from Benhorin and Medina.[139])

# Strategies to target hERG1 in cancer

✓ *Use of non cardiotoxic hERG1 blockers*

✓ *Targeting the molecular differences between "tumour" and "cardiac" hERG1:*

CCR Translations

Clinical Cancer Research

**hERG Channels: From Antitargets to Novel Targets for Cancer Therapy**

Annarosa Arcangeli[1] and Andrea Becchetti[2]

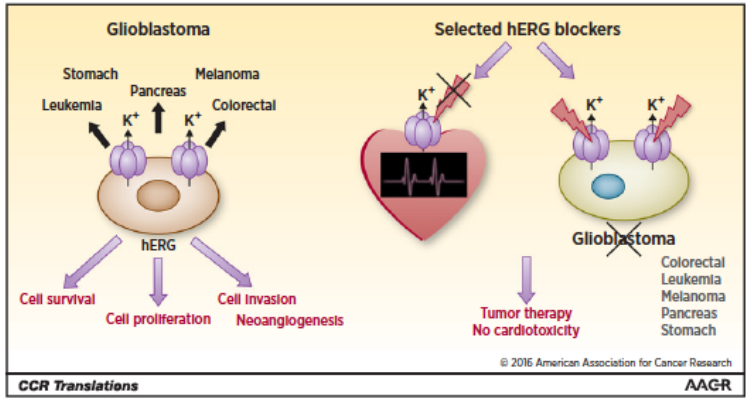



**Figure 1.**

Left, hERG is often overexpressed on the plasma membrane of different human cancer cells. It regulates tumor cell proliferation, survival, migration/invasiveness, and neoangiogenesis. Right, inhibiting hERG in different types of cancer cells (red lightning bolts) by using selective blockers that do not produce cardiac arrhythmia (as indicated by the black cross) is a possible strategy for anticancer therapy. The article by Pointer and colleagues (1) suggests that this is feasible in glioblastoma. Such a strategy may be effective in other cancers (shown in gray) in which hERG is overexpressed and has been shown to regulate neoplastic progression.

# *Strategies to target hERG1 in cancer*

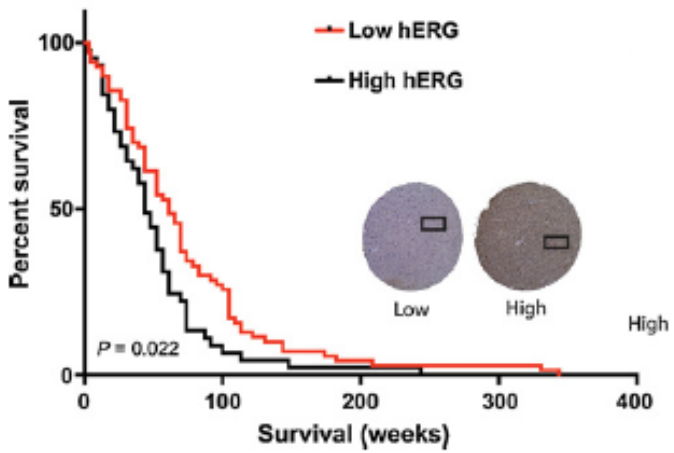
✓ *Use of non cardiotoxic (non torsadogenic) hERG1 blockers*

Personalized Medicine and Imaging

Clinical Cancer Research

# Administration of Non-Torsadogenic human Ether-à-go-go-Related Gene Inhibitors Is Associated with Better Survival for High hERG–Expressing Glioblastoma Patients

Kelli B. Pointer[1,2,3,4], Paul A. Clark[1], Kevin W. Eliceiri[4,5], M. Shahriar Salamat[6], Gail A. Robertson[7,8], and John S. Kuo[1,2,3,5,9]



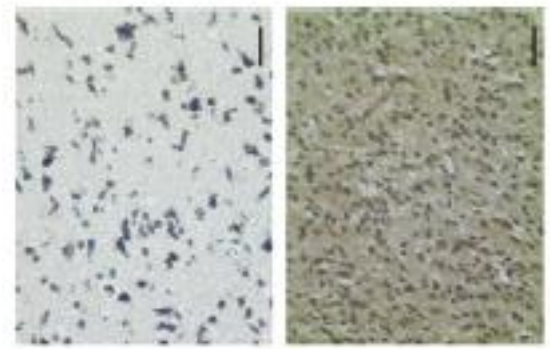



**Table 1.** Univariate and multivariate Cox regression analyses of glioblastoma hERG expression and confounding variables

| | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | *N* | Median survival, weeks (95% CI) | *P* (log-rank) | HR (95% CI) | *P* |
| hERG expression | | | 0.022 | 2.122 (1.247–3.610) | 0.003 |
| High | 45 | 43.5 (33.8–53.1) | | | |
| Low | 71 | 60.9 (48.2–73.6) | | | |
| Gender | | | 0.463 | | NS |
| Male | 81 | 52.2 (44.5–59.9) | | | |
| Female | 35 | 56.5 (34.5–78.5) | | | |
| Age | | | 0.957 | | NS |
| ≤55 | 51 | 52.2 (39.5–64.8) | | | |
| >55 | 58 | 52.2 (44.1–60.3) | | | |
| KPS | | | 0.024 | 1.143 (0.769–1.700) | 0.508 |
| ≤70 | 55 | 60.9 (48.3–73.5) | | | |
| >70 | 60 | 43.5 (29.7–57.3) | | | |
| Temozolomide | | | <0.001 | 2.845 (1.849–4.378) | <0.001 |
| Yes | 66 | 69.6 (61.6–77.6) | | | |
| No | 50 | 34.8 (27.3–42.3) | | | |
| Radiation | | | <0.001 | 2.122 (1.247–3.610) | 0.006 |
| Yes | 95 | 60.9 (9.7–25.1) | | | |
| No | 21 | 17.4 (49.6–72.2) | | | |
| Tobacco use | | | 0.519 | | NS |
| Yes | 46 | 52.2 (41.4–63.0) | | | |
| No | 70 | 52.2 (37.9–66.5) | | | |

Abbreviation: CI, confidence interval; NS, not significant.

Personalized Medicine and Imaging

Clinical Cancer Research

# Administration of Non-Torsadogenic human Ether-à-go-go-Related Gene Inhibitors Is Associated with Better Survival for High hERG–Expressing Glioblastoma Patients

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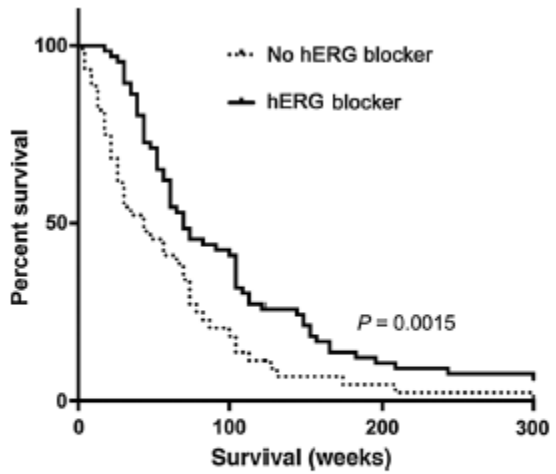



**Figure 4.**
Receipt of hERG blockers correlates with a better glioblastoma patient survival. Patients who received more than one hERG blocker were compared with patients who had not received hERG blockers, and there was a statistically significant difference in their survival ($P = 0.0015$).

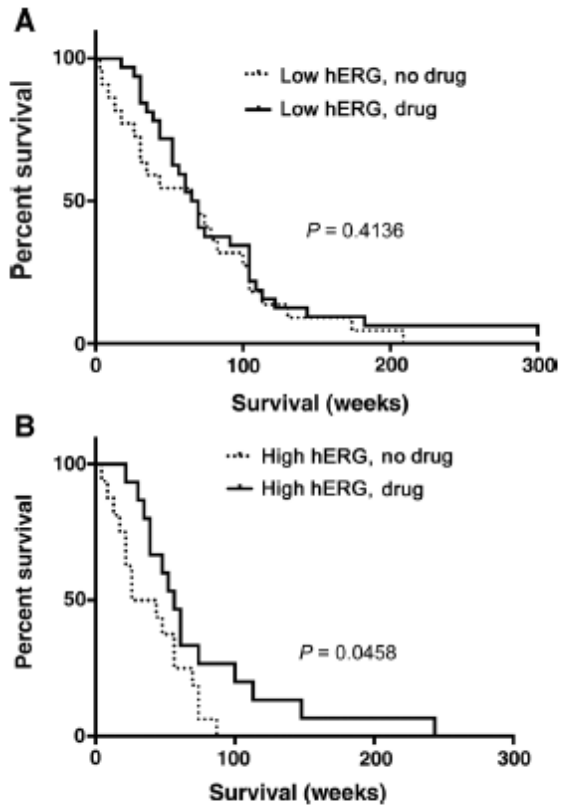
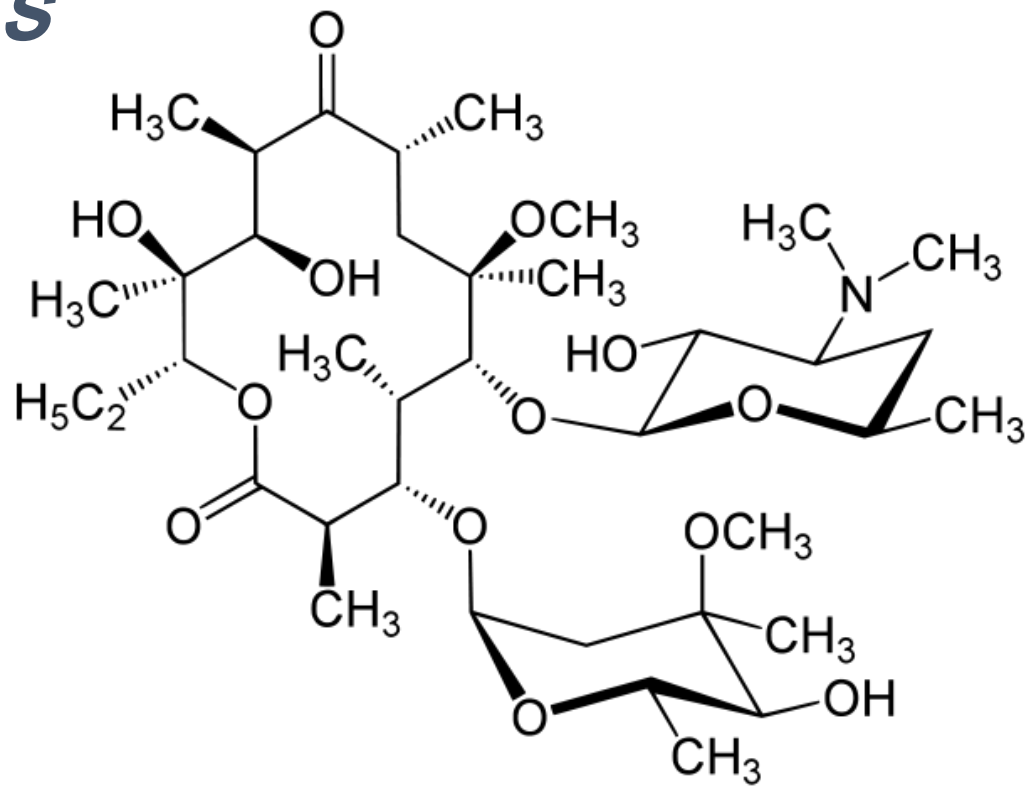



**Figure 5.**
Patient hERG expression levels correlate with the benefit of receiving an hERG. Patients were stratified based on whether or not they had high or low hERG expression levels. In each group, patients were further stratified on the basis of whether they had received an hERG blocker. **A,** No statistically significant difference was found in survival between patients who had low hERG expression levels ($P = 0.4136$). **B,** There was a statistically significant difference in survival for patients with high hERG expression levels based on whether they received an hERG blocker ($P = 0.0458$).

# Strategies to target hERG1 in cancer

## ✓ Use of non cardiotoxic (non torsadogenic) hERG1 blockers




Clarythromycin

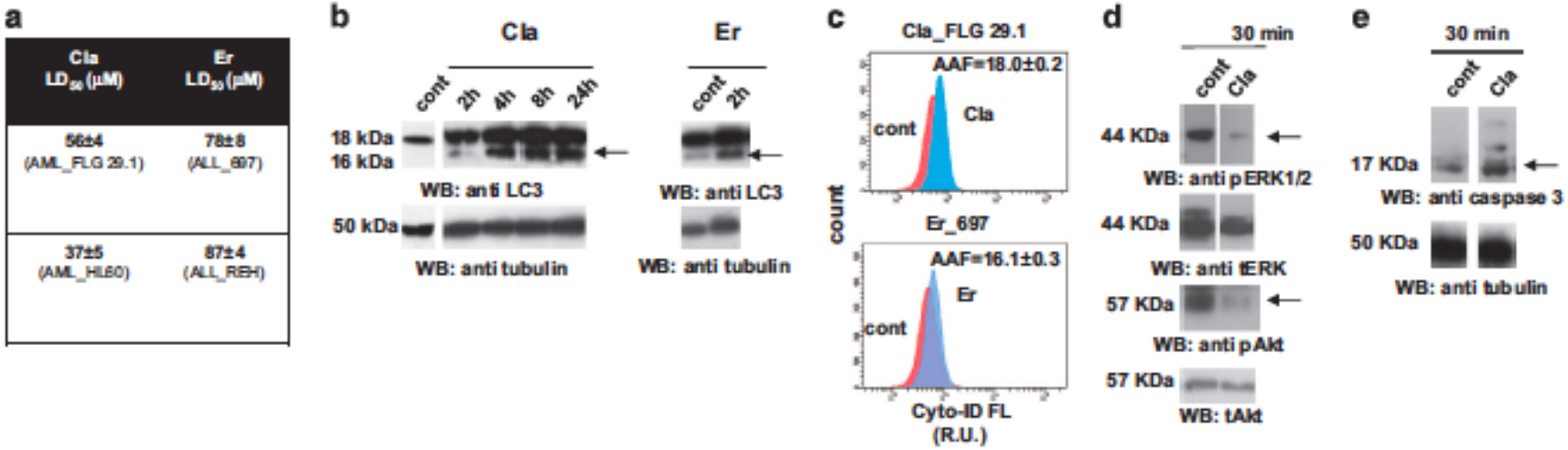
OPEN

Citation: Blood Cancer Journal (2016) 6, e423; doi:10.1038/bcj.2016.32

www.nature.com/bcj

LETTER TO THE EDITOR

# Macrolide antibiotics exert antileukemic effects by modulating the autophagic flux through inhibition of hERG1 potassium channels



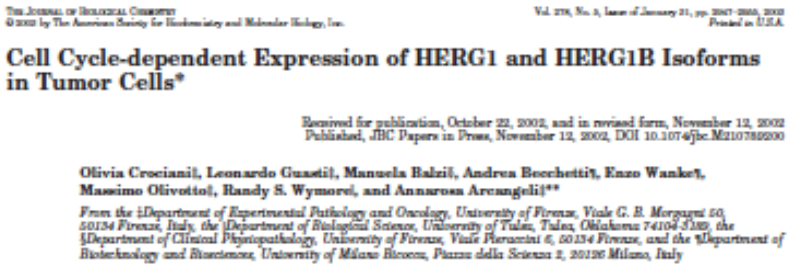
# *Strategies to target hERG1 in cancer*

✓ *Use of non cardiotoxic (non torsadogenic) hERG1 blockers*

✓ ***Targeting the molecular differences between "tumour" and "cardiac" hERG1:***

✓ *Preferential expression of hERG1B in leukemias (CD160130)*

# High hERG1B expression in leukemias



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**Cell Cycle-dependent Expression of HERG1 and HERG1B Isoforms in Tumor Cells***

Received for publication, October 22, 2002, and in revised form, November 12, 2002
Published, JBC Papers in Press, November 12, 2002, DOI 10.1074/jbc.M210788200

**Olivia Crociani‡, Leonardo Guasti‡, Manuela Balzi‡, Andrea Becchetti¶, Enzo Wanke¶, Massimo Olivotto‖, Randy S. Wymore§, and Annarosa Arcangeli‡****

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2003

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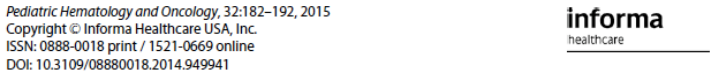
Leukemia (2014), 1–4

www.nature.com/leu

LETTER TO THE EDITOR

Differential expression of *hERG1A* and *hERG1B* genes in pediatric acute lymphoblastic leukemia identifies different prognostic subgroups

2014



*Pediatric Hematology and Oncology*, 32:182–192, 2015

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.3109/08880018.2014.949941

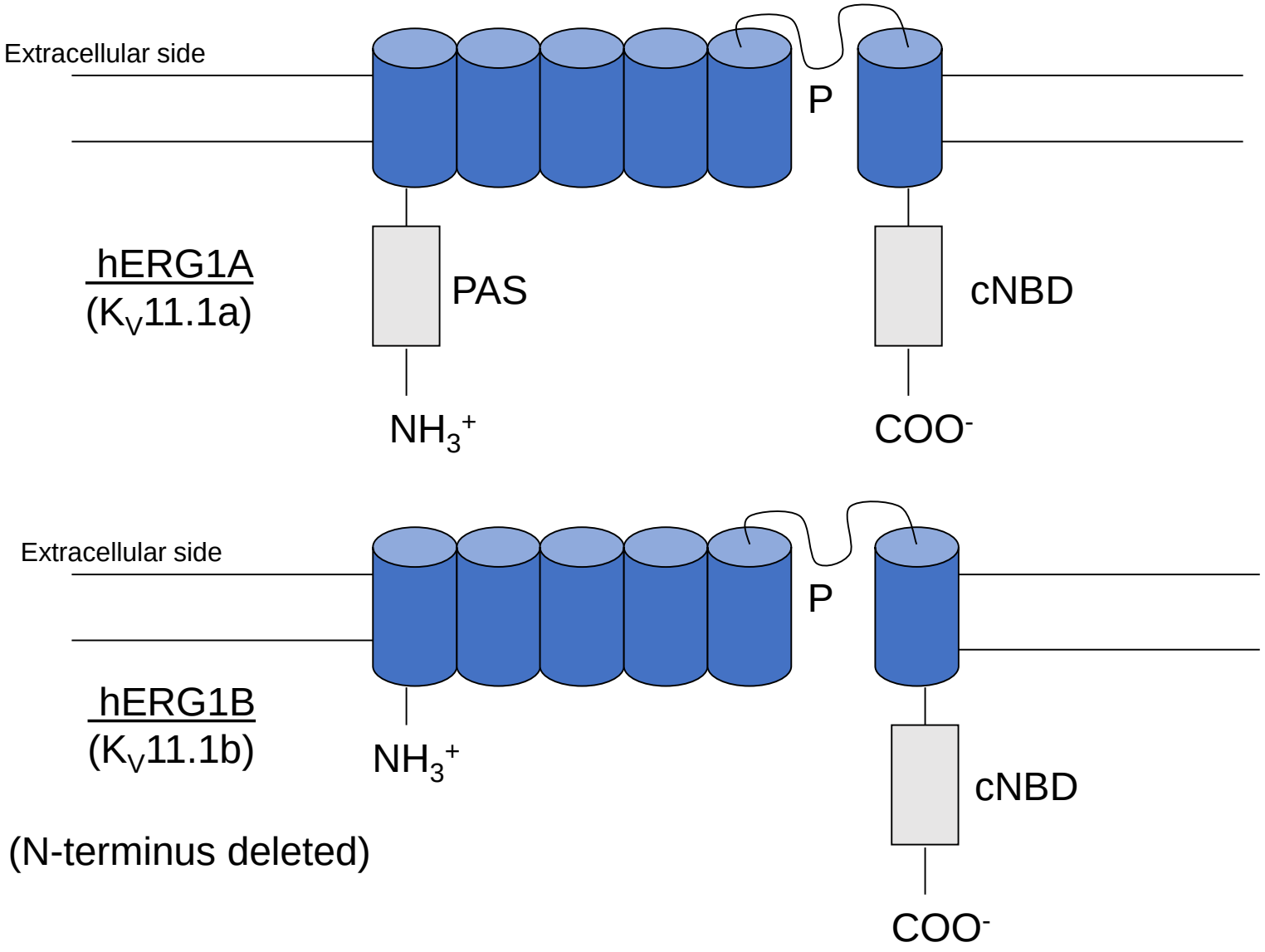
informa
healthcare

ORIGINAL ARTICLE

***herg1b* Expression as a Potential Specific Marker in Pediatric Acute Myeloid Leukemia Patients with HERG 897K/K Genotype**

Merve Erdem,[1,*] Tugce Ayca Tekiner,[1,2] Arta Fejzullahu,[1,3] Gokce Akan,[4] Sema Anak,[5] Ebru Tugrul Saribeyoglu,[5] Ugur Ozbek,[6] and Fatmahan Atalar[1]

2015


Extracellular side
P
hERG1A
($K_V$11.1a)
PAS
cNBD
$NH_3^+$
$COO^-$
Extracellular side
P
hERG1B
($K_V$11.1b)
$NH_3^+$
cNBD
$COO^-$
(N-terminus deleted)

1521-0111/87/2/183–196$25.00
MOLECULAR PHARMACOLOGY

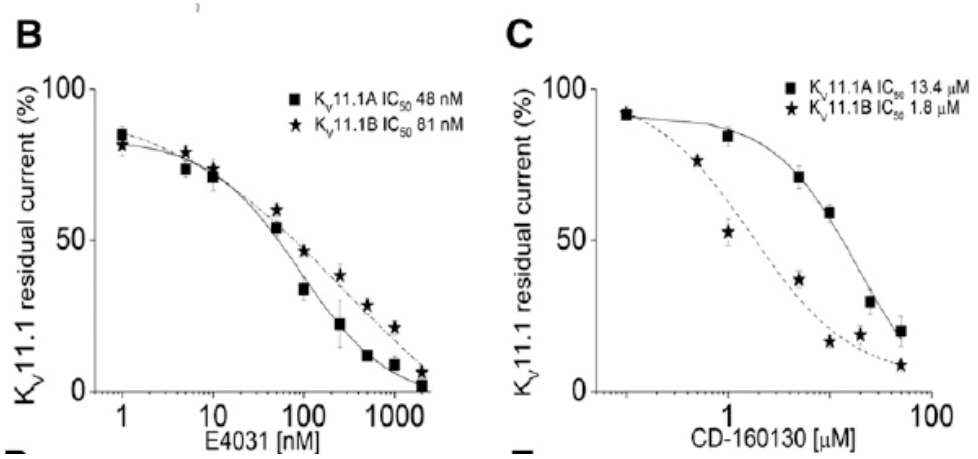
http://dx.doi.org/10.1124/mol.114.094920
Mol Pharmacol 87:183–196, February 2015

# New Pyrimido-Indole Compound CD-160130 Preferentially Inhibits the $K_V$11.1B Isoform and Produces Antileukemic Effects without Cardiotoxicity[S]

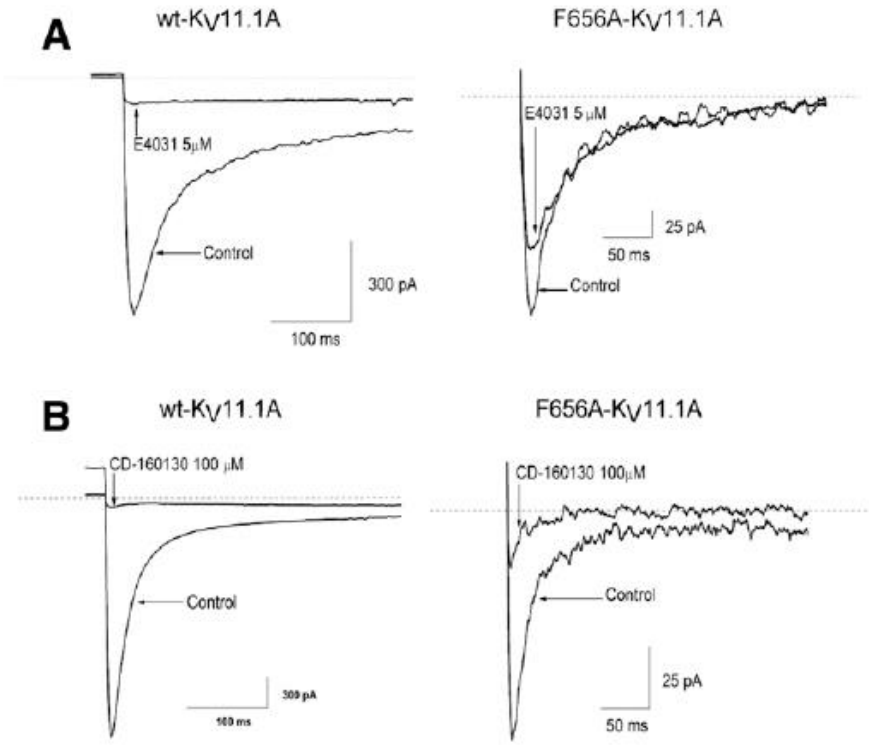
Luca Gasparoli, Massimo D'Amico, Marika Masselli, Serena Pillozzi, Rachel Caves, Rawan Khuwaileh, Wolfgang Tiedke, Kenneth Mugridge, Alessandro Pratesi, John S. Mitcheson, Giuseppe Basso, Andrea Becchetti, and Annarosa Arcangeli

