Carbonic IX inhibitors: a new class of targeted agents

Philippe Lambin

1. Radiotherapy + targeted agents = *Promising*

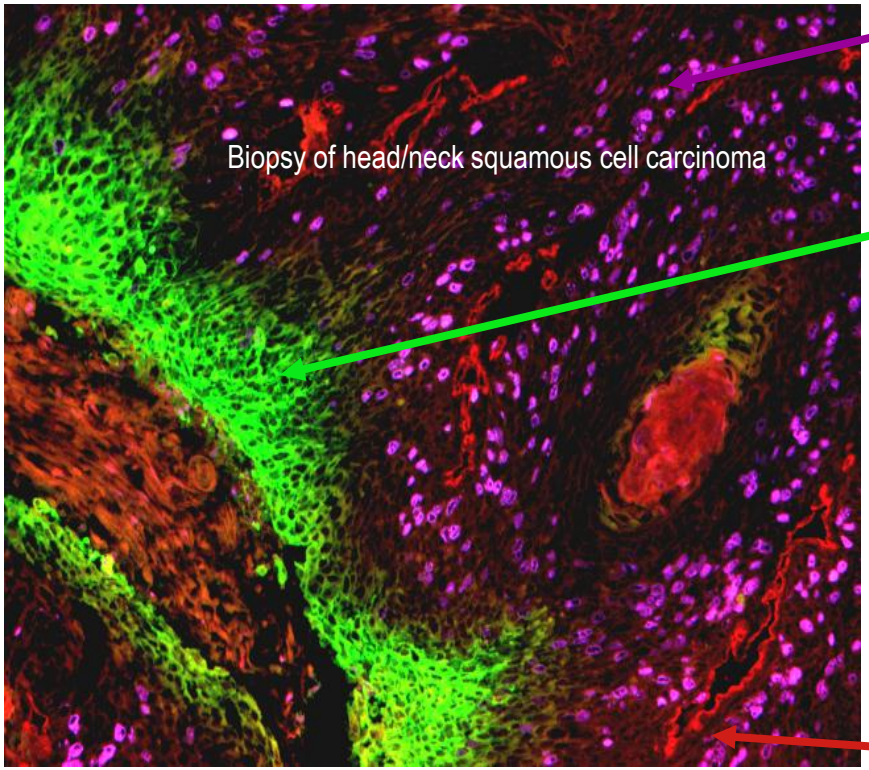
2. Collaboration of Radiation Oncology community + Pharmaceutical companies = *Suboptimal*

3. The Radiation Oncology community should invest in *the design & development* of « smart » radiosensitizers

What are the requiremenst of the « perfect drug »?

1. Supra-additive effect with radiation
2. Low toxicity, tumour specific
3. Oral administration
4. Biomarkers (tissues, blood, imaging…)
5. Known target
6. *Mutifunctional* with known mechanisms of actions

Hypoxia a feature of solid tumours



proliferating cells (IdUrd +)

Hypoxia (pimonidazole +)

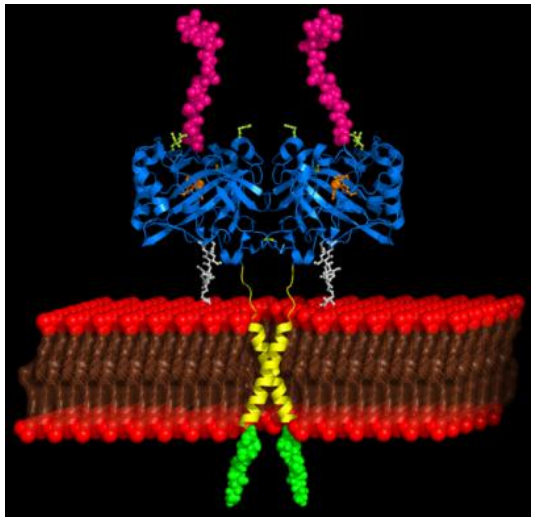
Consequences for the patient?