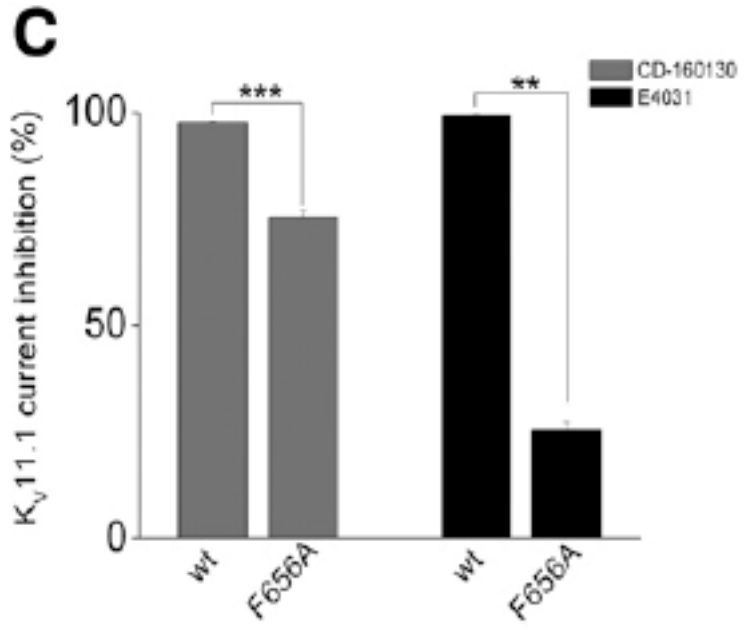
*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy (L.G., S.P., A.A.); Department of Chemistry "Ugo Schiff," University of Florence, Florence, Italy (M.M., A.P.); DI.V.A.L. Toscana srl, Sesto Fiorentino, Italy (M.D.A., M.M.); Department of Cell Physiology and Pharmacology, University of Leicester, Leicester, United Kingdom (R.C., R.K., J.S.M.); BlackSwan Pharma GmbH, Leipzig, Germany (W.T., K.M.); Oncohematology Laboratory, Department of Woman and Child Health, University of Padova, Padova, Italy (G.B.); and Department of Biotechnologies and Biosciences, University of Milano-Bicocca, Milan, Italy (A.B.)*

Received July 22, 2014; accepted November 19, 2014

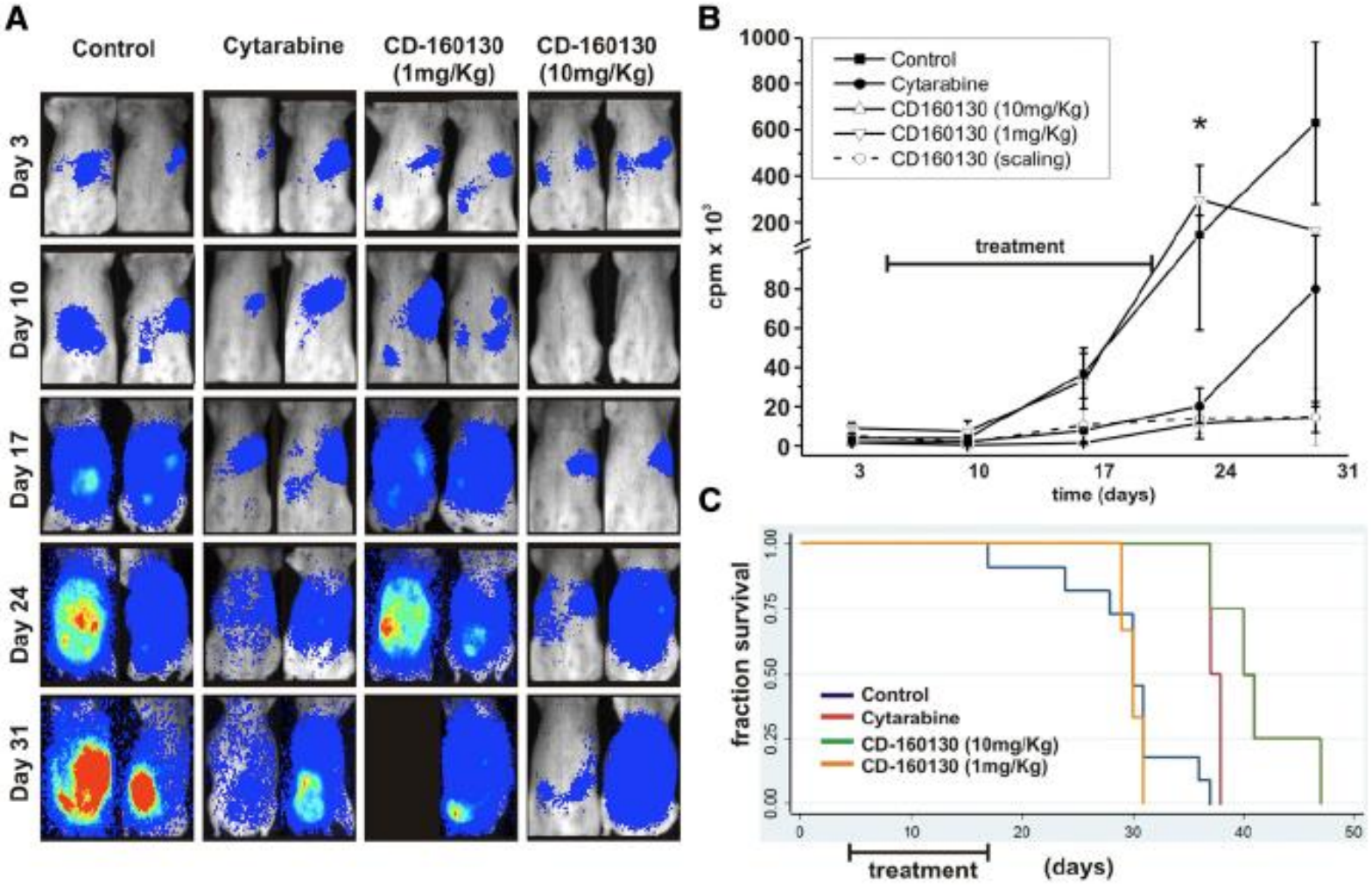


# *CD 160130 does not bind the F656 “canonical” binding site in hERG1*



# CD 160130 hampers leukemia burden in vivo



# *…without lengthening the QT interval (in guinea pigs)*

**B**

**ECG parameters**

| CD-160130 (n=5) 10mg/kg | QT ± SEM (ms) | HR ± SEM (beat/min) | QTc ± SEM | ΔQTc (% vs Pre-drug) |
|---|---|---|---|---|
| Pre-drug | 129.0 ± 4.2 | 237.1 ± 3.4 | 305.1 ± 7.5 | - |
| 0 min | 131.4 ± 5.4 | 241.1 ± 5.2 | 308.4 ± 7.4 | 1.1 ± 3.5 |
| 5 min | 127.5 ± 7.0 | 249.1 ± 1.1 | 298.8 ± 12.9 | -2.1 ± 4.9 |
| 10 min | 129.0 ± 5.8 | 240.5 ± 3.4 | 300.9 ± 11.2 | -1.4 ± 4.4 |
| 15 min | 128.0 ± 6.7 | 240.5 ± 2.8 | 298.6 ± 11.8 | -2.2 ± 4.6 |
| Sotalol (n=5) 3mg/kg | QT ± SEM (ms) | HR ± SEM (beat/min) | QTc ± SEM | ΔQTc (% vs Pre-drug) |
| Pre-drug | 115.4 ± 3.6 | 240.3 ± 3.7 | 276.4 ± 6.8 | - |
| 0 min | 115.4 ± 3.8 | 240.4 ± 5.1 | 273.7 ± 6.7 | -1.0 ± 3.4 |
| 5 min | 132.7 ± 4.0 | 240.4 ± 1.2 | 301.1 ± 6.2 | 8.9 ± 3.5 |
| 10 min | 135.3 ± 4.2 | 235.0 ± 3.1 | 304.3 ± 6.1 | 10.1 ± 3.5 |
| 15 min | 133.0 ± 5.8 | 232.2 ± 4.5 | 299.5 ± 8.0 | 8.4 ± 3.9 |

**CD-160130**

# Strategies to target hERG1 in cancer

✓ *Use of non cardiotoxic (non torsadogenic) hERG1 blockers*

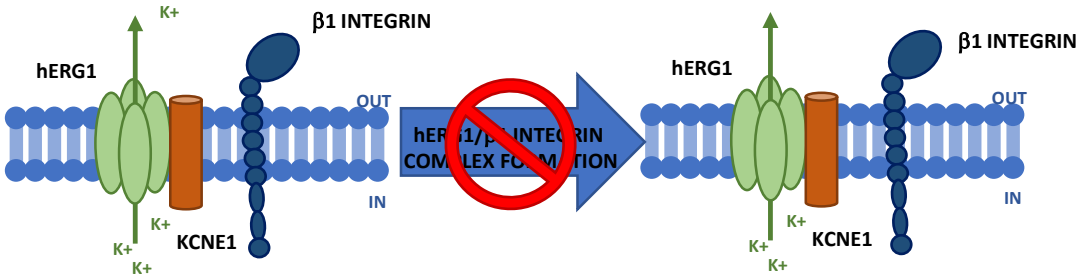
✓ ***Targeting the molecular differences between "tumour" and "cardiac" hERG1:***

✓ *Preferential expression of hERG1B in leukemias (CD160130)*

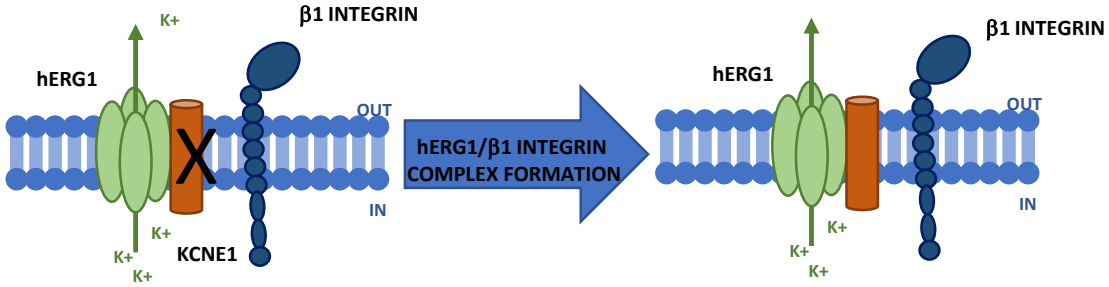
✓ *Formation of a hERG1/β1 integrin complex in tumour cells*

# hERG1 and β1 integrin associate in human cancer tissue but not cardiac tissue.

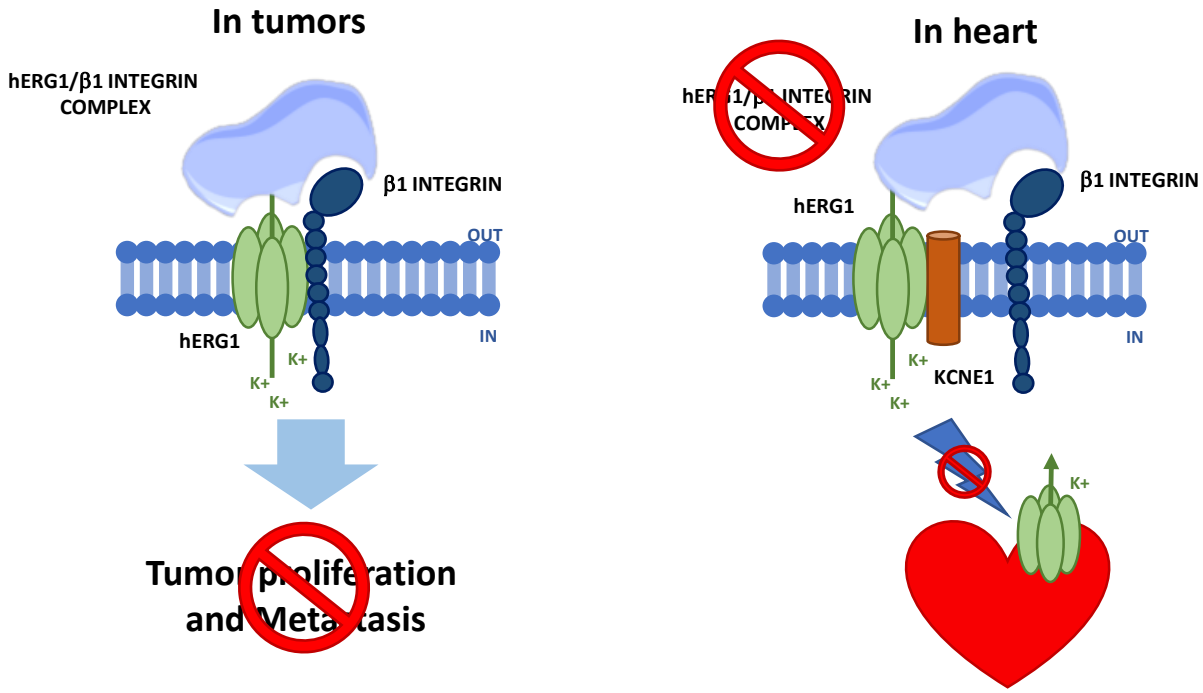
**In heart**

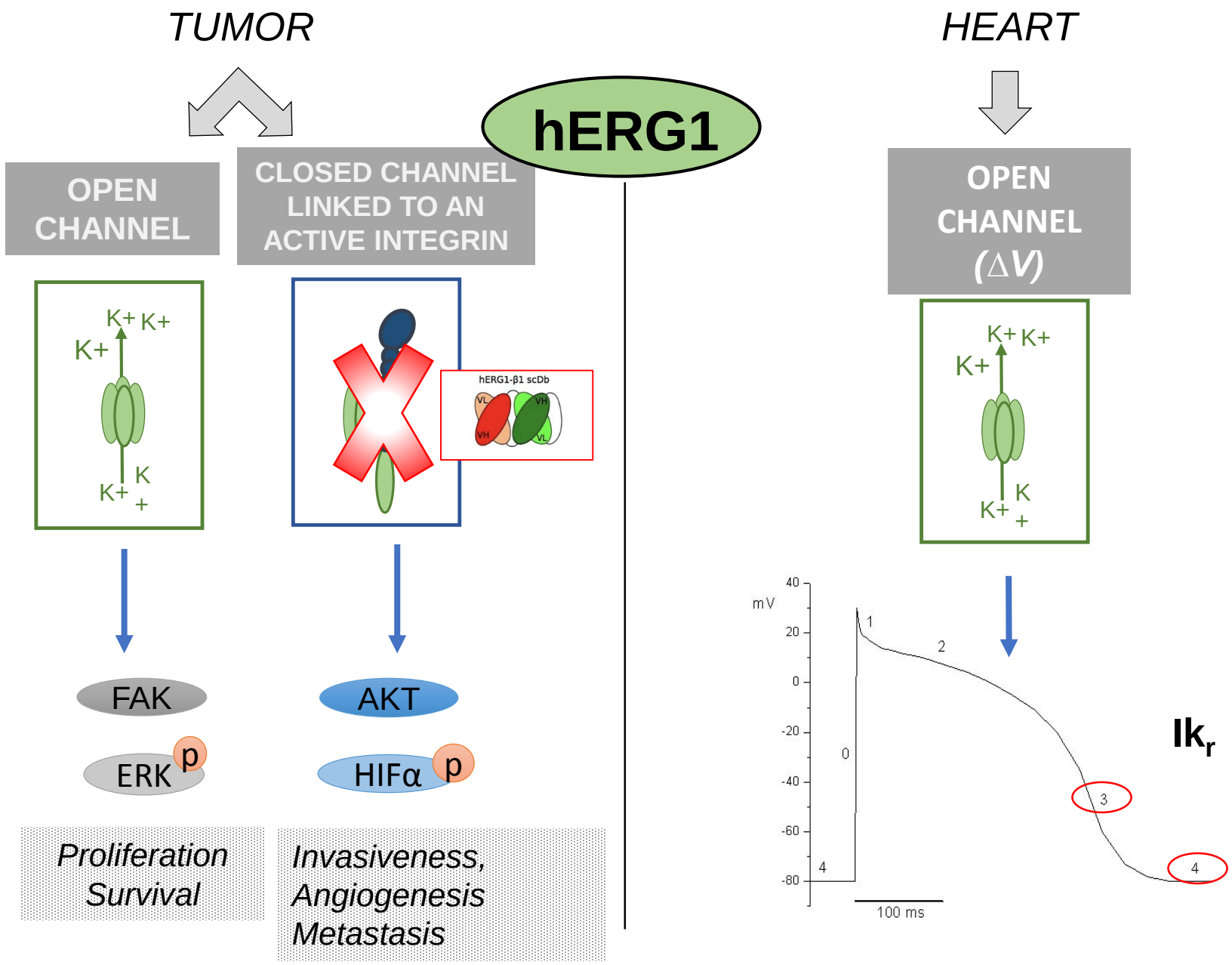



**In tumors**



# Future …. Blockade of the hERG1/β1 integrin complex

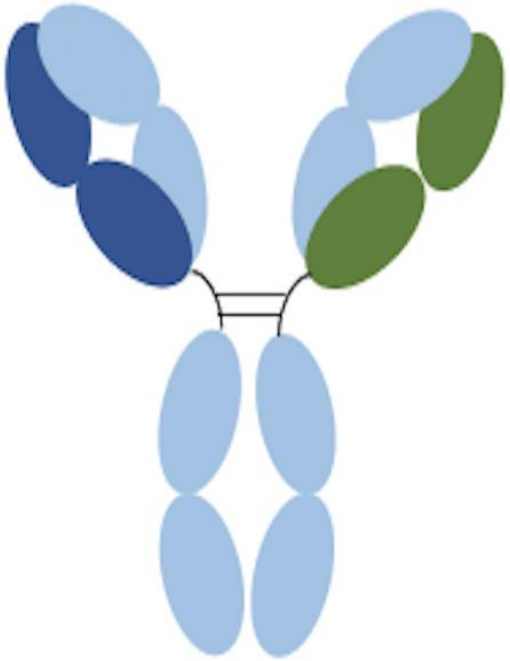



TUMOR
HEART
hERG1
OPEN CHANNEL
CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN
OPEN CHANNEL (ΔV)
K+
hERG1-β1 scDb
VL
VH
FAK
ERK
p
AKT
HIFα
Proliferation
Survival
Invasiveness,
Angiogenesis
Metastasis
mV
40
20
0
-20
-40
-60
-80
100 ms
Ikr

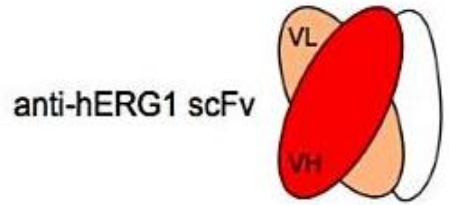
# PIPELINE DEVELOPMENT STRATEGY

## DESIGNING NEXT GENERATION ANTIBODY MOLECULES FROM BIG MONOCLONAL ANTIBODY TO SMALL BISPECIFIC DIABODY

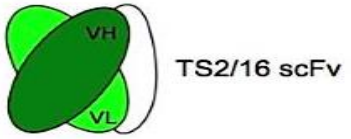
MCK THERAPEUTICS



***Anti-hERG1 Monoclonal Antibody (M.W. 160 kDa)***

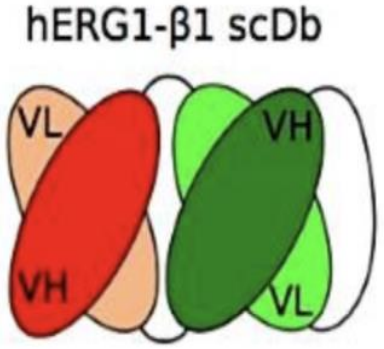



***Anti-hERG1 Single-Chain Variable Fragment (Sc-FV)***




***Anti-integrin beta1 Single-Chain Variable –Fragment (Sc-fV)***

***Anti hERG1/integrin beta1 BISPECIFIC Antibody (DIABODY) (M.W. 50 kDa)***

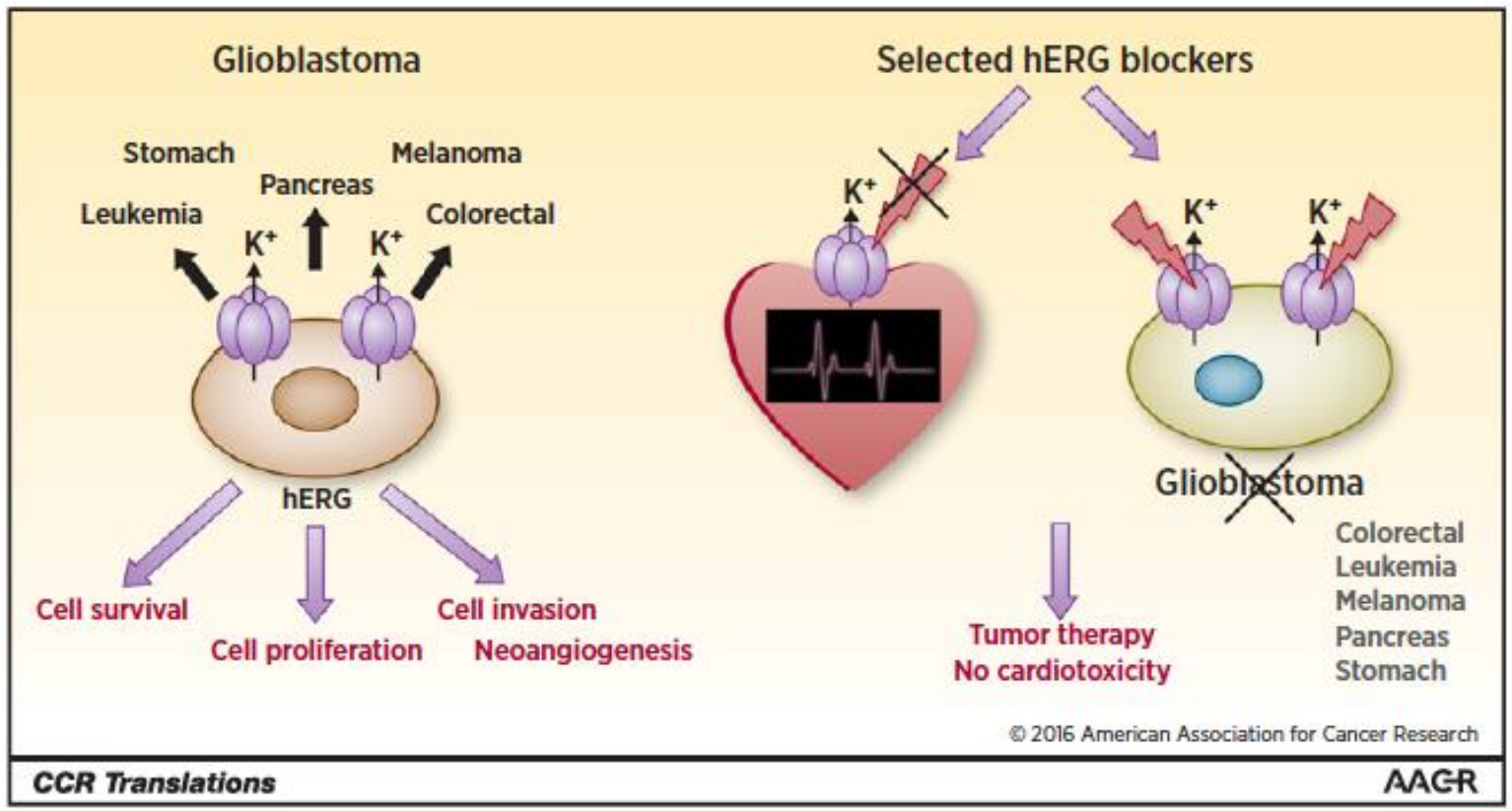



**THERAPEUTIC ANTIBODY**
**MCKAA2017THE01**

Clinical Cancer Research

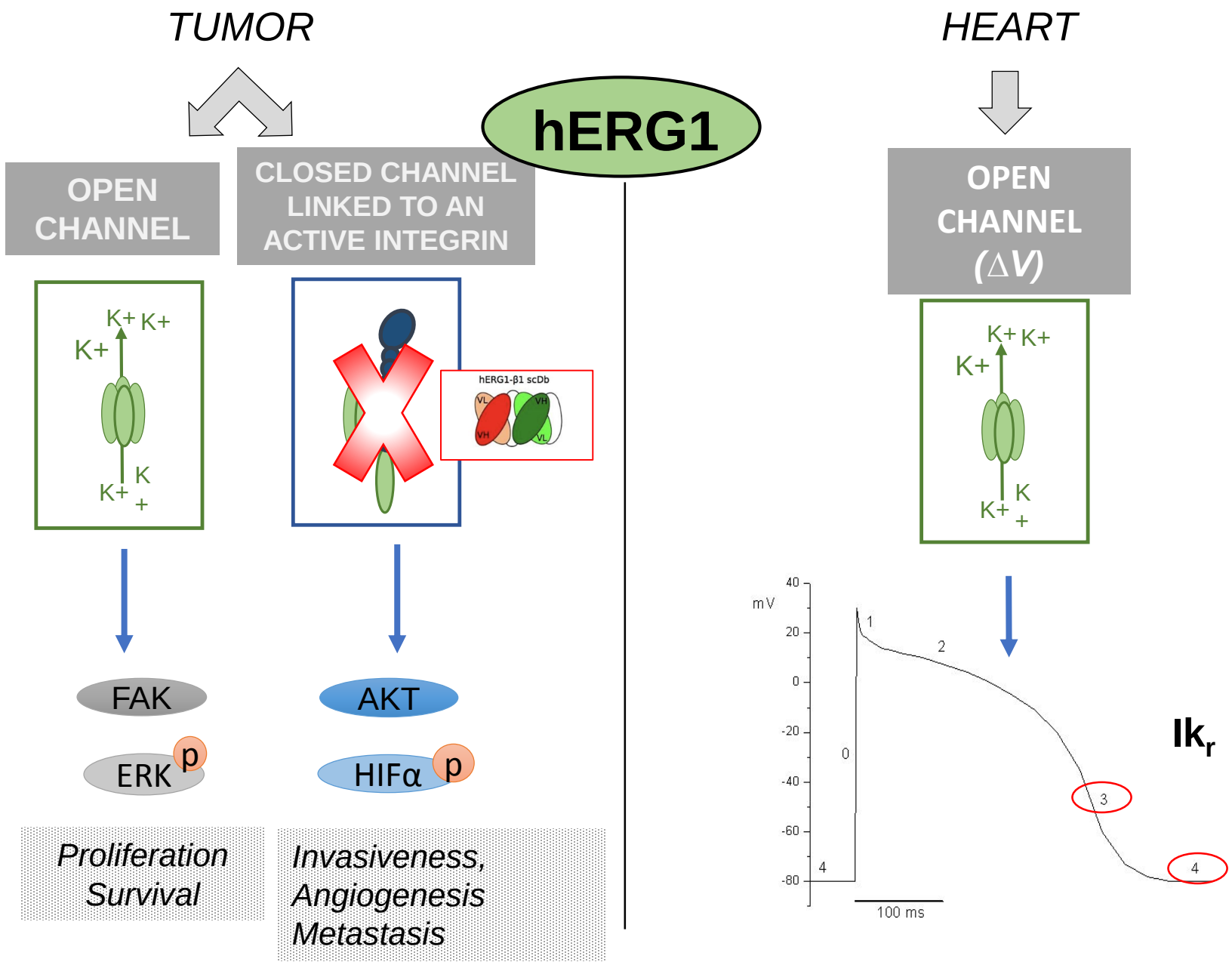
# hERG Channels: From Antitargets to Novel Targets for Cancer Therapy

Annarosa Arcangeli[1] and Andrea Becchetti[2]



# CONCLUSIONS

- Ion channels are relevant in cancer (biomarkers!)
- Ion channels can exert both conductive and non conductive effects in cancer cells
- hERG1 has both conductive (ion flux-mediated) and non conductive (once bound to integrin receptors in the closed state) activities
- hERG1 mediates tumour progression (e.g. proliferation, invasion, angiogenesis, metastasis….)
- hERG1 can be considered a novel cancer biomarker
- The hERG1/beta1 integrin complex can be considered a therapeutic target in cancer and can be targeted through newly developed bispecific antibodies


TUMOR
HEART
hERG1
OPEN CHANNEL
CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN
OPEN CHANNEL (ΔV)
K+
hERG1-β1 scDb
VL
VH
FAK
ERK
p
AKT
HIFα
Proliferation
Survival
Invasiveness,
Angiogenesis
Metastasis
mV
40
20
0
-20
-40
-60
-80
100 ms
Ikr

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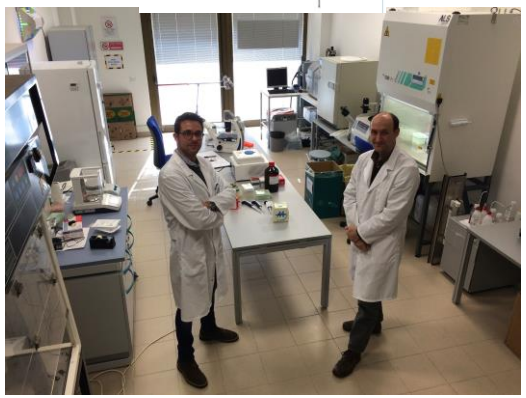
University of Milano Bicocca, Italy

Prof. F. Di Costanzo
Dept. Medical Oncology
AOUC Firenze

Prof. R. Coppola
Dept. General Surgery
Campus Biomedico Rome



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Con la ricerca, contro il cancro

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# TARGETING POTASSIUM CHANNELS TO OVERCOME CHEMORESISTANCE IN CRC

# (1) OVERCOMING CISPLATIN RESISTANCE BY TARGETING Kv11.1 AND KCa 3.1 CHANNELS

# The networking of Potassium channels

FULL PAPER

BJC

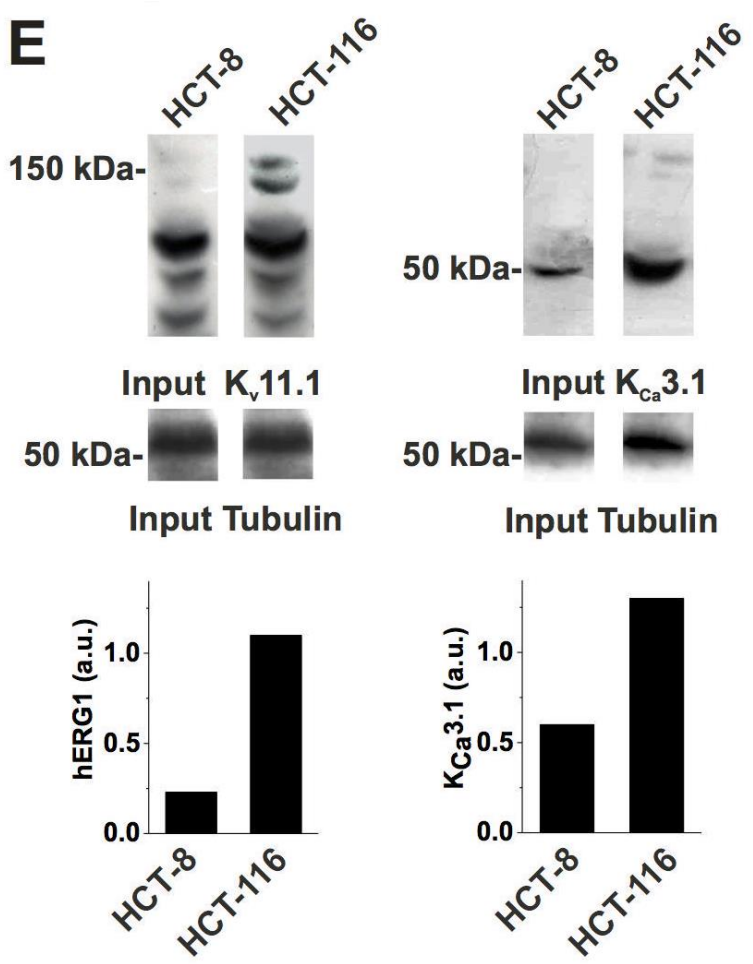
British Journal of Cancer (2017), 1–13 | doi: 10.1038/bjc.2017.392

Keywords: Riluzole; SKA-31; E4031; Cisplatin uptake; preclinical mouse models

## The combined activation of $K_{Ca}3.1$ and inhibition of $K_v11.1$/hERG1 currents contribute to overcome Cisplatin resistance in colorectal cancer cells

Serena Pillozzi[1,9], Massimo D'Amico[2,9], Gianluca Bartoli[1], Luca Gasparoli[1], Giulia Petroni[1], Olivia Crociani[1], Tiziano Marzo[3,4], Angela Guerriero[1], Luigi Messori[3], Mirko Severi[5], Roberto Udisti[5], Heike Wulff[6], K George Chandy[7], Andrea Becchetti[8] and Annarosa Arcangeli[*,1]

# Cisplatin-resistant CRC cells (HCT116) express high levels of $K_{Ca}3.1$ and hERG1 channels



**In resistant cells, KCa3.1 activators (SKA-31) and Kv11.1 inhibitors (E4031) had a synergistic action with Cisplatin in triggering apoptosis and inhibiting proliferation.**

**Table 1B. Combination index and percentage (%) of apoptotic HCT-116 cells after different treatment combinations**

| | | | Apoptosis | |
|---|---|---|---|---|
| Drug (concentration μM) | Combination index at $IC_{50}$ | Effect | Early apoptotic cells (%) | Late apoptotic cells (%) |
| Cisplatin (25) | — | | 5.9 ± 1.0 | 5.3 ± 1.5 |
| Cisplatin (25) + Riluzole (10) | 0.70 ± 0.08 | S | 10.6 ± 1.3 $P = 0.021$ | 17.6 ± 3.3 $P = 0.016$ |
| Cisplatin (25) + SKA-31 (5) | 0.64 ± 0.11 | S | 12.5 ± 3.9 | 10.1 ± 2.4 |
| Cisplatin (25) + TRAM-34 (25) | 2.66 ± 0.78 | A | 13.8 ± 3.6 $P = 0.016$ | 8.7 ± 1.6 |
| Cisplatin (25) + E4031 (7) | 0.68 ± 0.07 | S | 8.0 ± 0.3 | 13.2 ± 3.4 $P = 0.042$ |
| Cisplatin (25) + Riluzole (10) + E4031 (7) | 0.47 ± 0.05 | S | ND | ND |
| Cisplatin (25) + SKA-31 (5) + E4031 (7) | 0.69 ± 0.14 | S | ND | ND |
| Oxaliplatin (60) + Riluzole (10) | 0.98 ± 0.01 | S | ND | ND |
| Oxaliplatin (60) + SKA-31 (5) | 0.71 ± 0.05 | S | ND | ND |
| Oxaliplatin (60) + TRAM-34 (25) | 3.36 ± 0.34 | A | ND | ND |
| Oxaliplatin (60) + E4031 (7) | 0.83 ± 0.01 | S | ND | ND |

Abbreviation: ND = not determined. CI > 1, antagonism (A); CI = 1, additivity (Ad); CI < 1, synergy (S). HCT-116 cells were exposed to Cisplatin or Oxaliplatin in combination with Riluzole, SKA-31, TRAM-34 and E4031 for 24 h as described in Pillozzi et al, 2011. All the drugs were used at drug concentrations indicated in the first column. Data are means ± s.e.m. of three independent experiments, each carried out in triplicate. CI values were calculated using the Calcusyn software Version 2 (Biosoft). For statistical analysis, Student's t-test was applied.

# Cisplatin uptake into resistant cells depend on $K_{Ca}3.1$ channel activity, and is potentiated by either $K_{Ca}3.1$ activators or hERG1 inhibitors

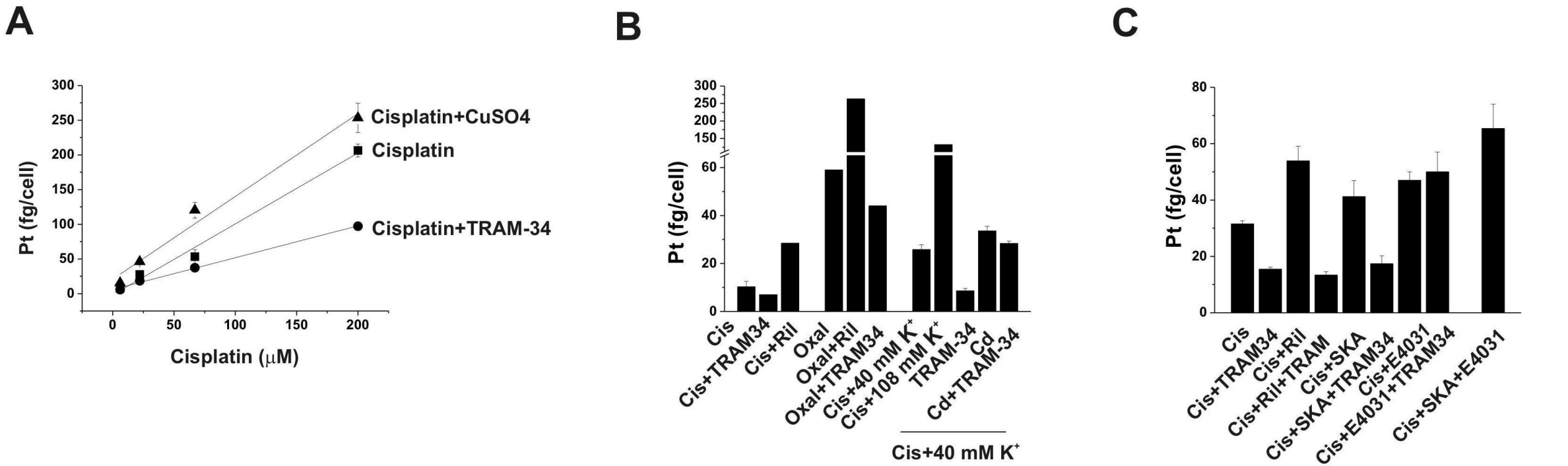


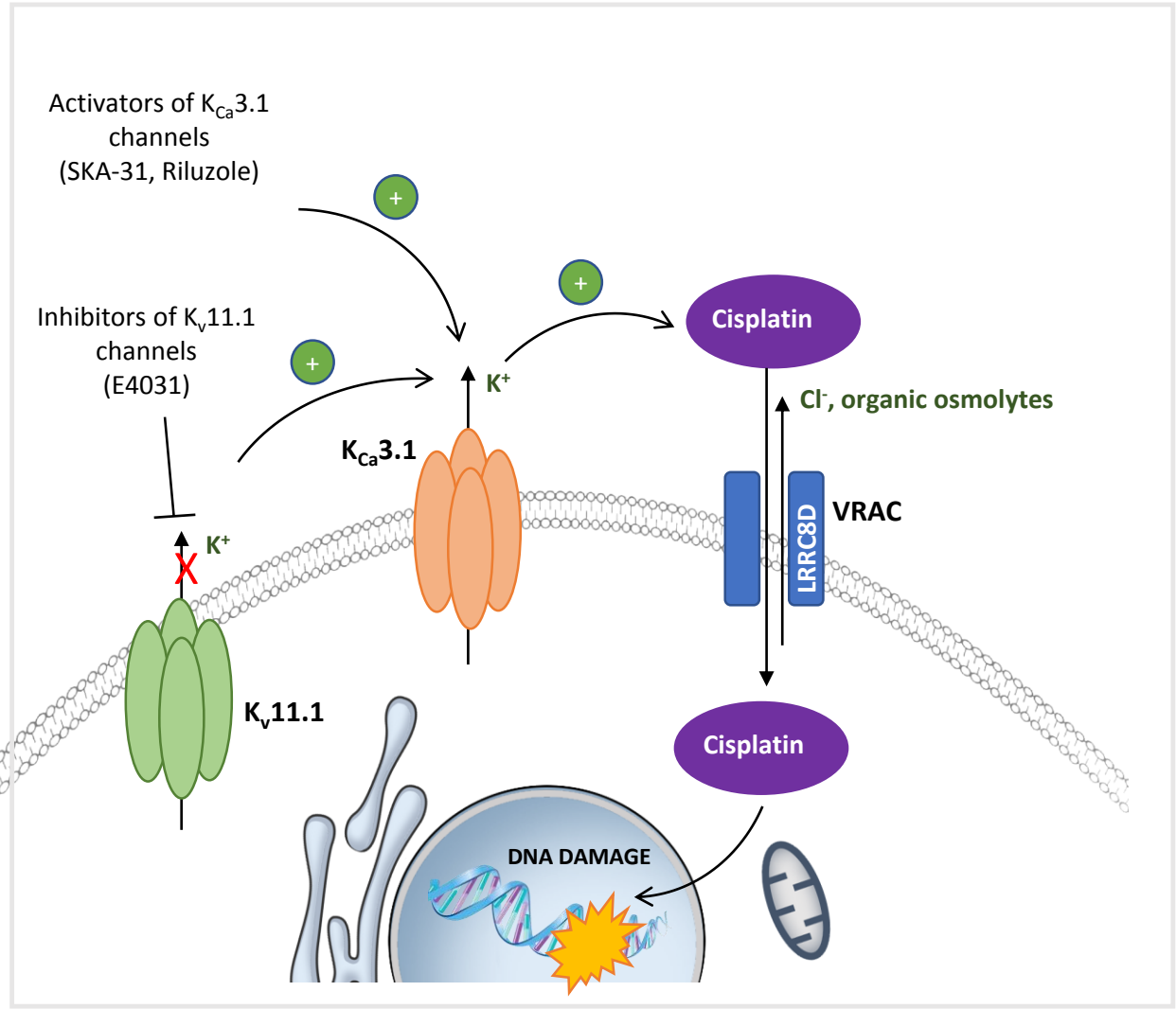
**Table 2. Summary of the effects of $K^+$ channel modulators (Riluzole, SKA-31, TRAM-34 and E4031) on different biological processes of HCT-116 cells**

| | | Cisplatin | | | |
|---|---|---|---|---|---|
| **Drug** | **$V_{REST}$** | **Platinum uptake** | **Cell viability** | **Apoptosis** | **Cell cycle** |
| Riluzole | Hyperpolarisation | ↑ | ↓ (S) | ↑↑ | ↑↑ % of cells in G2/M |
| SKA-31 | Hyperpolarisation | ↑ | ↓ (S) | ↑↑ | ↑ % of cells in G2/M |
| TRAM-34 | Depolarisation | ↓ | (A) | ↑↑ | ↑↑ % of cells in G2/M |
| E4031 | Depolarisation | ↑ | ↓ (S) | ↑↑ | ↑ % of cells in G2/M |

Abbreviations: ↓ = decrease, ↑ = increase, ↑↑ = strong increase, (A) = antagonism, (S) = synergy. $V_{REST}$ was determined in cells treated with the single $K^+$ channel modulators alone; Platinum uptake, cell viability, apoptosis and cell cycle data are relative to treatments in combination with Cisplatin (25μM). Experimental data and concentrations used are from Table 1B, Figures 2–4 and Supplementary Table S7.

**The activation of $K_{Ca}3.1$ modulates the VRAC-dependent uptake of Cisplatin (Jentsch et al, 2016).**

**Blocking hERG1 increases the uptake of Cisplatin, which relies on the activity of $K_{Ca}3.1$ channels.**

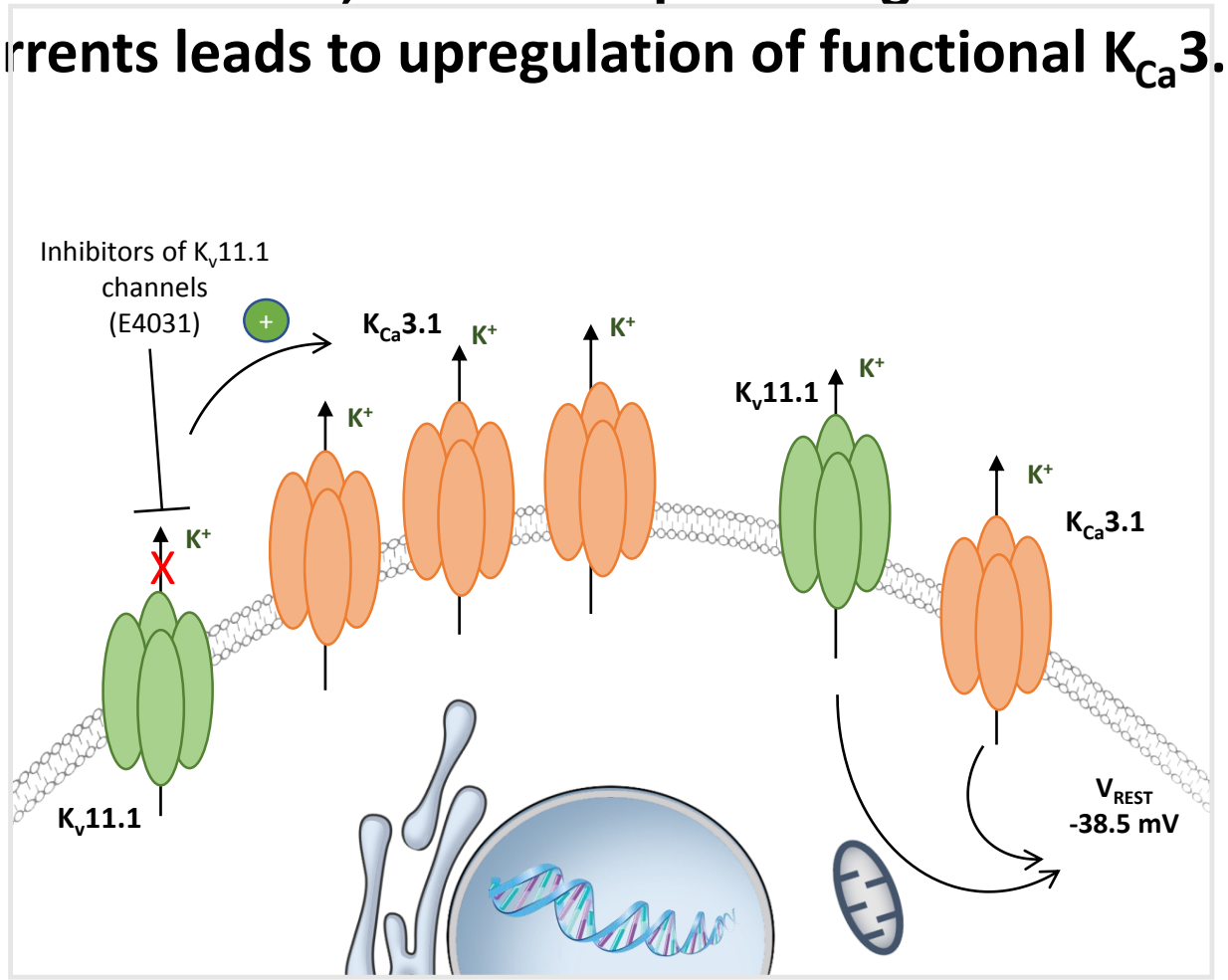


**Cisplatin-resistant cells exhibit higher functional expression of $K_{Ca}3.1$ and hERG1 channels, compared with Cisplatin-sensitive cells.**

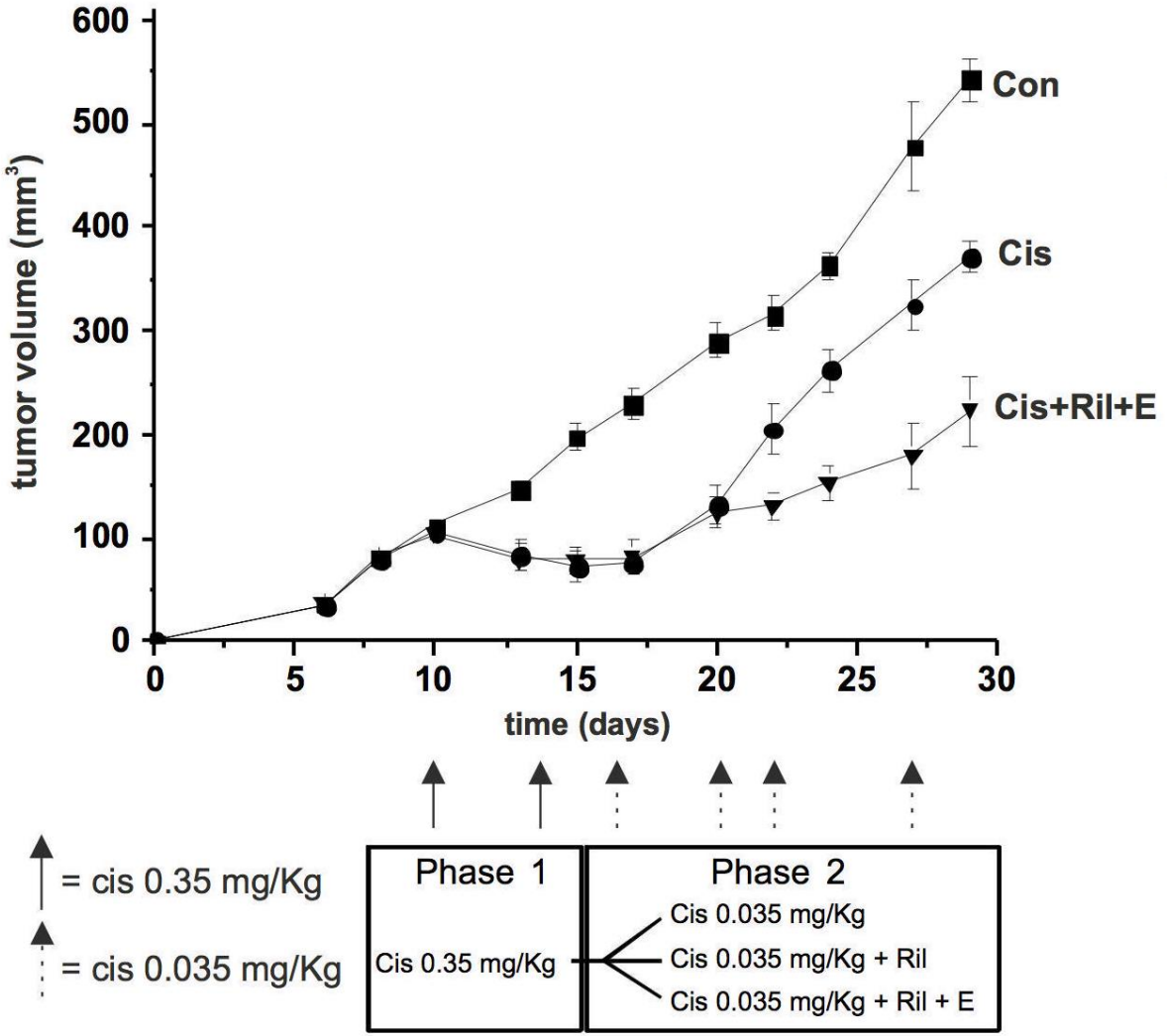
**The two channels are functionally related in these cells:**

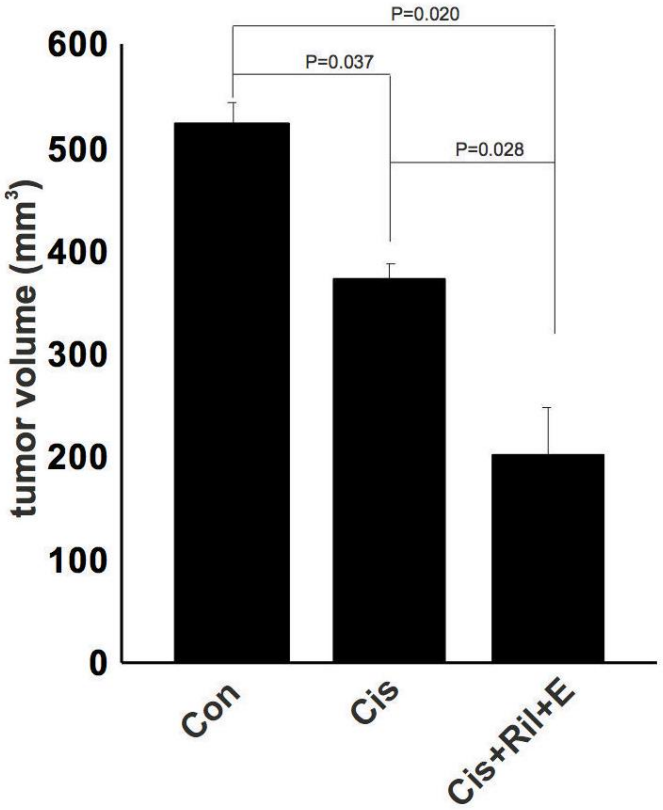
**(1)they set VREST to more hyperpolarised values;**

**(2)their expression is coordinated, one compensating for the other: prolonged (24h) inhibition of hERG1 currents leads to upregulation of functional $K_{Ca}3.1$ channels.**

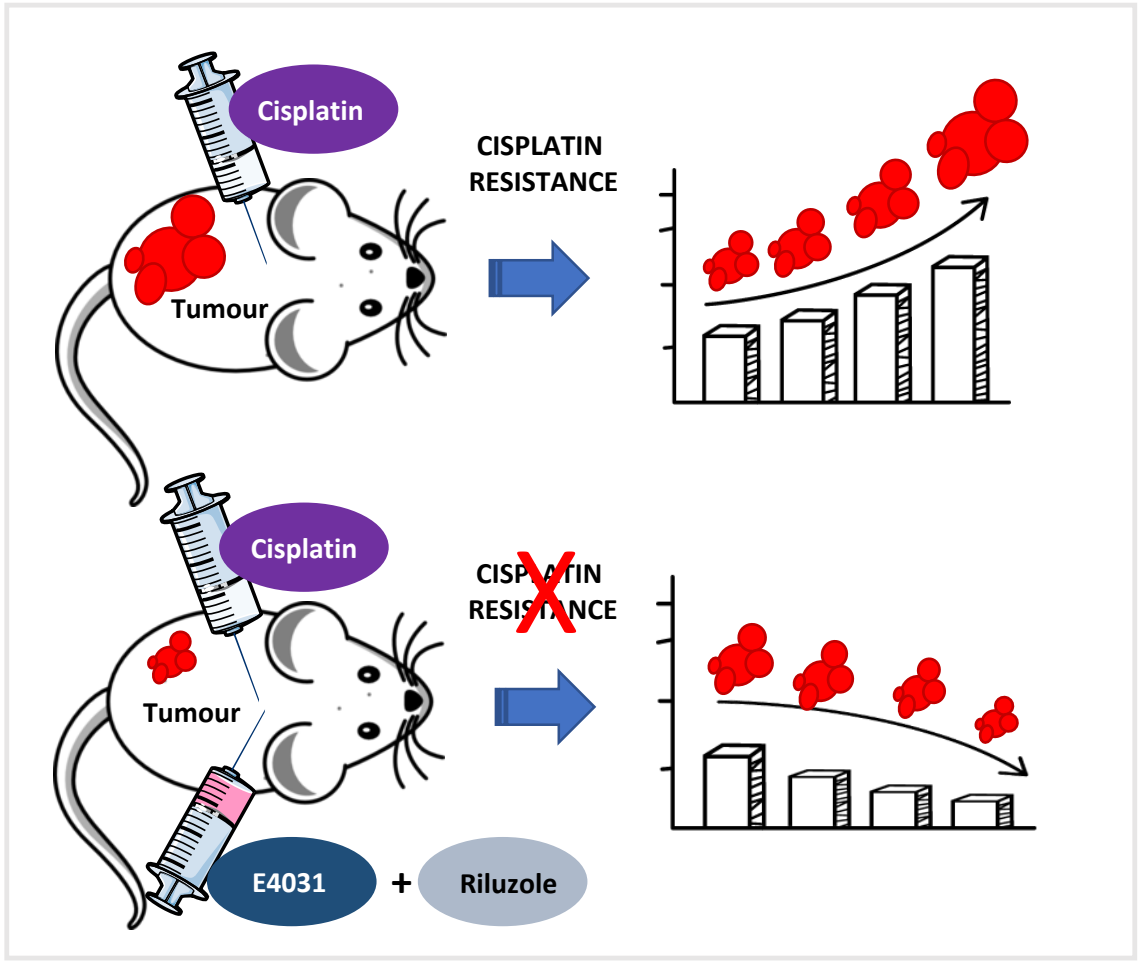


# The concomitant activation of $K_{Ca}3.1$ and inhibition of hERG1 potentiates the pro-apoptotic activity of Cisplatin, *in vivo*, hence contributing to overcome Cisplatin resistance.



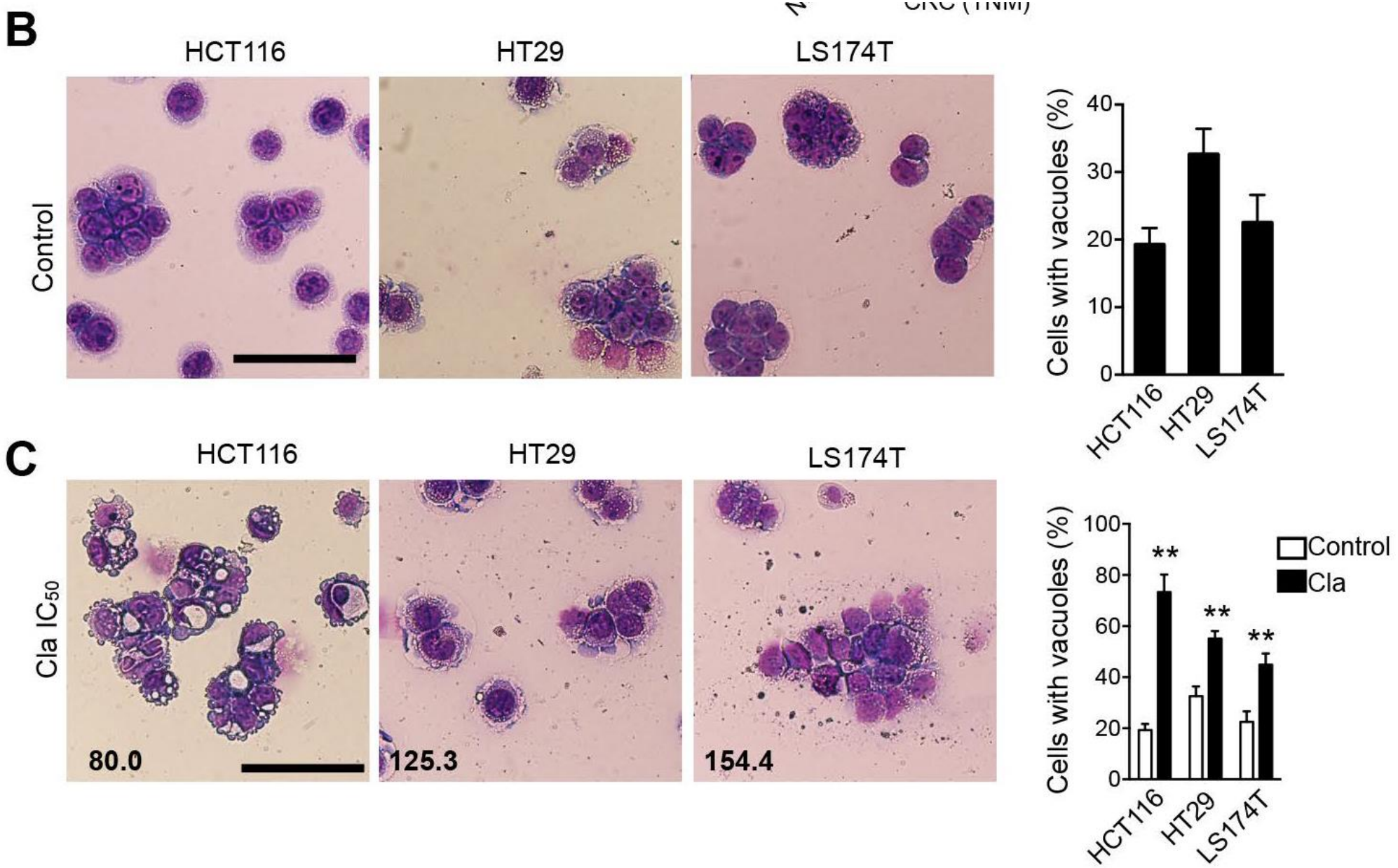
**The concomitant activation of $K_{Ca}3.1$ and inhibition of hERG1 potentiates the pro-apoptotic activity of Cisplatin, *in vivo*, hence contributing to overcome Cisplatin resistance.**



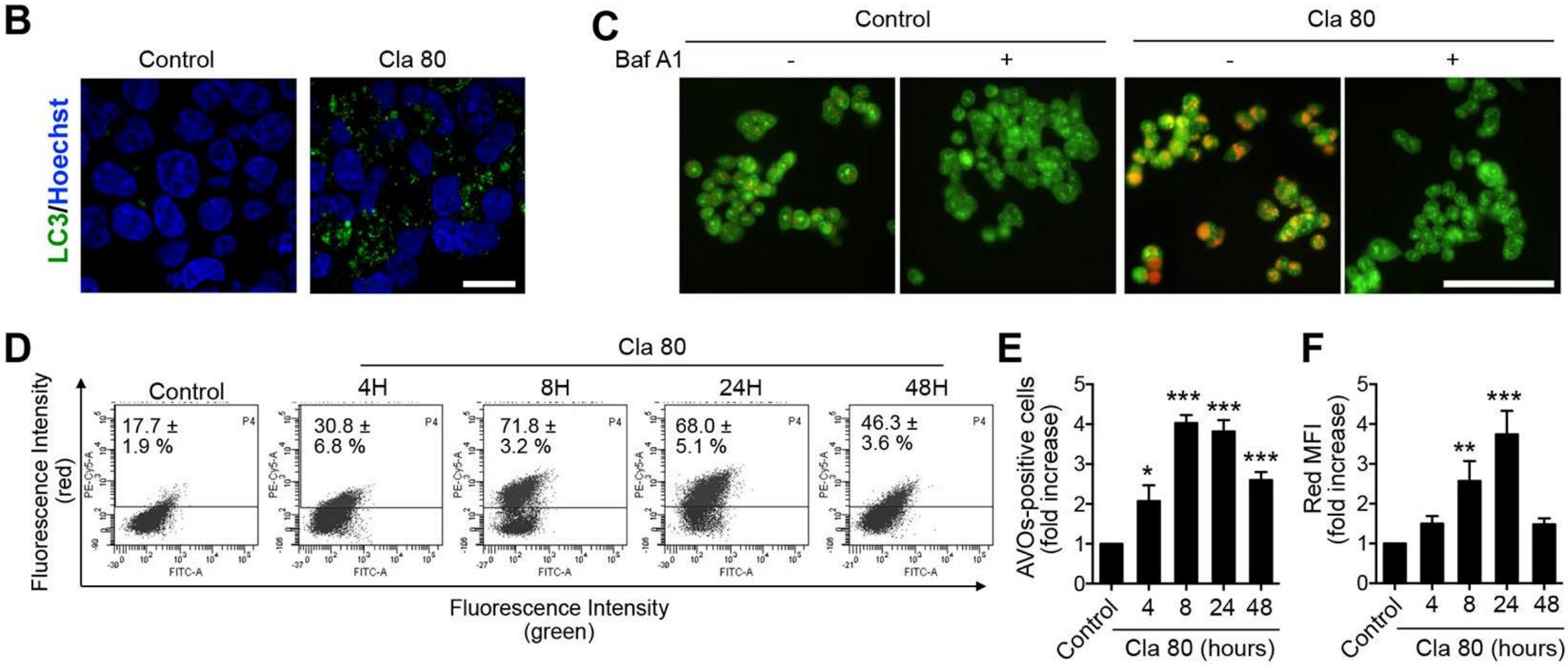
# (2) Effects of Clarithromycin on hERG1: sinergy with 5-FU

(unpublished)

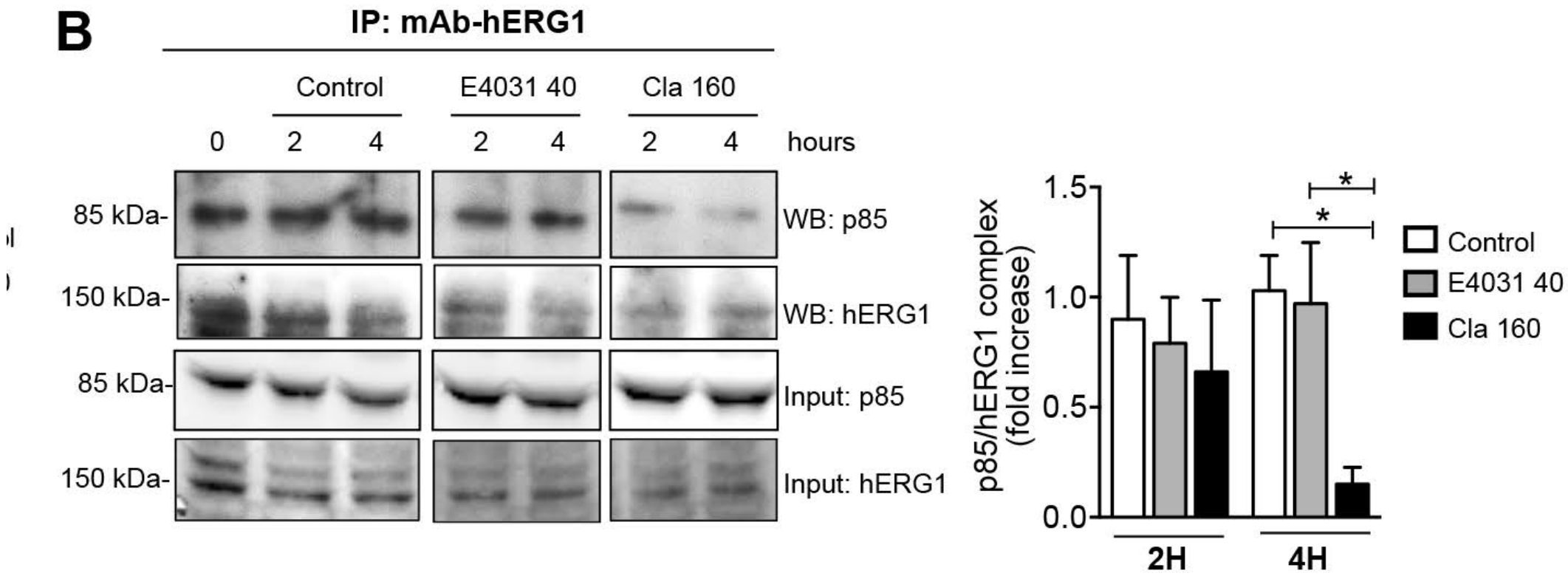
# Clarithromycin triggers autophagy in CRC cells



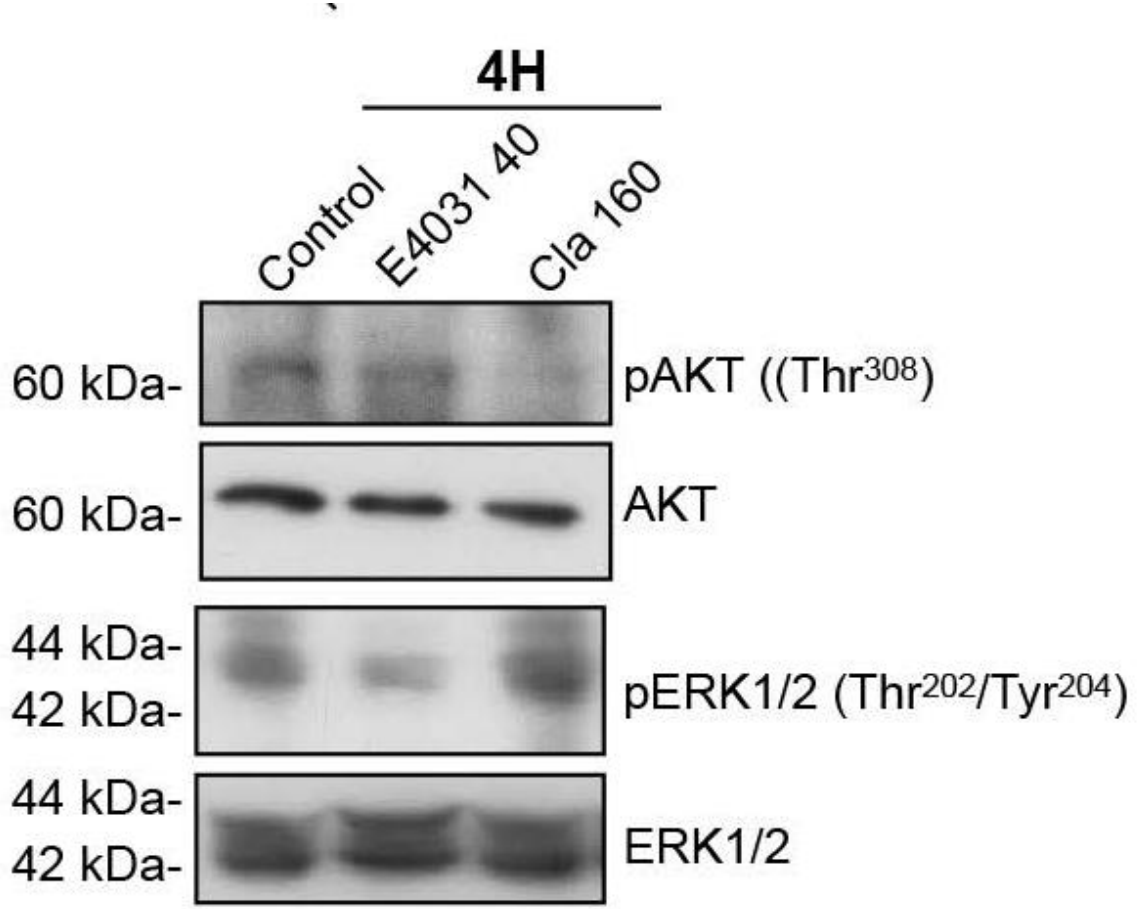
# Clarithromycin triggers autophagy in CRC cells



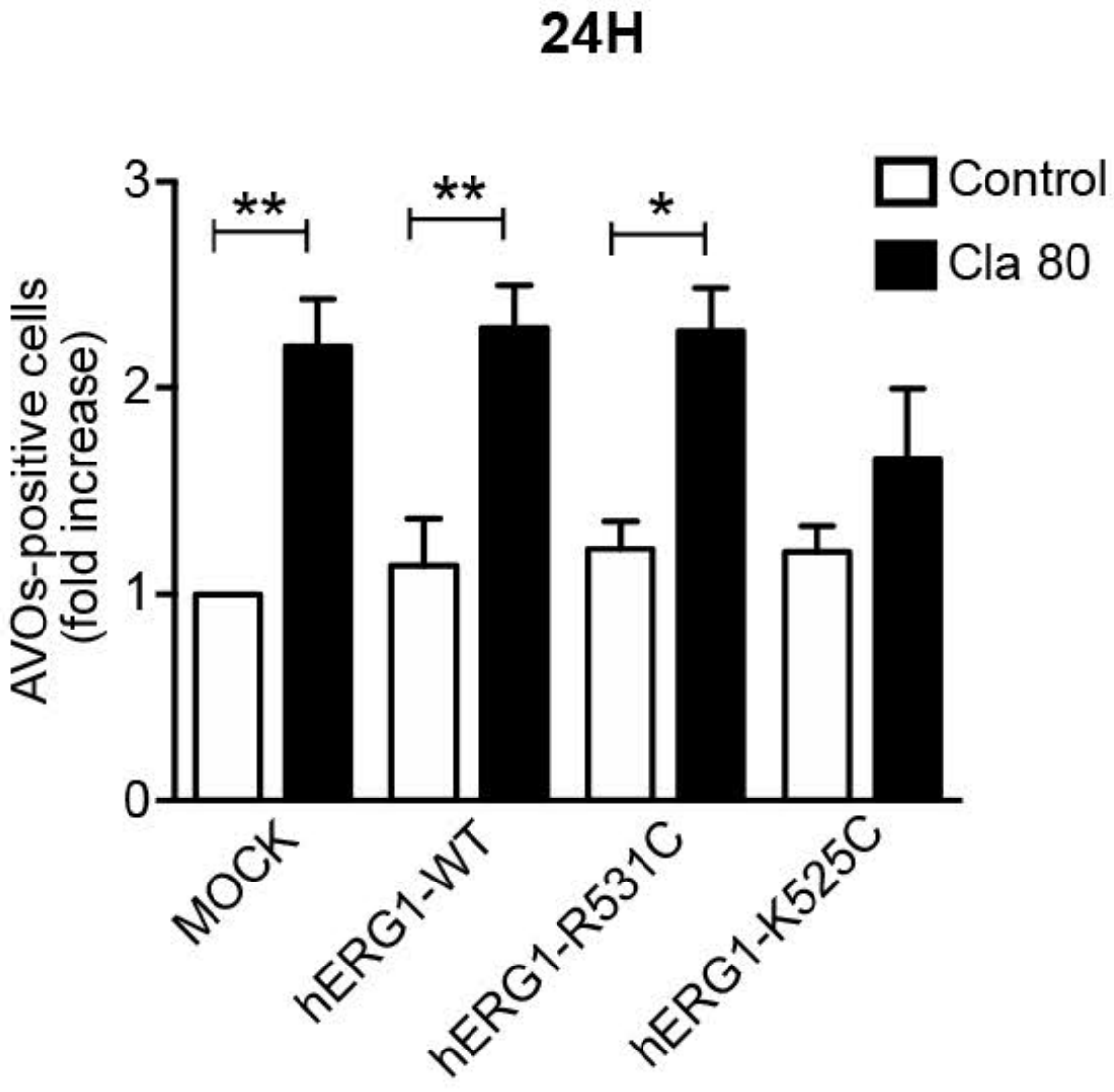
# Chlarithromycin dissociates the hERG1/PI3k (p85 subunit) complex

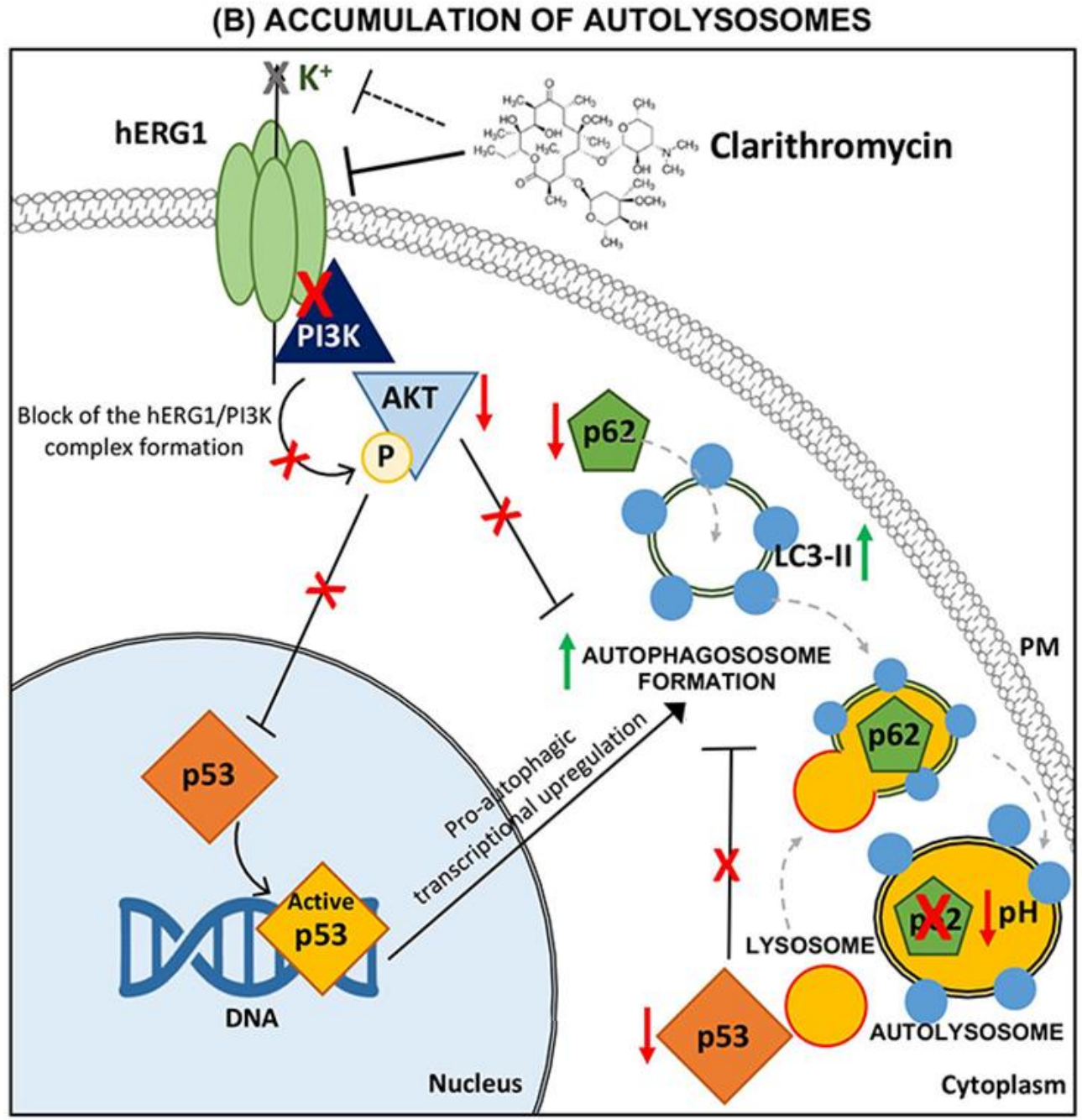


# Different effects of E4031 and Clarithromycin on Akt phosphorylation

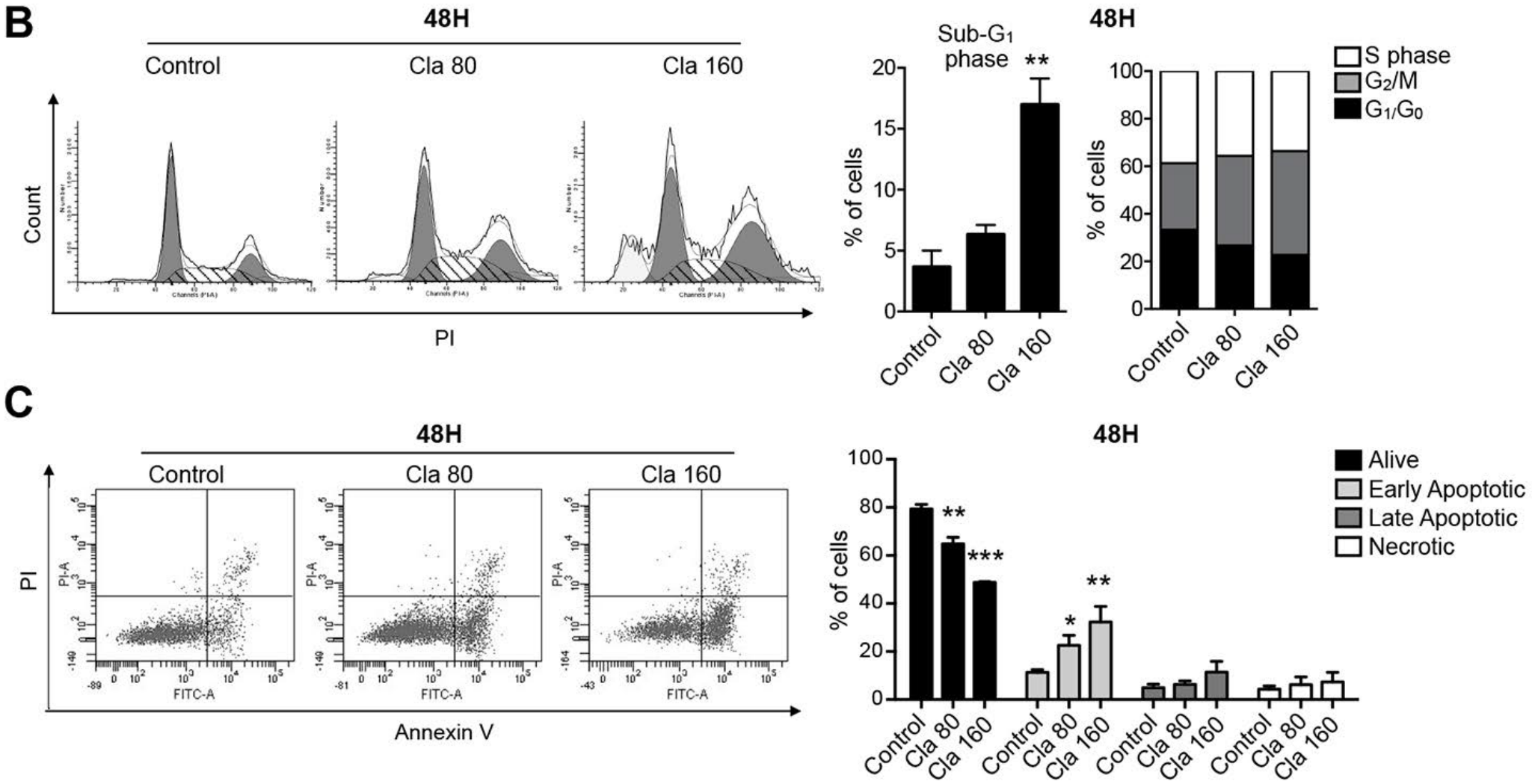


# Clarithromycin effects depend on the conformational state of hERG1 channels , occurring when the channel is prefrentially in the closed state

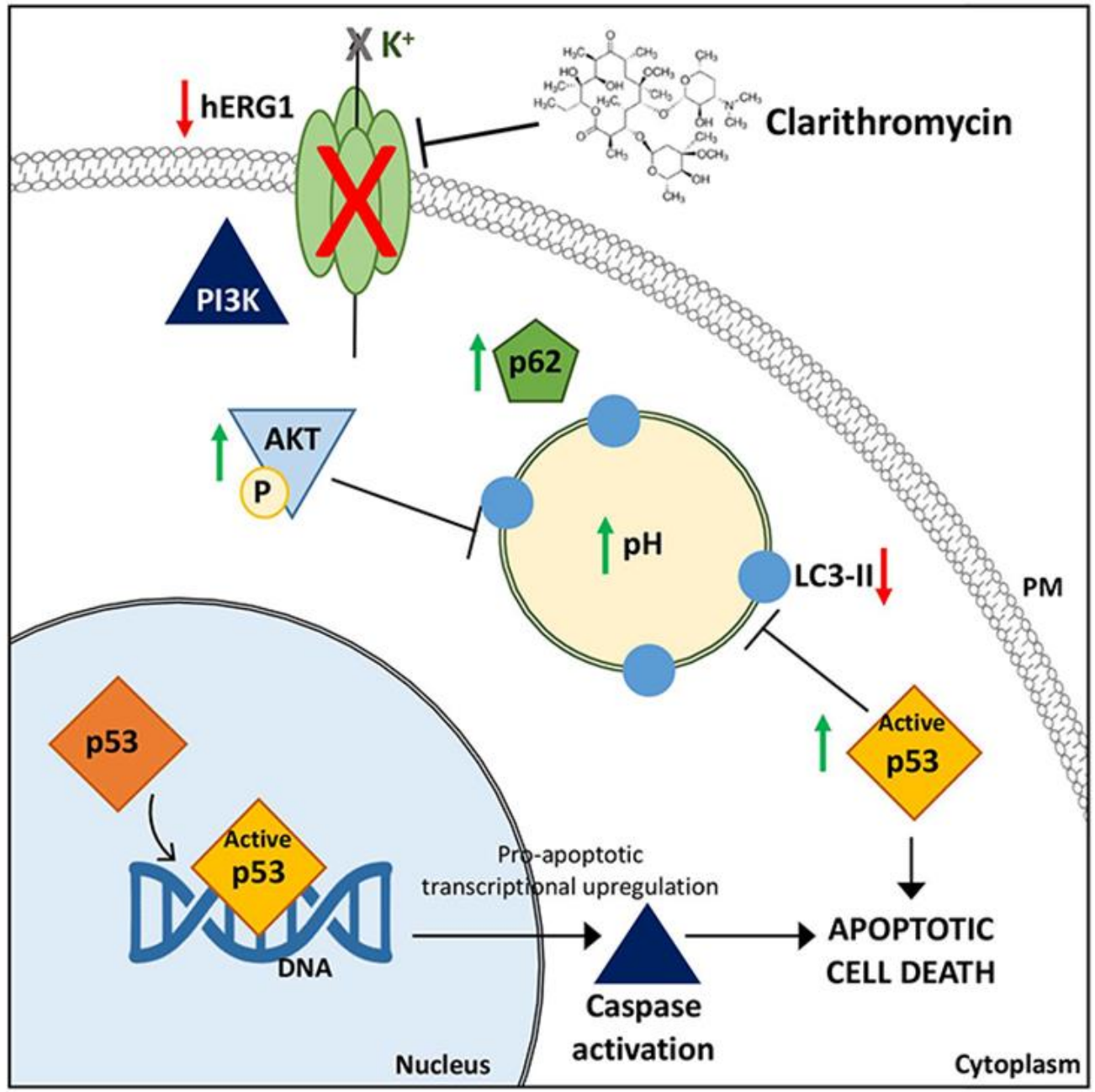



(B) ACCUMULATION OF AUTOLYSOSOMES
K+
hERG1
Clarithromycin
PI3K
AKT
P
Block of the hERG1/PI3K
complex formation
p62
LC3-II
AUTOPHAGOSOSOME
FORMATION
PM
p53
Active
p53
DNA
Pro-autophagic
transcriptional upregulation
p62
LYSOSOME
pH
p53
AUTOLYSOSOME
Nucleus
Cytoplasm

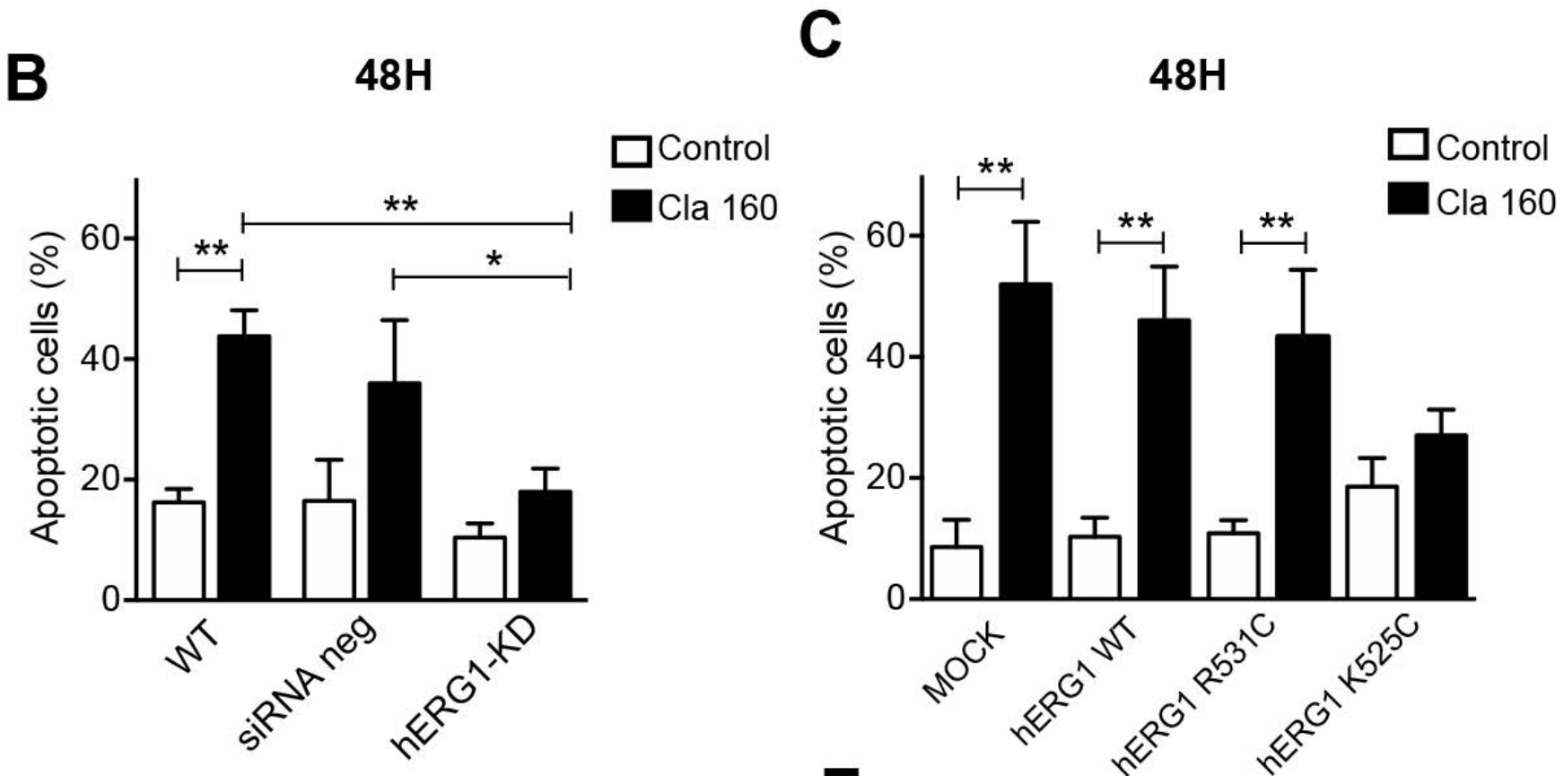
# Clarithromycin triggers apoptotic cell death



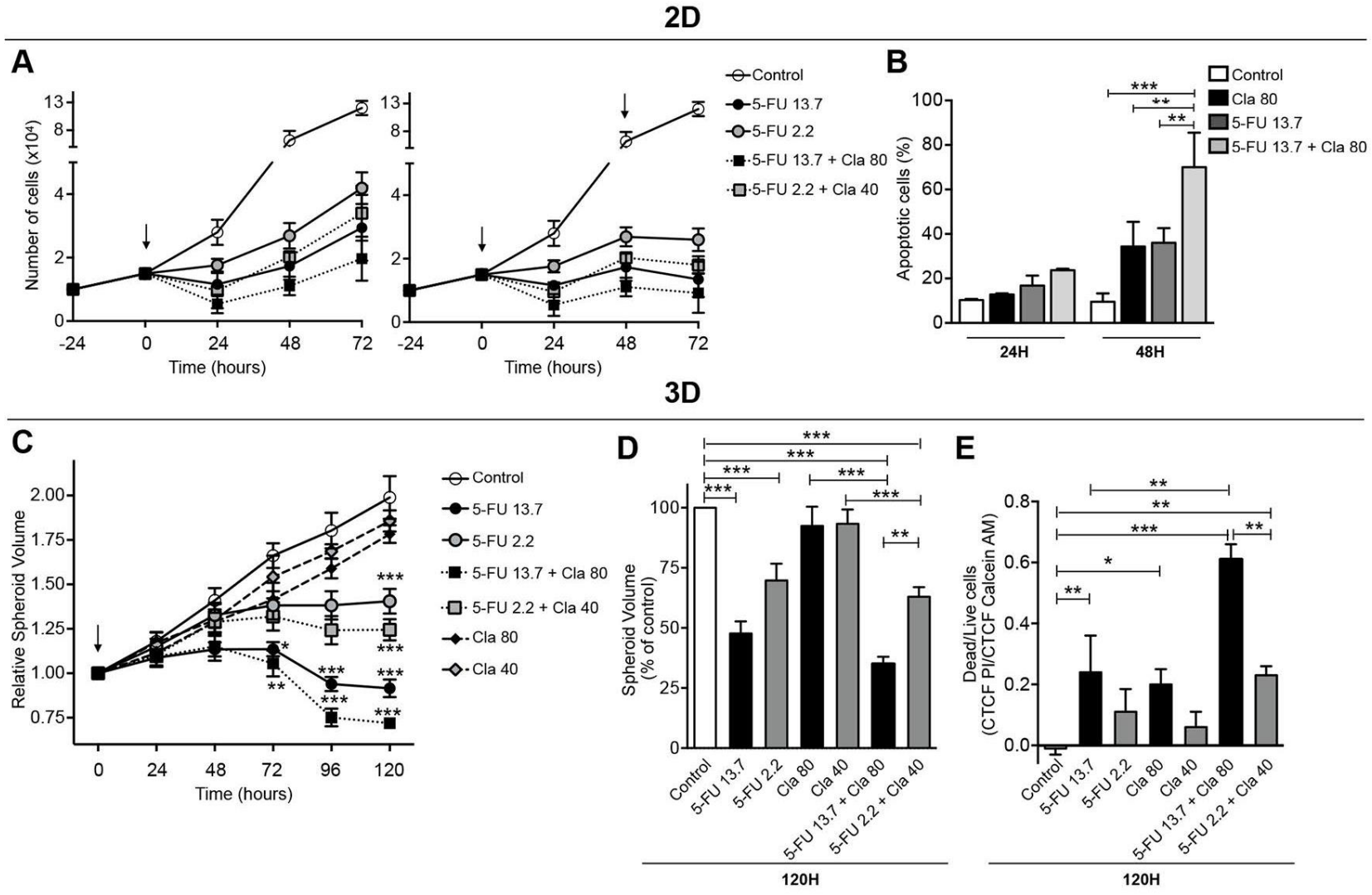
## (C) EXHAUSTION OF AUTOPHAGY AND APOPTOTIC CELL DEATH



# The hERG1 conformational state determines (the closed state favours) the pro-apoptotic effects of Clarithromycin



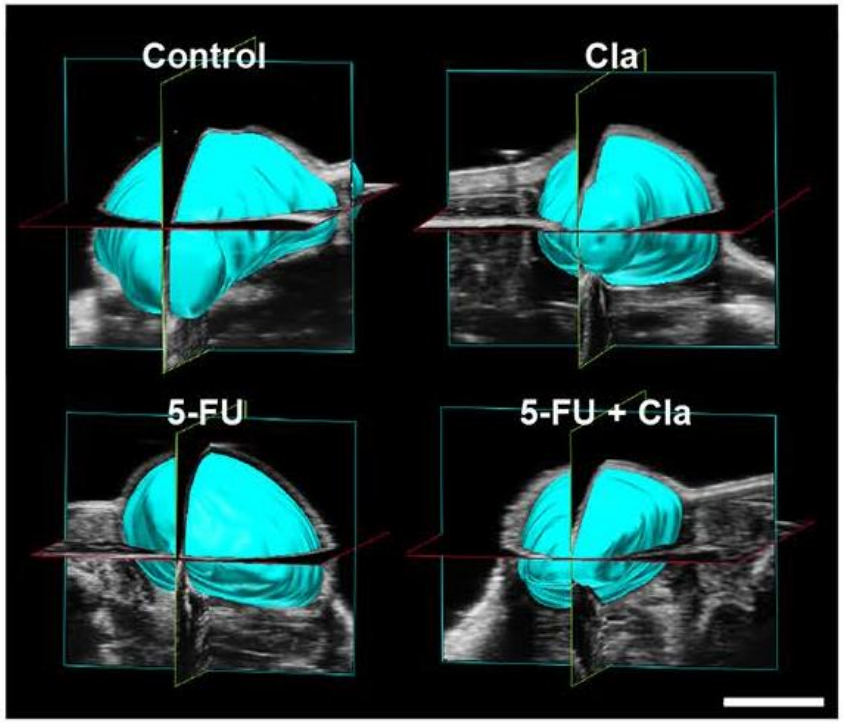
# Clarithromycin has a synergic, anti-proliferative, effect with 5-FU

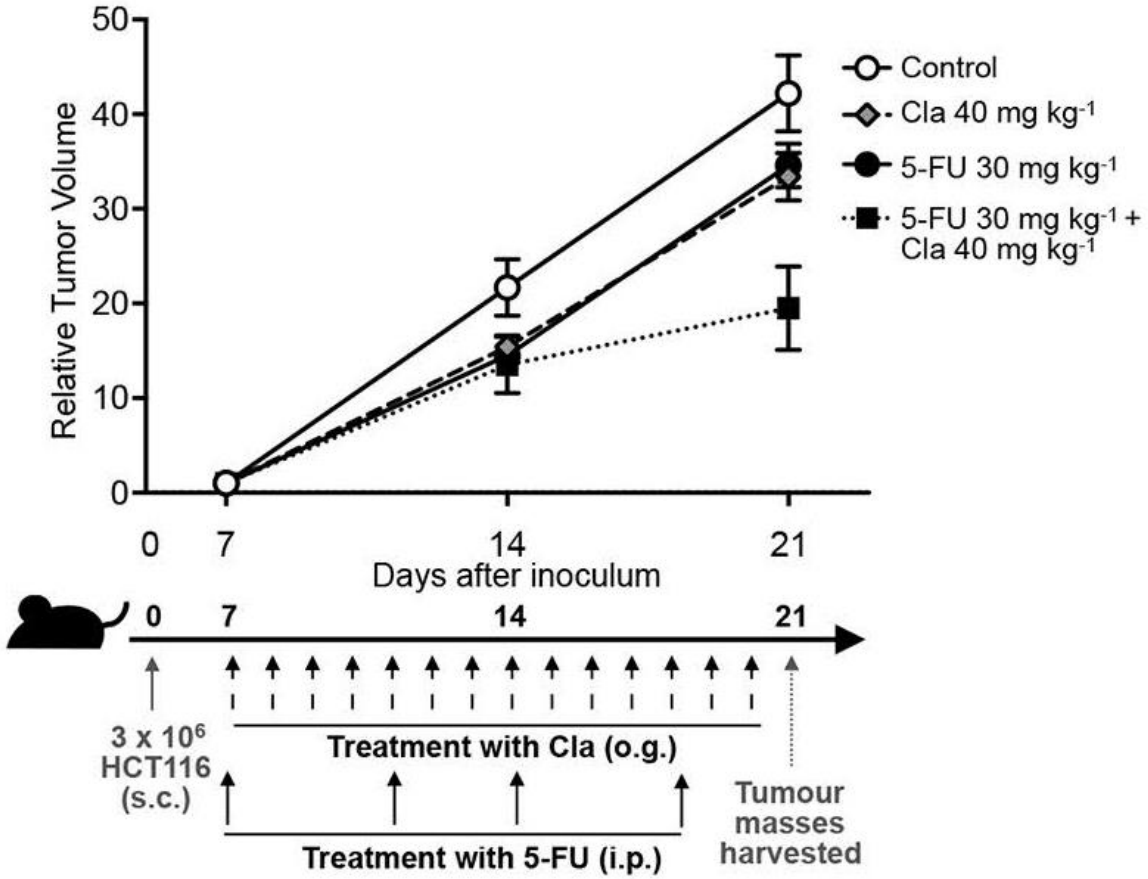


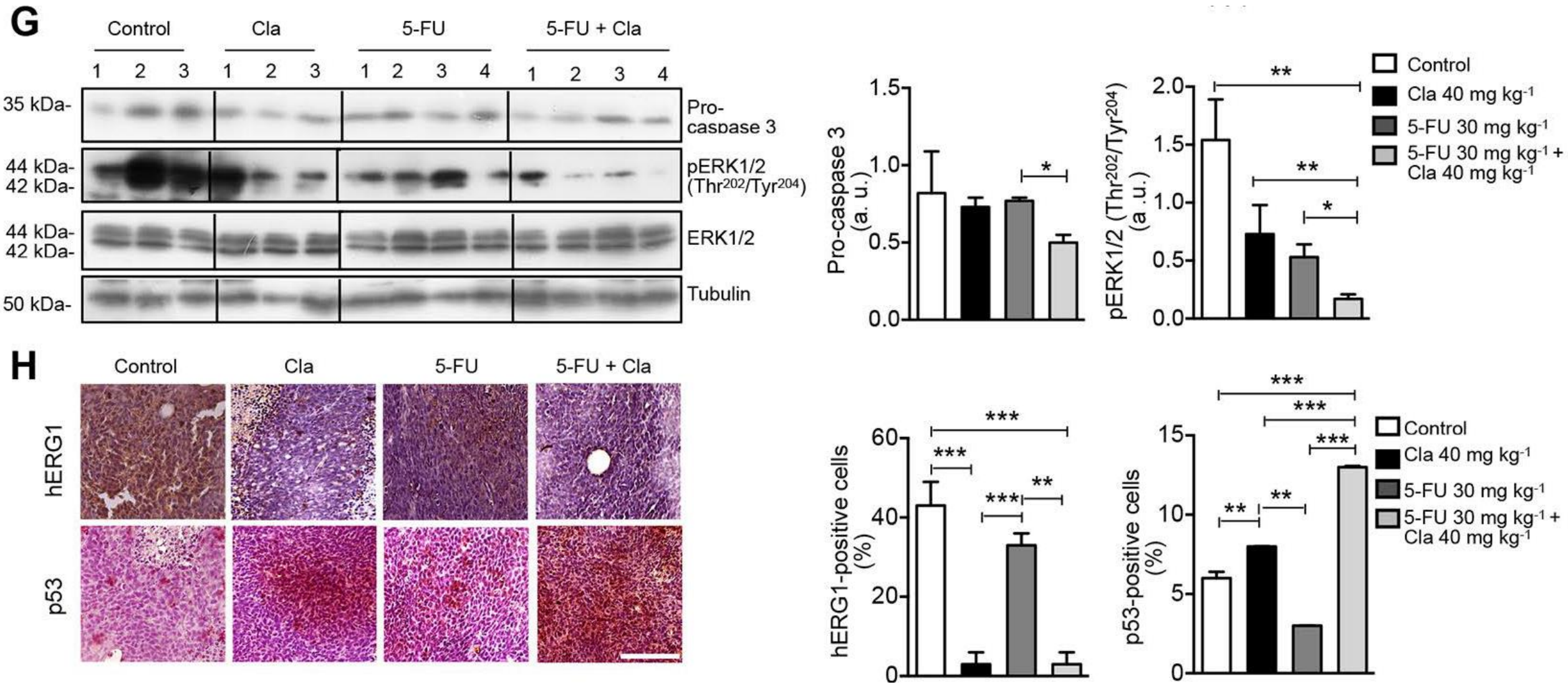
# ….overcoming 5-FU resistance in vivo, in a preclinical xenograft mouse model

## in vivo

F




G
Control
Cla
5-FU
5-FU + Cla
1 2 3 1 2 3 1 2 3 4 1 2 3 4
35 kDa-
Pro-caspase 3
44 kDa-
42 kDa-
pERK1/2 (Thr202/Tyr204)
44 kDa-
42 kDa-
ERK1/2
50 kDa-
Tubulin
H
Control
Cla
5-FU
5-FU + Cla
hERG1
p53
Pro-caspase 3 (a. u.)
pERK1/2 (Thr202/Tyr204) (a .u.)
hERG1-positive cells (%)
p53-positive cells (%)
Control
Cla 40 mg kg-1
5-FU 30 mg kg-1
5-FU 30 mg kg-1 + Cla 40 mg kg-1

# The hERG1/β1 integrin complex in CRC

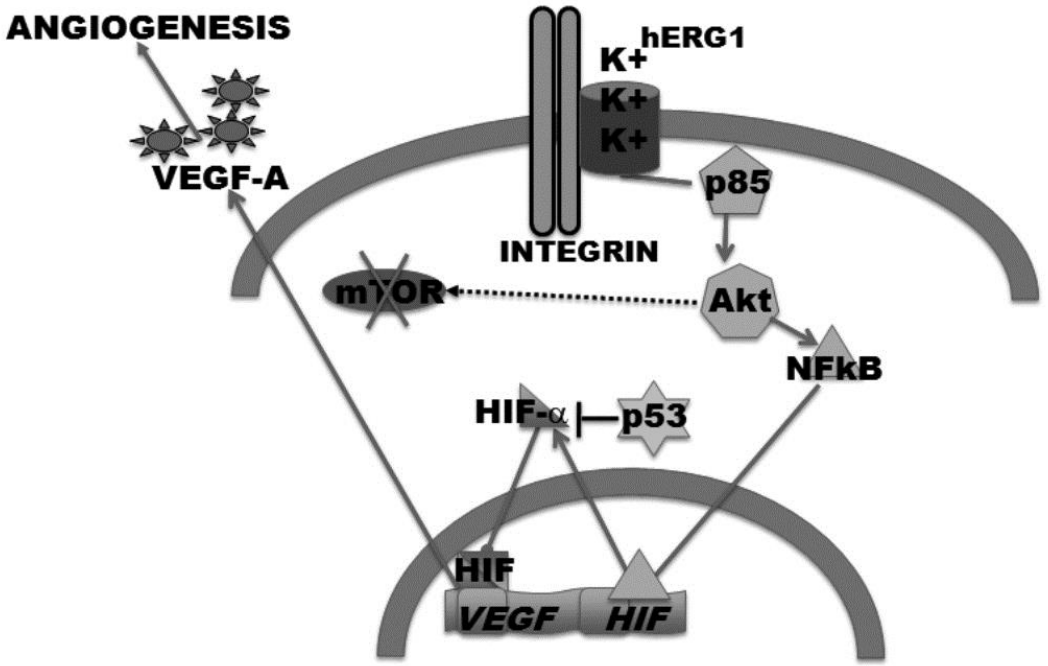
SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
COLORECTAL CANCER
INTEGRIN SIGNALLING
TUMOUR ANGIOGENESIS
ION CHANNEL SIGNALLING

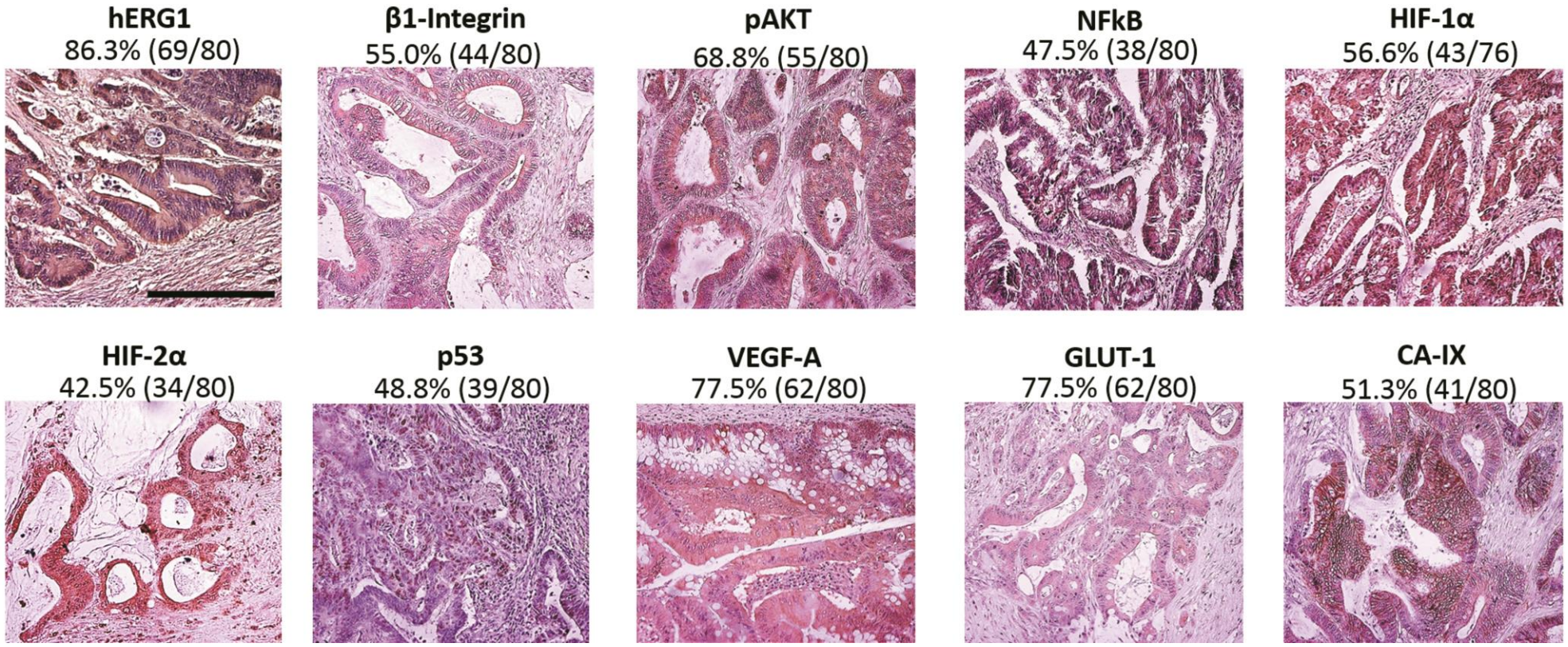
## hERG1 channels modulate integrin signaling to trigger angiogenesis and tumor progression in colorectal cancer

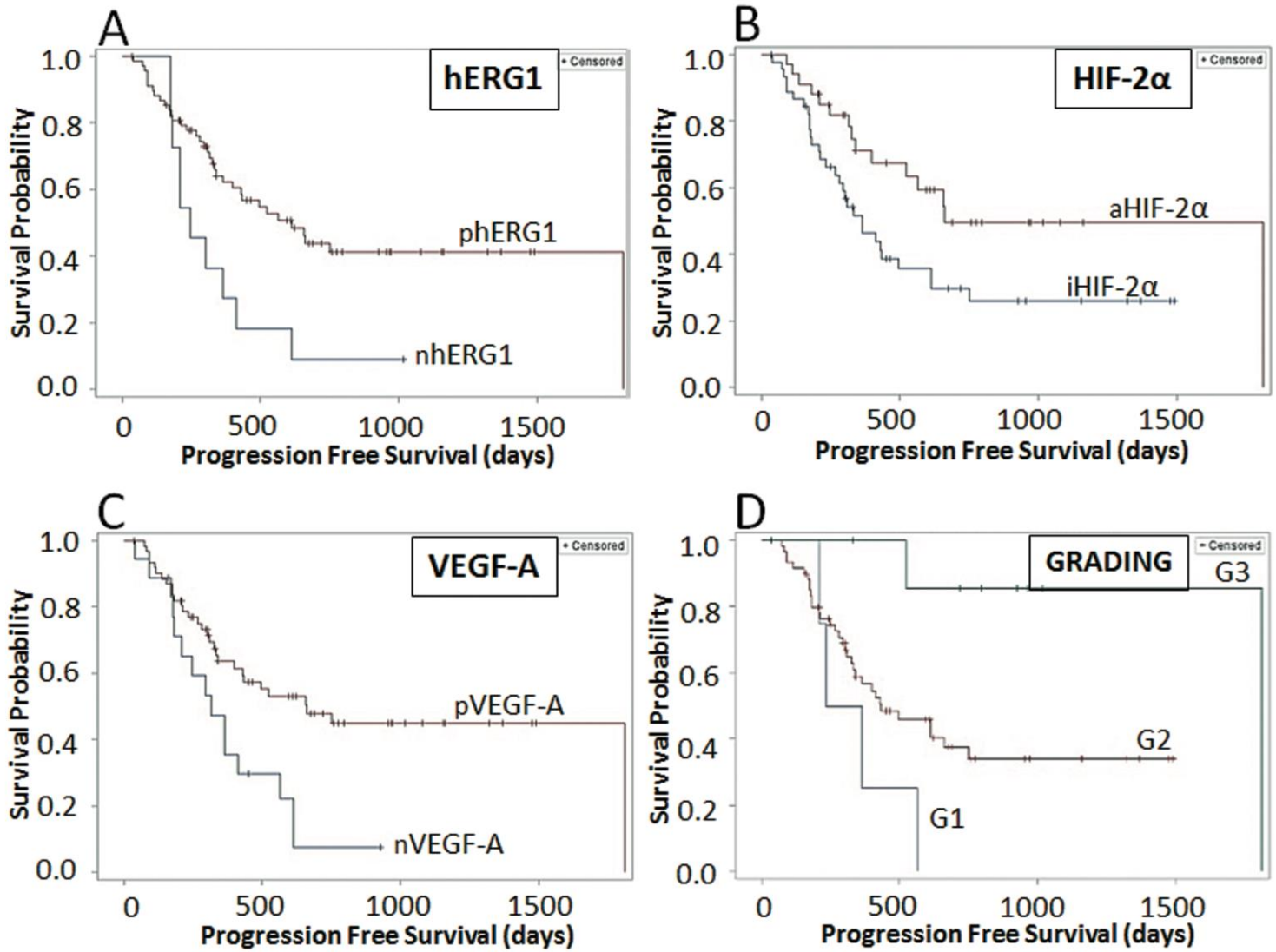
Olivia Crociani[1], Francesca Zanieri[1], Serena Pillozzi[1], Elena Lastraioli[1], Matteo Stefanini[1], Antonella Fiore[1], Angelo Fortunato[1], Massimo D'Amico[1], Marika Masselli[1], Emanuele De Lorenzo[1], Luca Gasparoli[1], Martina Chiu[2], Ovidio Bussolati[2], Andrea Becchetti[3] & Annarosa Arcangeli[1]



# Expression of hERG1 and several angiogenesis-related proteins in metastatic CRC samples from patients treated in first line with Bevacizumab

FIGURE 1




A
hERG1
Censored
phERG1
nhERG1
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
Progression Free Survival (days)
B
HIF-2α
Censored
aHIF-2α
iHIF-2α
Survival Probability
Progression Free Survival (days)
C
VEGF-A
Censored
pVEGF-A
nVEGF-A
Survival Probability
Progression Free Survival (days)
D
GRADING
Censored
G3
G2
G1
Survival Probability
Progression Free Survival (days)

# hERG1 and HIF-2α expression have a significant positive impact on PFS

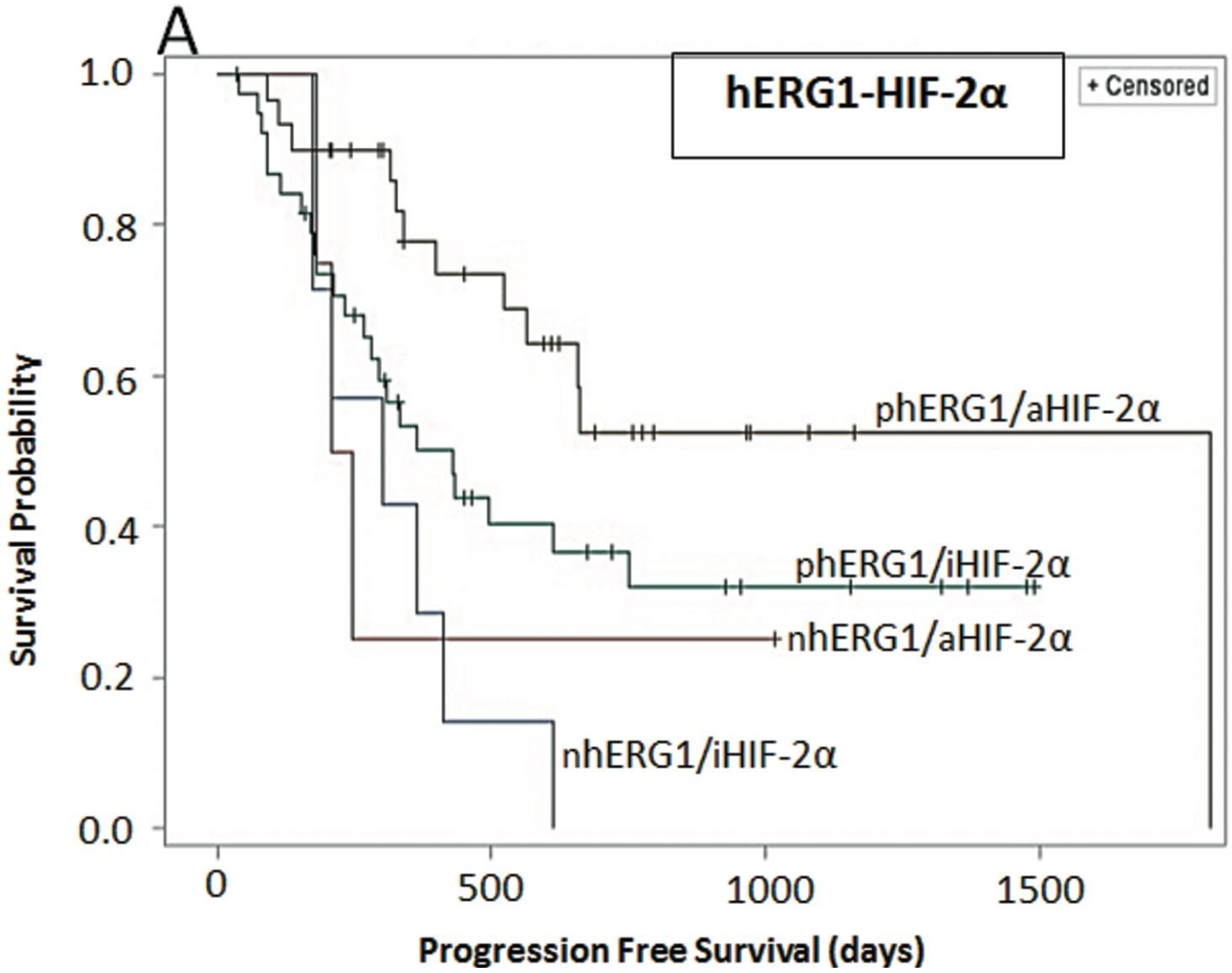
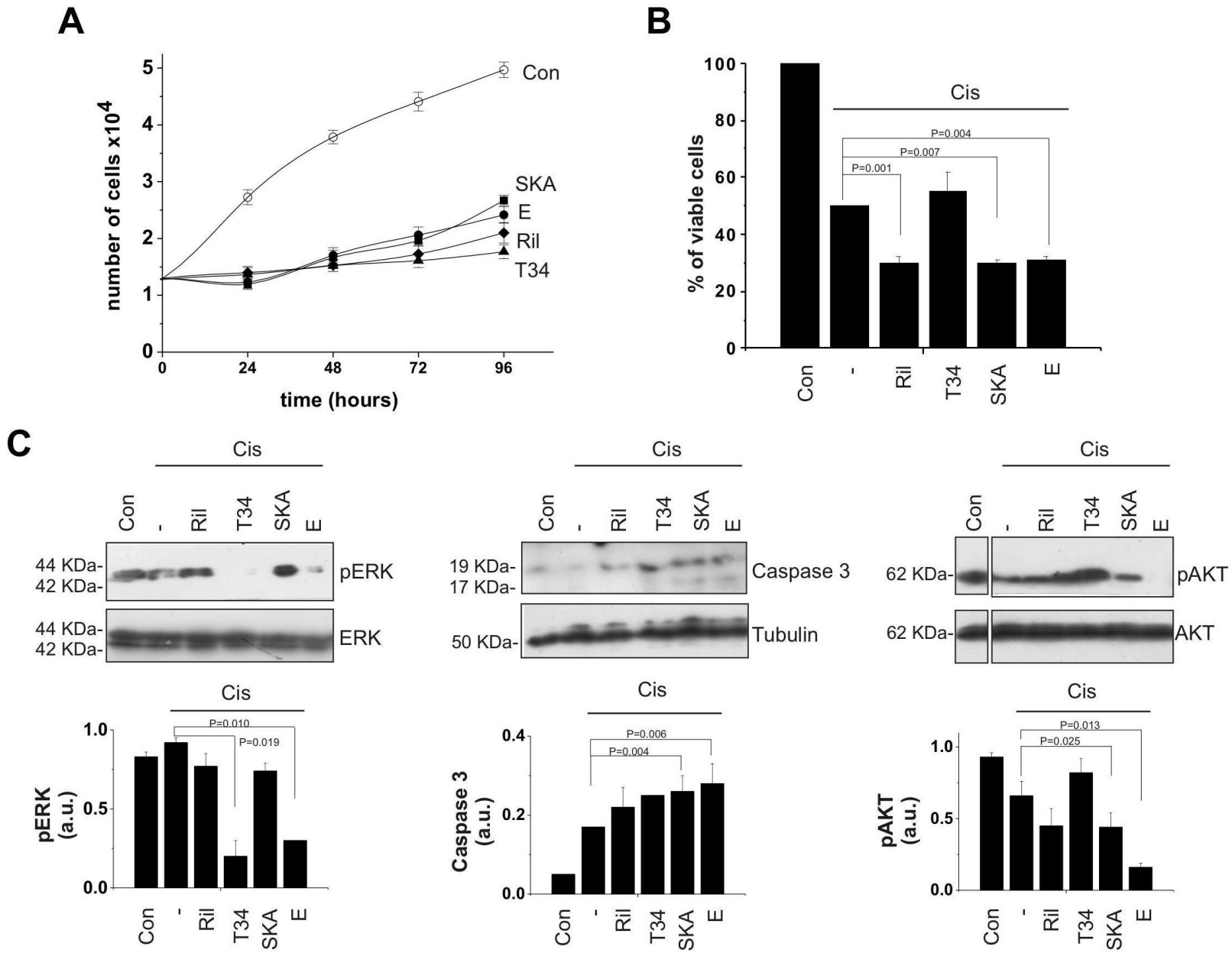
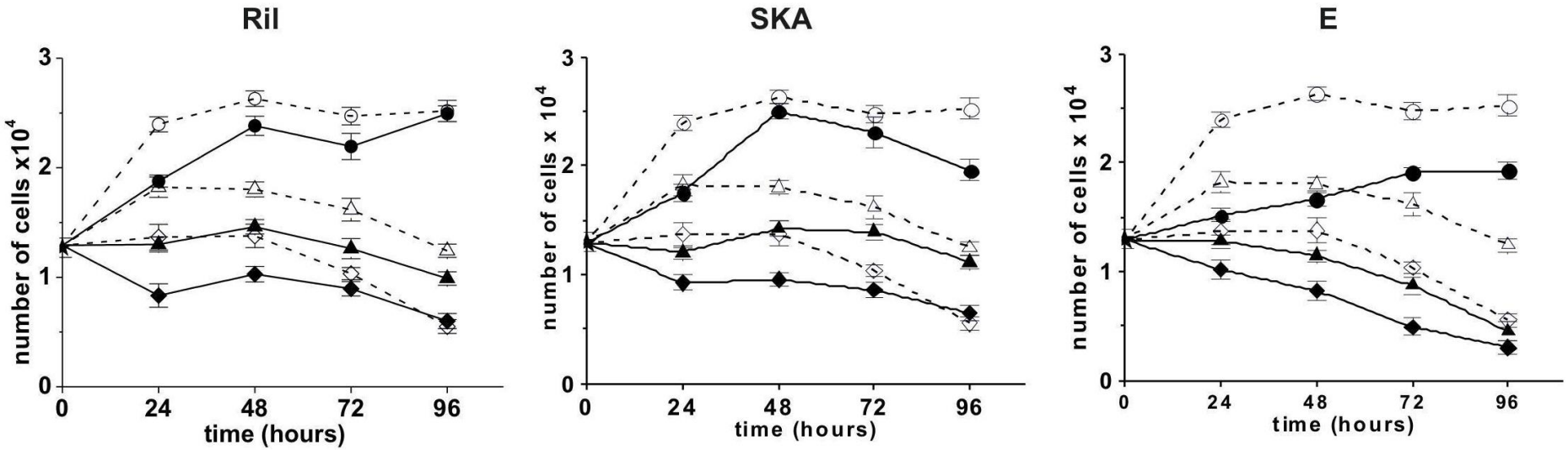


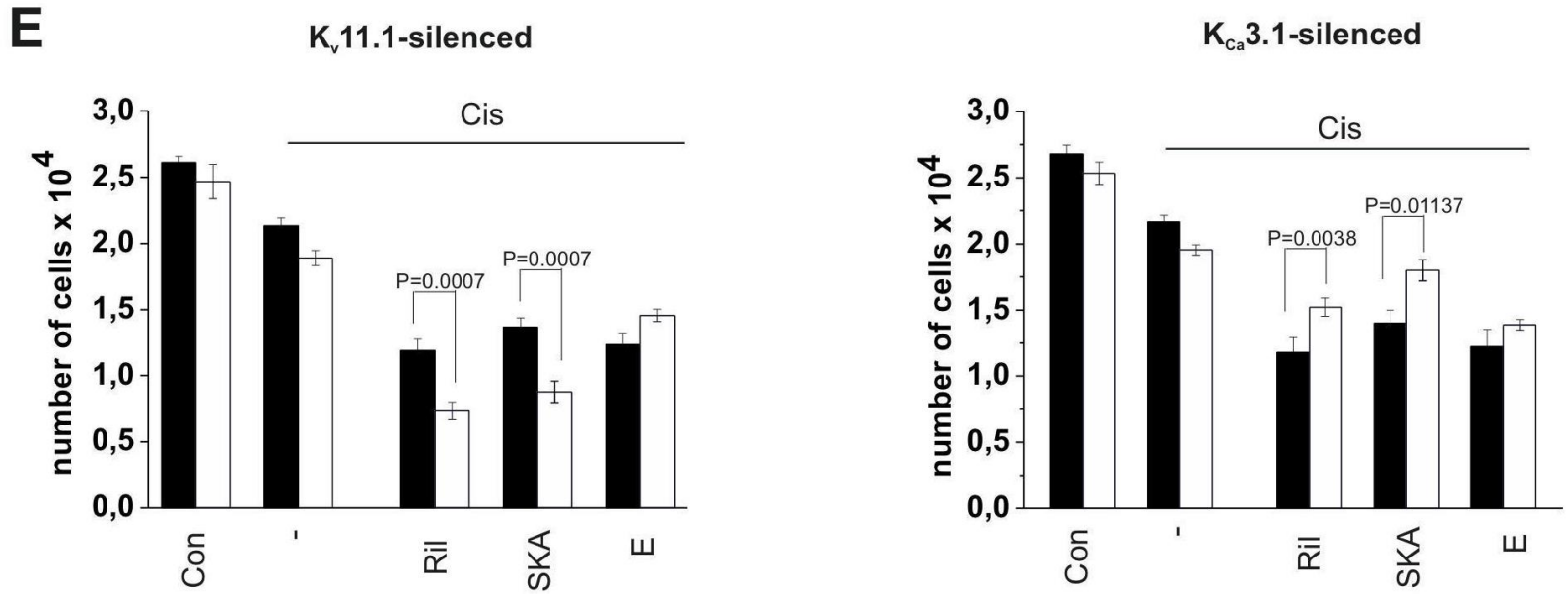
**Table 1A. $IC_{50}$ values and effects on apoptosis and cell cycle distribution of Cisplatin, Riluzole, SKA-31, TRAM-34 and E4031 in HCT-116 and HCT-8 cells**

| | | | Apoptosis | | Cell cycle | | |
|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | Concentration of the drug (μM) | Early apoptosis (%) | Late apoptosis (%) | G0/G1 (%) | S (%) | G2/M (%) |
| **HCT-116** | | | | | | | |
| Control | — | | 1.0 ± 0.6 | 0.8 ± 0.5 | 27.7 ± 7.7 | 55.5 ± 3.7 | 16.9 ± 6.4 |
| Cisplatin | 25.2 ± 2.1 | 25 | 5.9 ± 1.0 | 5.3 ± 1.5 | 48.0 ± 4.5 | 39.2 ± 2.5 | 12.8 ± 3.3 |
| | | | $P = 0.001$ | $P = 0.009$ | $P = 0.009$ | $P = 0.018$ | |
| Riluzole | 9.5 ± 1.0 | 10 | 13.6 ± 3.7 | 10.3 ± 4.1 | 51.6 ± 6.0 | 26.1 ± 8.8 | 22.3 ± 3.1 |
| | | | $P = 0.004$ | $P = 0.028$ | $P = 0.004$ | $P = 0.021$ | |
| SKA-31 | 5.3 ± 0.3 | 5 | 7.0 ± 0.9 | 3.5 ± 0.9 | 55.3 ± 2.4 | 31.4 ± 7.7 | 13.3 ± 5.7 |
| | | | $P = 0.000$ | $P = 0.015$ | $P = 0.006$ | $P = 0.028$ | |
| TRAM-34 | 24.4 ± 1.8 | 25 | 9.6 ± 2.1 | 6.6 ± 0.9 | 50.5 ± 3.2 | 34.2 ± 5.0 | 15.4 ± 7.6 |
| | | | $P = 0.001$ | $P = 0.000$ | | | |
| E4031 | 6.6 ± 1.6 | 7 | 5.2 ± 1.2 | 4.1 ± 1.0 | 51.4 ± 4.3 | 26.4 ± 4.1 | 22.3 ± 6.1 |
| | | | $P = 0.005$ | $P = 0.010$ | $P = 0.010$ | $P = 0.012$ | |
| **HCT-8** | | | | | | | |
| Control | — | | 0.8 ± 0.3 | 1.2 ± 0.3 | 30.7 ± 2.4 | 53.8 ± 3.3 | 15.5 ± 1.9 |
| Cisplatin | 8.7 ± 1.4 | 9 | 5.5 ± 1.4 | 13.4 ± 7.2 | 46.9 ± 1.3 | 43.4 ± 1.9 | 9.7 ± 3.1 |
| | | | $P = 0.008$ | $P = 0.018$ | $P = 0.019$ | $P = 0.002$ | $P = 0.041$ |
| Riluzole | 12.9 ± 0.7 | 13 | 3.6 ± 0.9 | 4.0 ± 1.2 | 8.5 ± 3.4 | 6.7 ± 4.3 | 84.6 ± 5.6 |
| | | | $P = 0.008$ | $P = 0.035$ | $P = 0.003$ | $P = 0.000$ | $P = 0.000$ |
| SKA-31 | 46.9 ± 1.4 | 45 | 3.0 ± 0.4 | 10.2 ± 5.0 | 47.5 ± 3.4 | 39.9 ± 8.7 | 12.6 ± 10.0 |
| | | | $P = 0.001$ | $P = 0.011$ | $P = 0.021$ | $P = 0.046$ | |
| TRAM-34 | 20.1 ± 1.1 | 20 | 3.2 ± 1.2 | 2.7 ± 1.0 | 62.9 ± 3.2 | 28.9 ± 4.5 | 8.2 ± 7.6 |
| | | | $P = 0.012$ | $P = 0.019$ | $P = 0.026$ | $P = 0.026$ | |
| E4031 | 13.3 ± 1.3 | 13 | 2.8 ± 1.9 | 2.8 ± 0.7 | 25.2 ± 0.4 | 57.1 ± 2.2 | 17.7 ± 2.4 |
| | | | | $P = 0.015$ | $P = 0.012$ | | |

$IC_{50}$ values were determined after 24 h of treatment by the Trypan Blue exclusion test, using the Origin Software. Apoptosis and cell cycle distributions were evaluated by treating the cells with the drug concentrations indicated in the third column for 24 h. The percentage of cells in early (Annexin +/PI − cells) and late apoptosis (Annexin +/PI + cells) was determined by Annexin/PI assay as detailed in the Materials and Methods section. Cell cycle distribution was assessed by flow cytometry after staining the cells with propidium iodide (PI) and is indicated as the percentage of cells in the different cell cycle phases. Data are means ± s.e.m. of three independent experiments, each carried out in triplicate. For statistical analysis, Student's *t*-test was applied.


A
number of cells x10^4
time (hours)
Con
SKA
E
Ril
T34
B
% of viable cells
Cis
P=0.004
P=0.007
P=0.001
Con
-
Ril
T34
SKA
E
C
Cis
Con
-
Ril
T34
SKA
E
44 KDa-
42 KDa-
pERK
ERK
19 KDa-
17 KDa-
Caspase 3
50 KDa-
Tubulin
62 KDa-
pAKT
AKT
P=0.010
P=0.019
pERK (a.u.)
P=0.006
P=0.004
Caspase 3 (a.u.)
P=0.013
P=0.025
pAKT (a.u.)
D

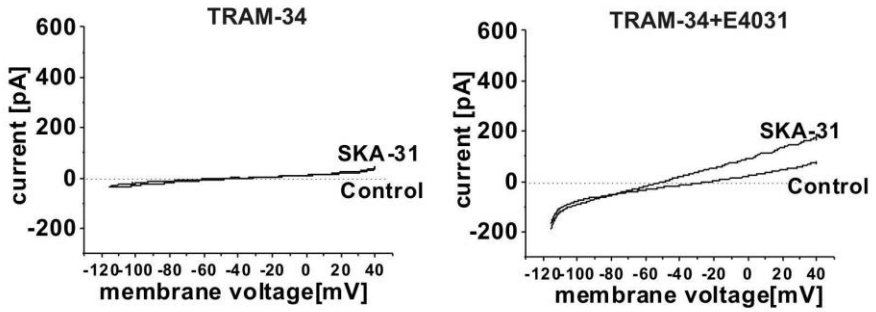

Ril
SKA
E
number of cells x10^4
number of cells x 10^4
time (hours)
0
24
48
72
96
1
2
3


E
K_v11.1-silenced
K_Ca3.1-silenced
Cis
number of cells x 10^4
P=0.0007
P=0.0007
P=0.0038
P=0.01137
Con
-
Ril
SKA
E
0,0
0,5
1,0
1,5
2,0
2,5
3,0

# hERG1 blockade leads to increased $K_{Ca}3.1$ expression and thereby stimulated Cisplatin uptake.

D

| | Number of cells with active $K_{Ca}3.1$ current/total cells (%) | Slope fold variation | Current density (pA/pF) |
|---|---|---|---|
| Control | 16/18 (89%) | 3.4 ± 0.6 | 22.1 ± 2.4 |
| E4031 | 14/18 (78%) | 5.1 ± 0.6 | 42.2 ± 2.5 |
| TRAM-34 | 0/11 (0%) | - | - |
| TRAM-34 +E4031 | 5/7 (71%) | 1.7 ± 0.2 | 3.2 ± 0.8 |







E

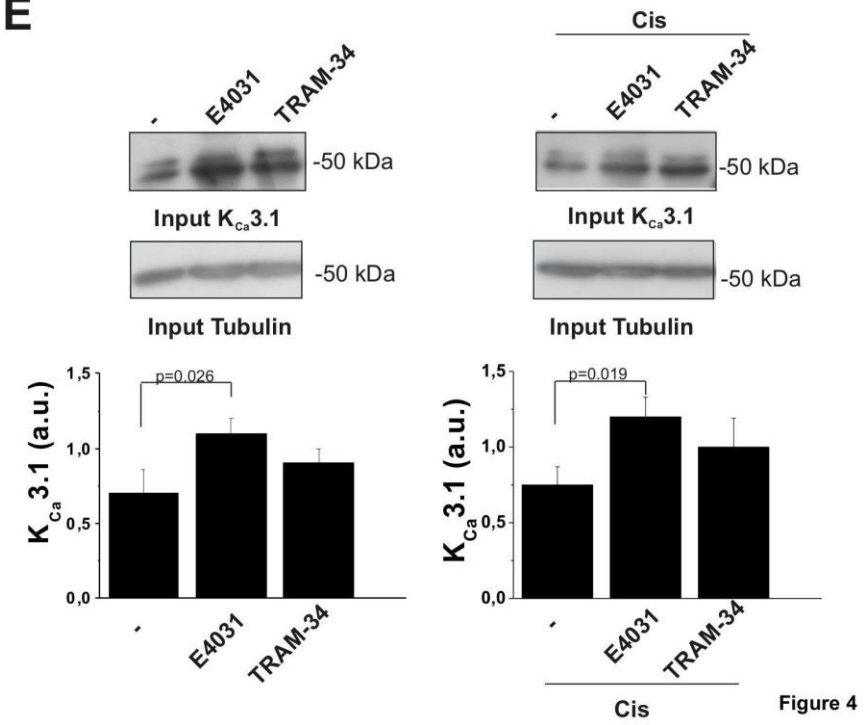
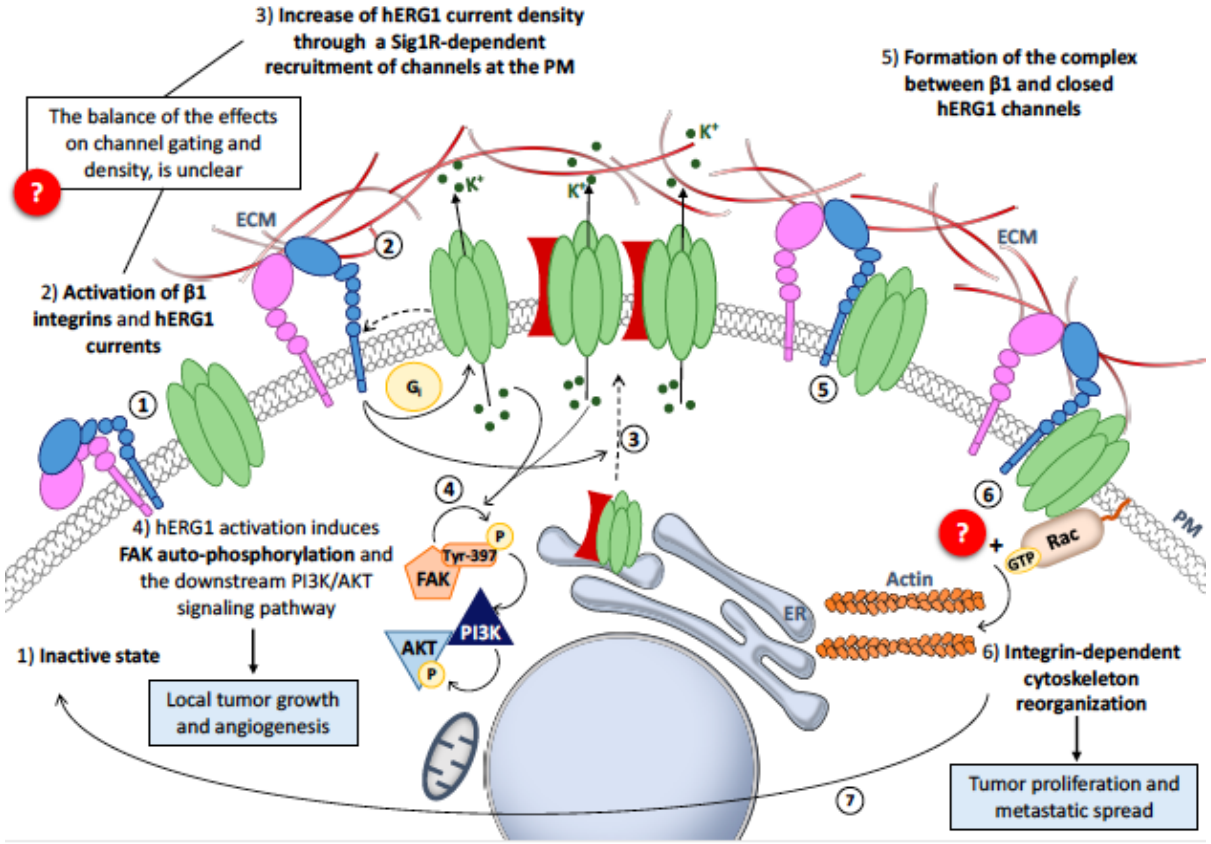
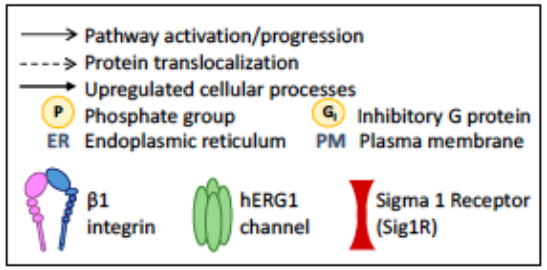



Figure 4

# hERG1 /β1

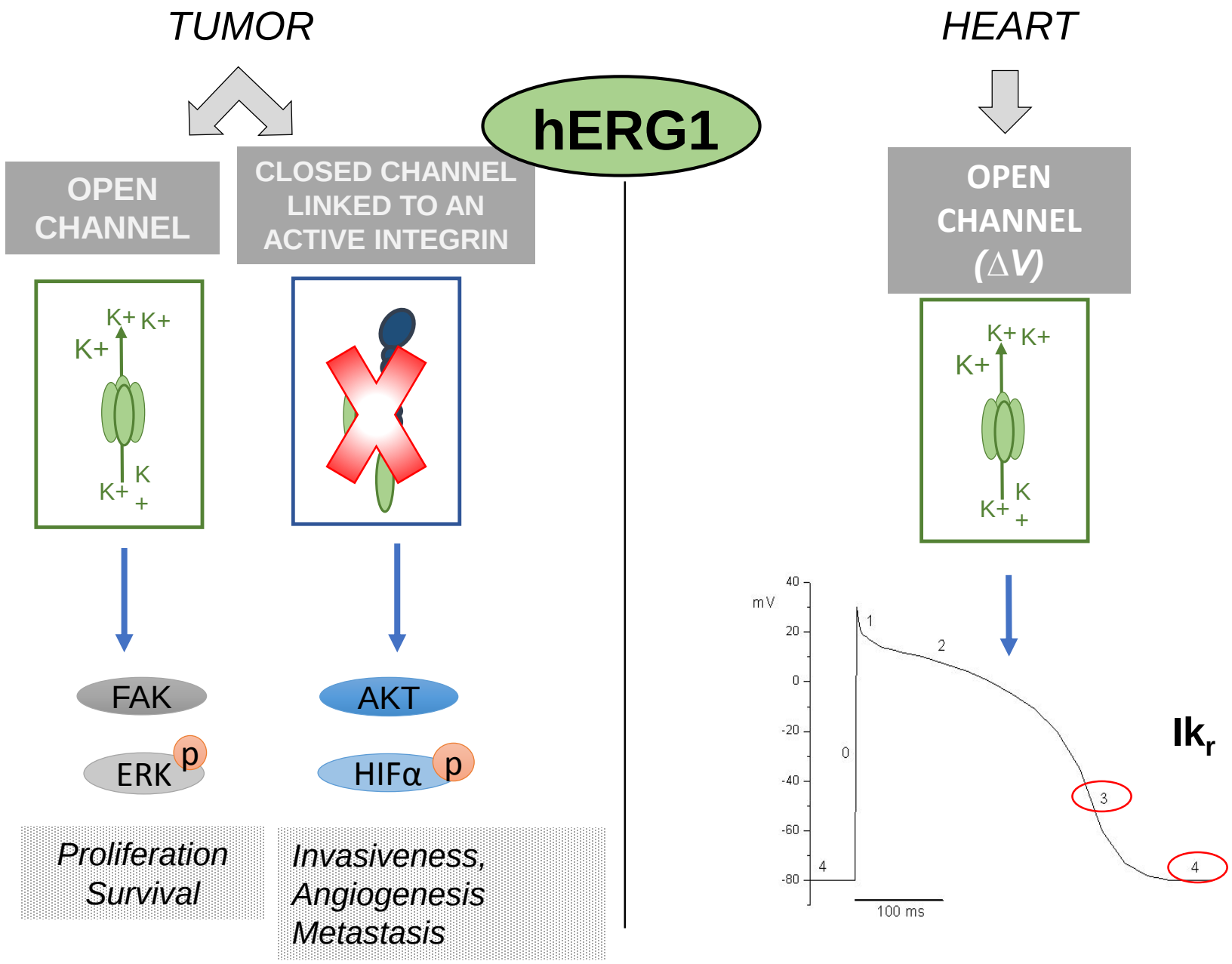


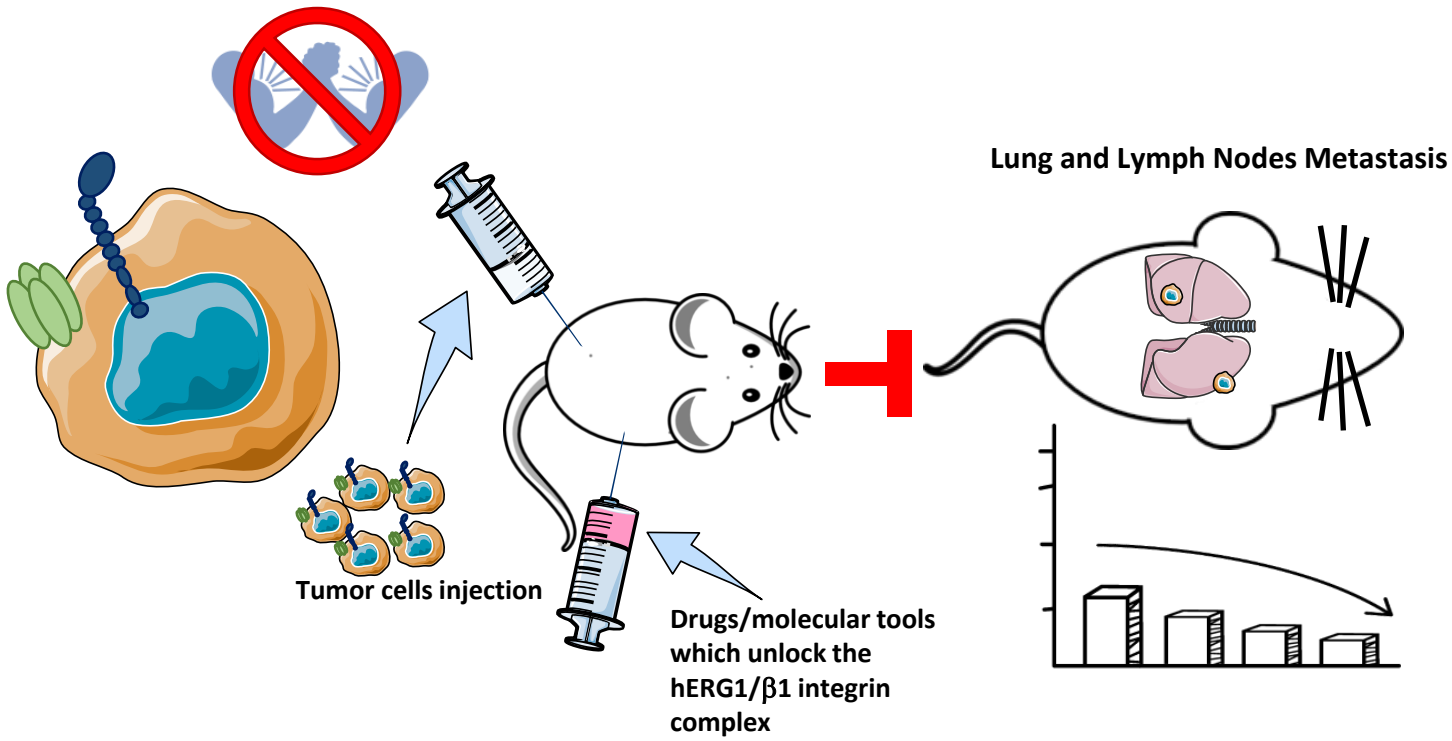

**hERG1/β1 activates cancer-specific intracellular pathways (PI3K/AKT, HIF 1/2 α, small GTPases (Rac1), f-actin remodeling)**

Becchetti A et al., Science Signaling, 2017
Becchetti A, Petroni G and Arcangeli A, Trends Cell Biol., 2019

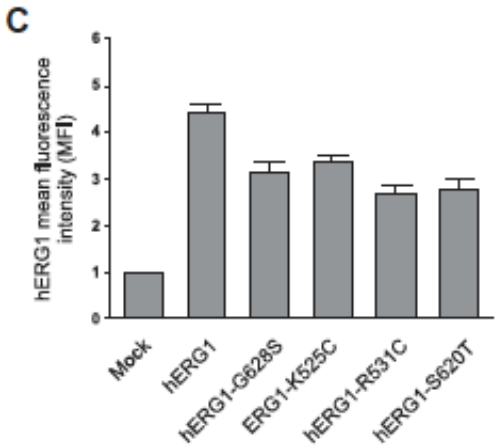
https://www.youtube.com/watch?v=eqj2NXzvmps

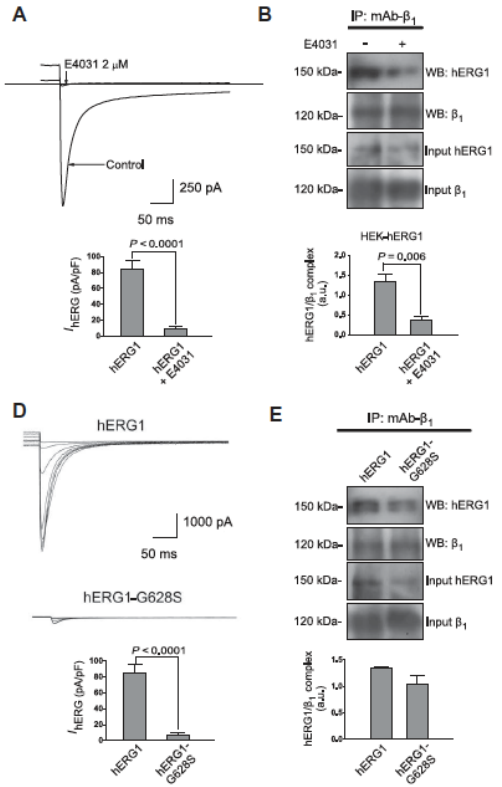

TUMOR
HEART
hERG1
OPEN CHANNEL
CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN
OPEN CHANNEL (ΔV)
K+
FAK
ERK
p
AKT
HIFα
p
Proliferation
Survival
Invasiveness,
Angiogenesis
Metastasis
mV
40
20
0
-20
-40
-60
-80
0
1
2
3
4
4
100 ms
Ikr

# Disrupting the hERG1/β1 integrin complex inhibits tumor metastasis



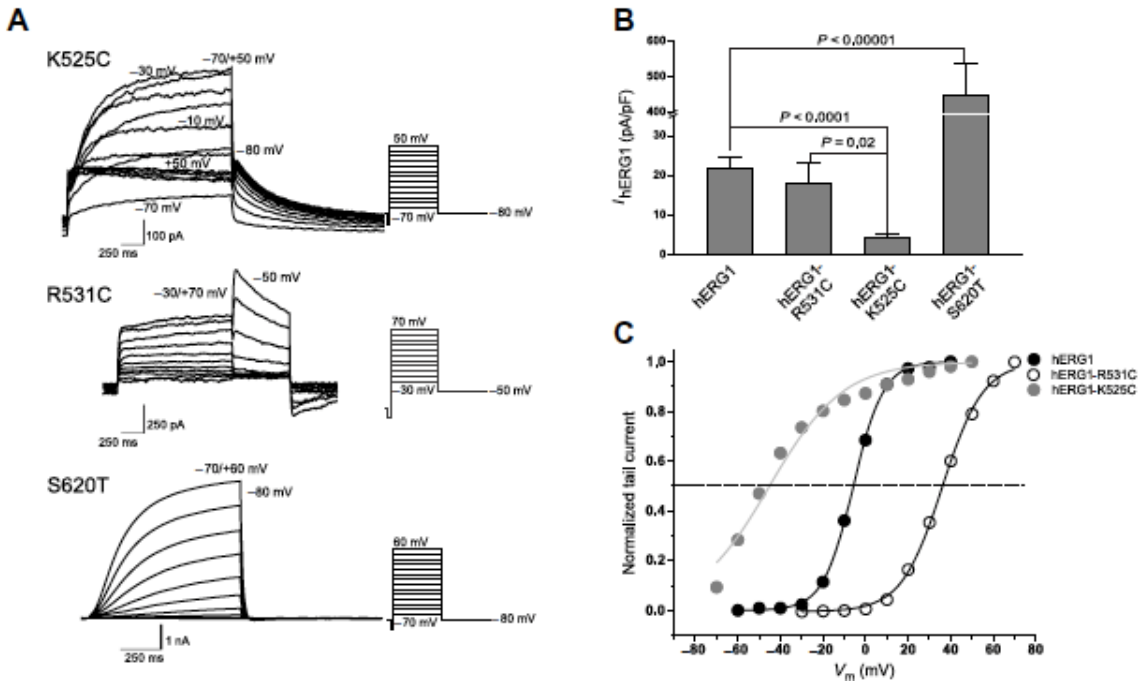
# *The hERG1 conformational state determines (the closed state favours) integrin association*



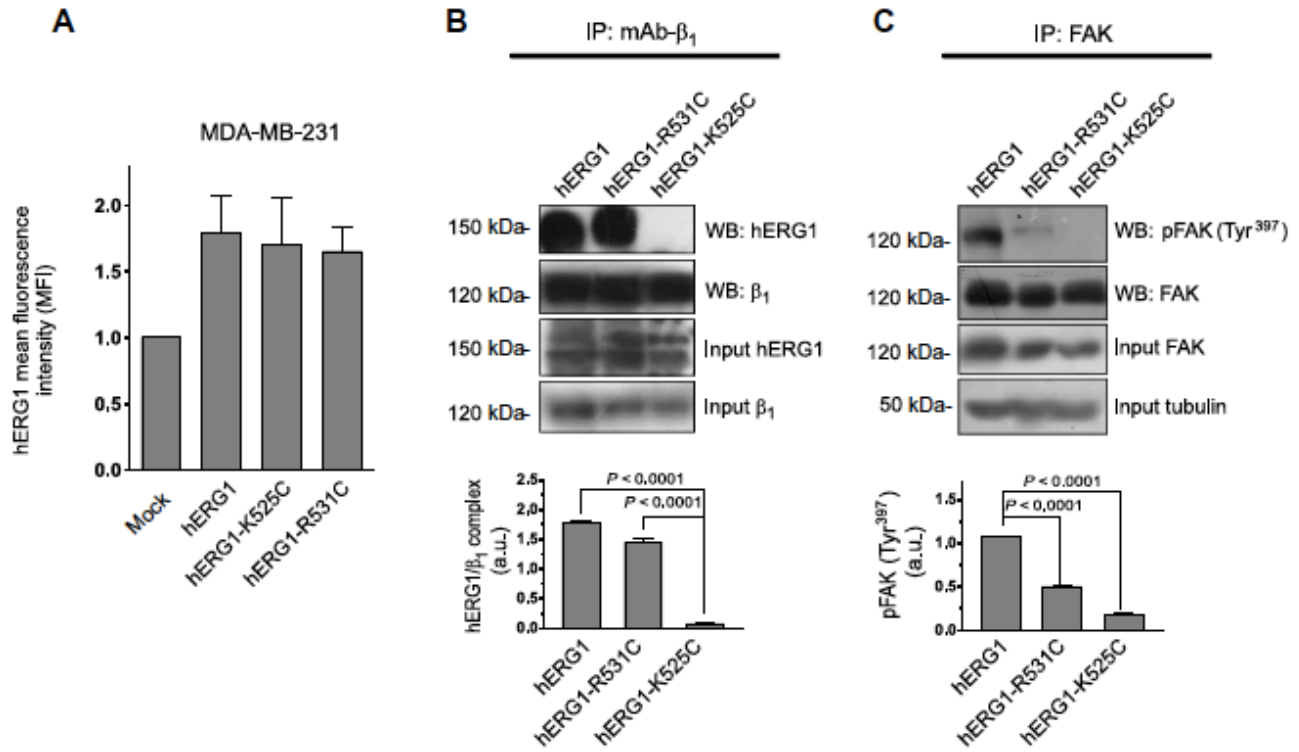




*hERG1 mutants:*
*G628S: non conductive*
*S620T: non inactivating*
*K525C: S4 (voltage sensor) mutant**
*R531C: S4 (voltage sensor) mutant**
**=alterations of gating*

# *The hERG1 conformational state determines (the closed state favours) integrin association*



*The hERG1 conformational state determines (the closed state favours) integrin association: MDA-MB-231 breast cancer cells*

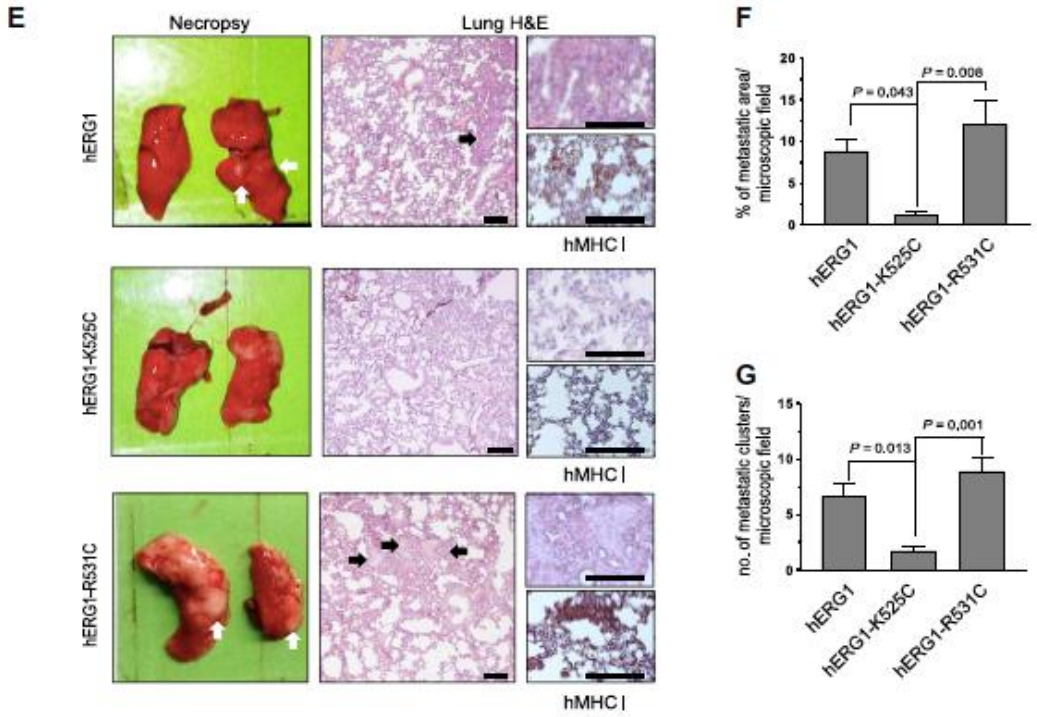


*The hERG1 conformational state determines (the closed state favours) integrin association…...and tumor metastasis (MDA-MB-231 breast cancer cells)*

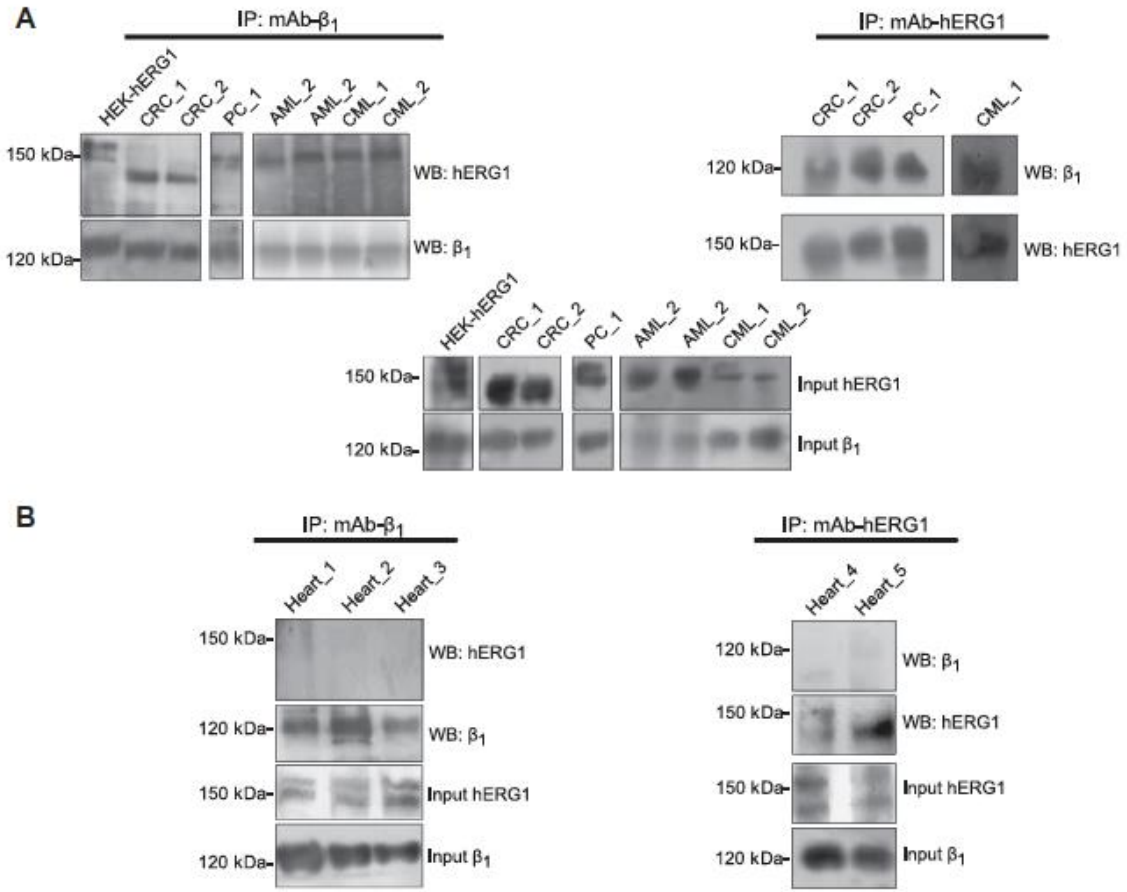
**D**

| MDA-MB-231 | hERG1 | hERG1-K525C | hERG1-R531C |
|---|---|---|---|
| Local tumor growth | | | |
| Number of tumor masses (%) | 9/10 (90%) | 10/10 (100%) | 9/10 (90%) |
| Median tumor volume ($mm^3$) | 150 (19–300) | 122 (33–300) | 212 (33–300) |
| Metastases | | | |
| Inguinal lymph nodes<br>Number of mice with macroscopic metastases (%) | 2/5 (40%) | 0/5 (0%) | 3/5 (60%) |
| Lung<br>Number of mice with macroscopic metastases (%) | 2/5 (40%) | 0/5 (0%) | 4/5 (80%) |

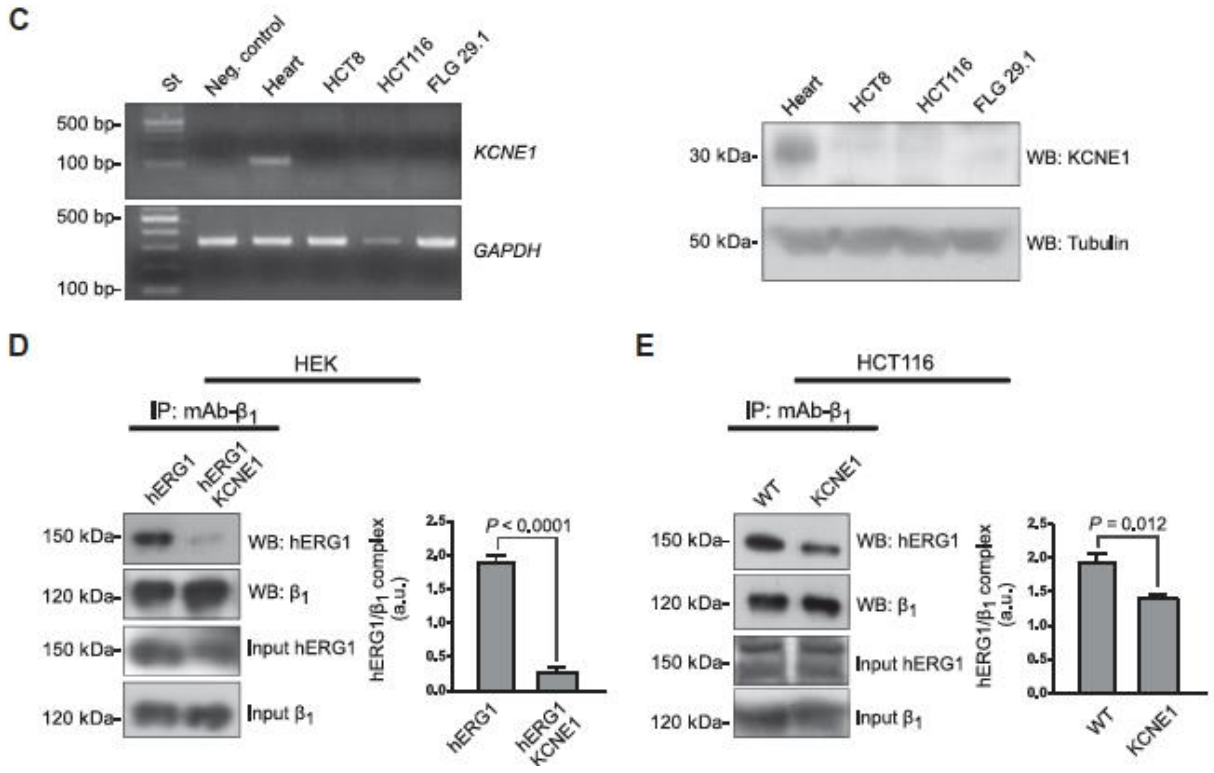
*The hERG1 conformational state determines (the closed state favours) integrin association……and tumor metastasis (MDA-MB-231 breast cancer cells)*



# *The hERG1/β1 complex occurs in tumour cells, but not in the heart*



# … because tumour cells do not express “canonical” (KCNE1) beta subunits



# PIPELINE DEVELOPMENT STRATEGY

## DESIGNING NEXT GENERATION ANTIBODY MOLECULES FROM BIG MONOCLONAL ANTIBODY TO SMALL BISPECIFIC DIABODY

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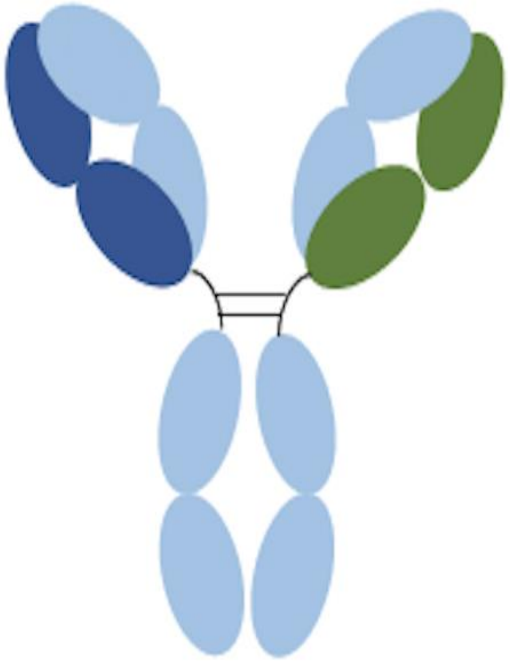
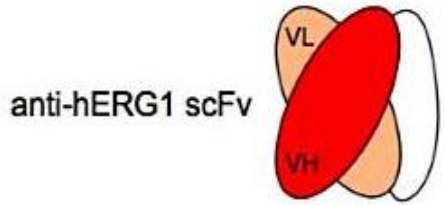
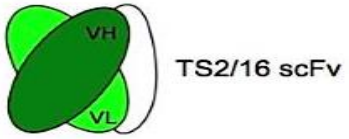


***Anti-hERG1 Monoclonal Antibody (M.W. 160 kDa)***

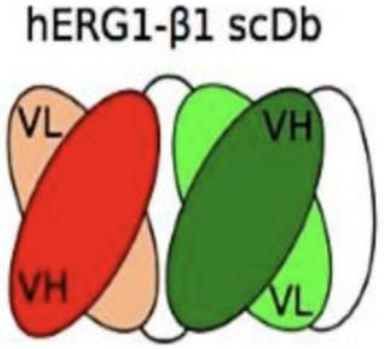



***Anti-hERG1 Single-Chain Variable Fragment (Sc-FV)***

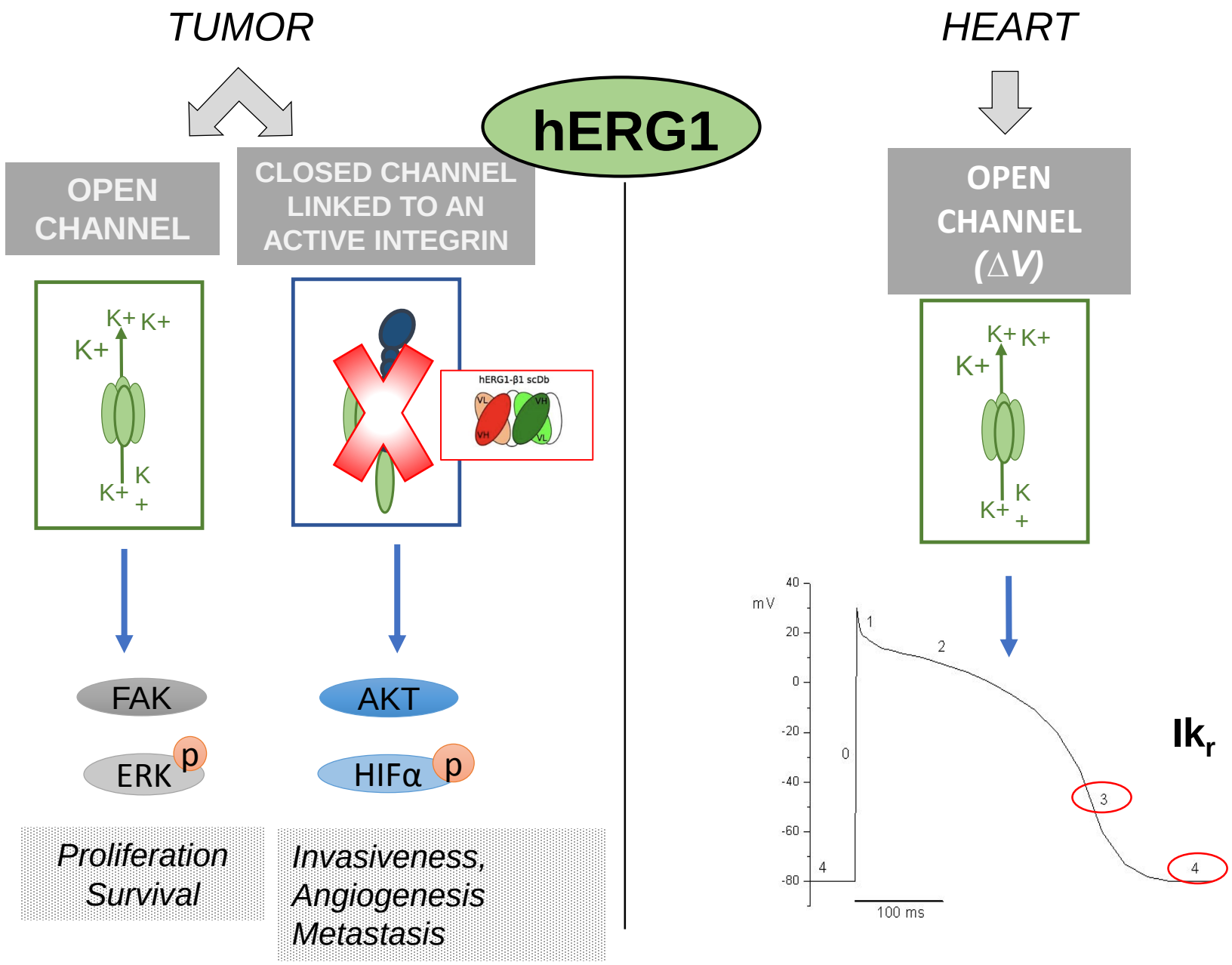



***Anti-integrin beta1 Single-Chain Variable –Fragment (Sc-fV)***

***Anti hERG1/integrin beta1 BISPECIFIC Antibody (DIABODY) (M.W. 50 kDa)***




**THERAPEUTIC ANTIBODY**
MCKAA2017THE01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40
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-20
-40
-60
-80
100 ms
Ikr

# CONCLUSIONS

- Ion channels are relevant in cancer (biomarkers!)
- Ion channels can exert both conductive and non conductive effects in cancer cells
- hERG1 has both conductive (ion flux-mediated) and non conductive (once bound to integrin receptors in the closed state) activities
- hERG1 mediates tumour progression (e.g. proliferation, invasion, angiogenesis, metastasis….)
- hERG1 can be considered a novel cancer biomarker
- The hERG1/beta1 integrin complex can be considered a therapeutic target in cancer and can be targeted through newly developed bispecific antibodies

# Acknowledgements:

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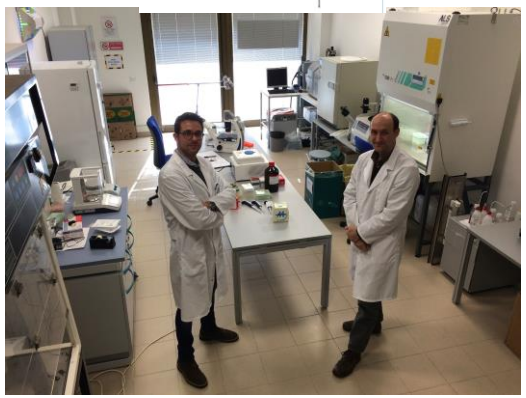
Dept. Medical Oncology
AOUC Firenze

Prof. R. Coppola
Dept. General Surgery
Campus Biomedico Rome



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Di.V.A.L. TOSCANA

ITT Istituto Toscano Tumori

AIRC ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO
Con la ricerca, contro il cancro

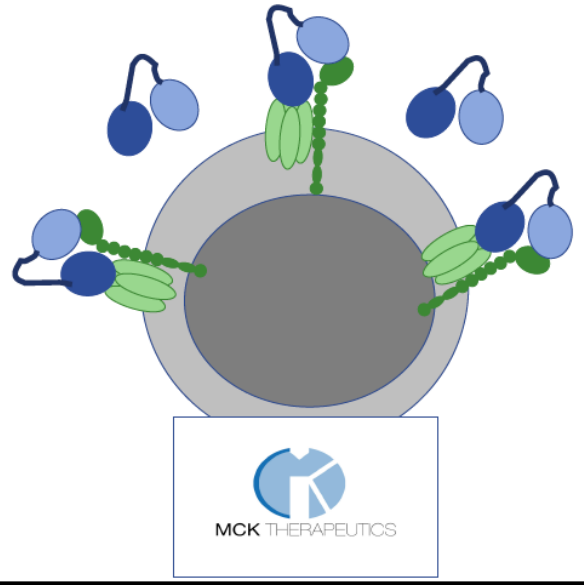
Supported By:
ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH

# MCKAA2017THE01

**MCKAA2017THE01** is **a highly innovative BISPECIFIC SINGLE CHAIN DIABODY molecule able to target the hERG1/β1 biomarker in pancreatic cancer.**

**The DIABODY MCKAA2017THE01 has a double antigenic binding sites within the pancreatic cancer-specific antigen hERG1/β1,**

**while DOES NOT bind hERG1 in cardiac myocytes**



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# Besides **MCKAA2017THE01**

## Novel companion diagnostics linked to MCKAA2017THE01

# MCK THERAPEUTICS APPROACH TO PRECISION CARE OF PANCREATIC CANCER

## DEVELOPING A FULL SET OF PRODUCTS FOR THERAPY, SCREENING & EARLY DETECTION OF PDAC

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### DIAGNOSTIC COMPANIONS

**MCKAA2018DIA03**

IN VIVO MOLECULAR IMAGING

**MCKAA2017DIA01**

IN VITRO (IHC)

**MCKAA2017DIA02**

IN VITRO (IF)

### THERAPEUTICS

MICROMETASTASIS

PANCREATIC CANCER

**MCKAA2017THE01**

SINGLE/COMBINATION TREATMENT AGENT

EARLY/ADVANCED (METASTATIC) STAGES

# *Acknowledgements:*


**Prof.A. Becchetti University of Milano Bicocca, Italy**

**Prof. F. Di Costanzo Dept. Medical Oncology AOUC Firenze**

**Prof. R. Coppola Dept. General Surgery Campus Biomedico Rome**

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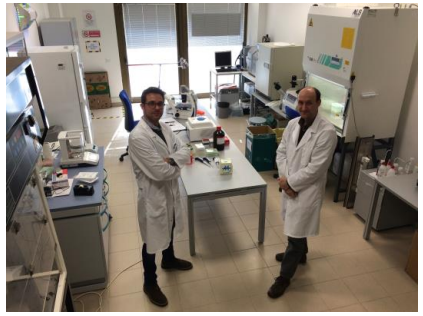

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IMPROVING **HEALTH & QUALITY OF LIFE** OF PATIENTS WITH **HUGE UNMET MEDICAL NEEDS**