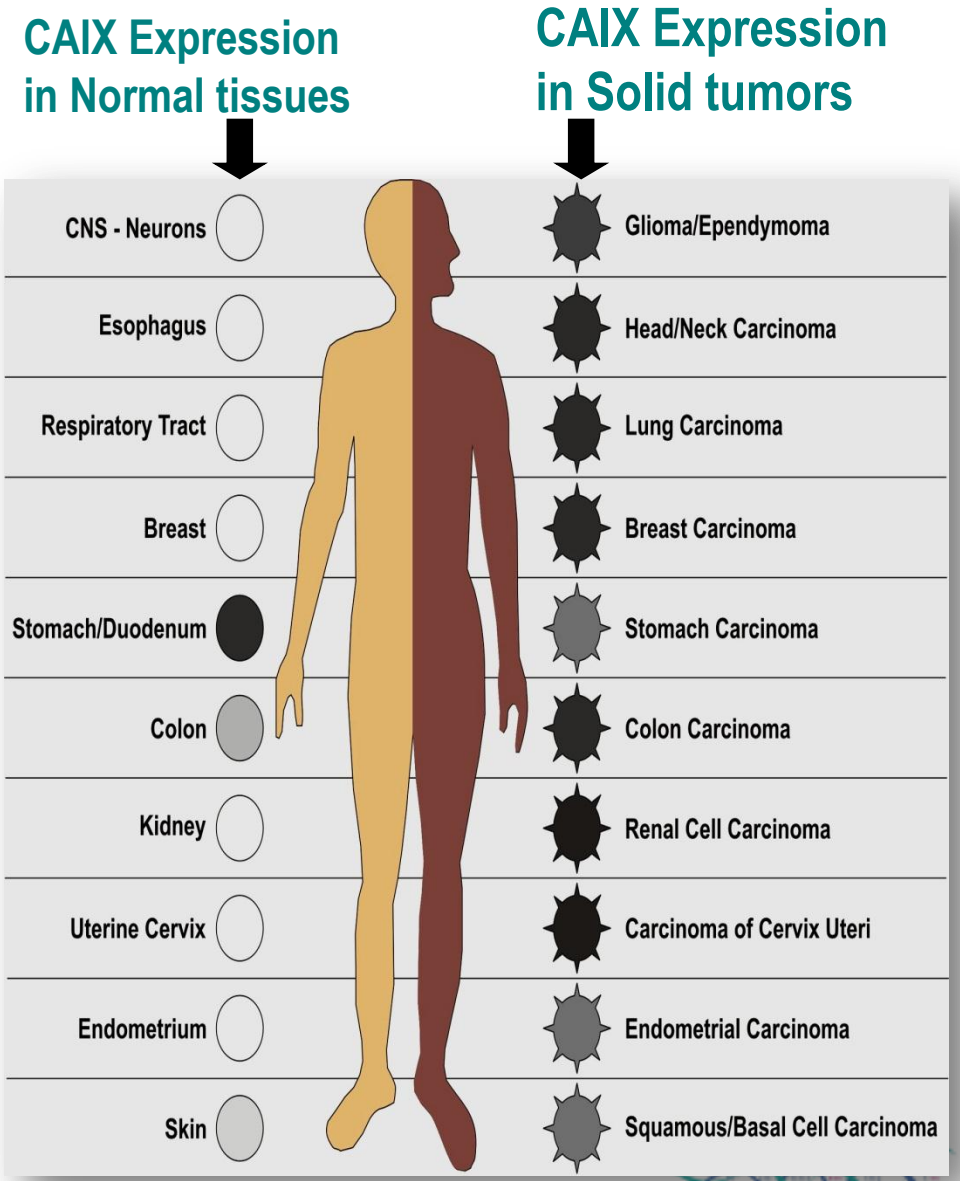
- less sensitive to therapy
- more metastasis
- increased mortality

blood vessels

Carbonic Anhydrase IX: a therapeutic target?

Hypoxic CA IX
= functional + Dimer

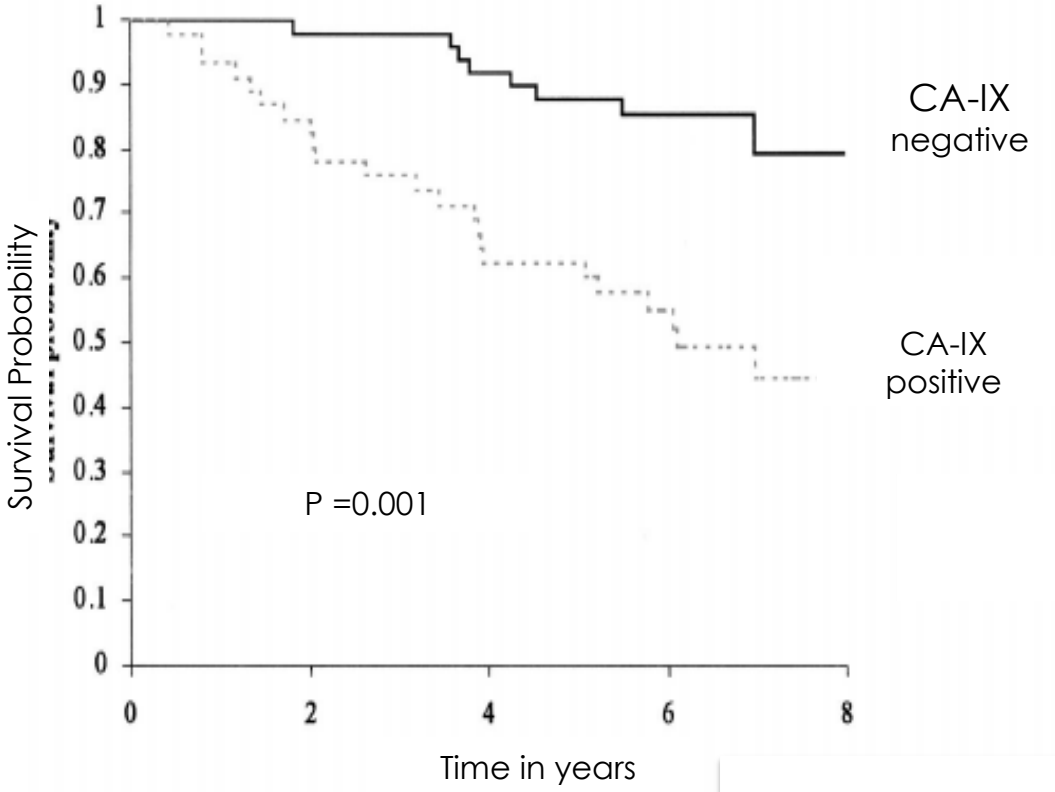




Oosterwijk et al, 1986-
Parkkila et al, 1997-
Harris et al, 2000-
>400 papers

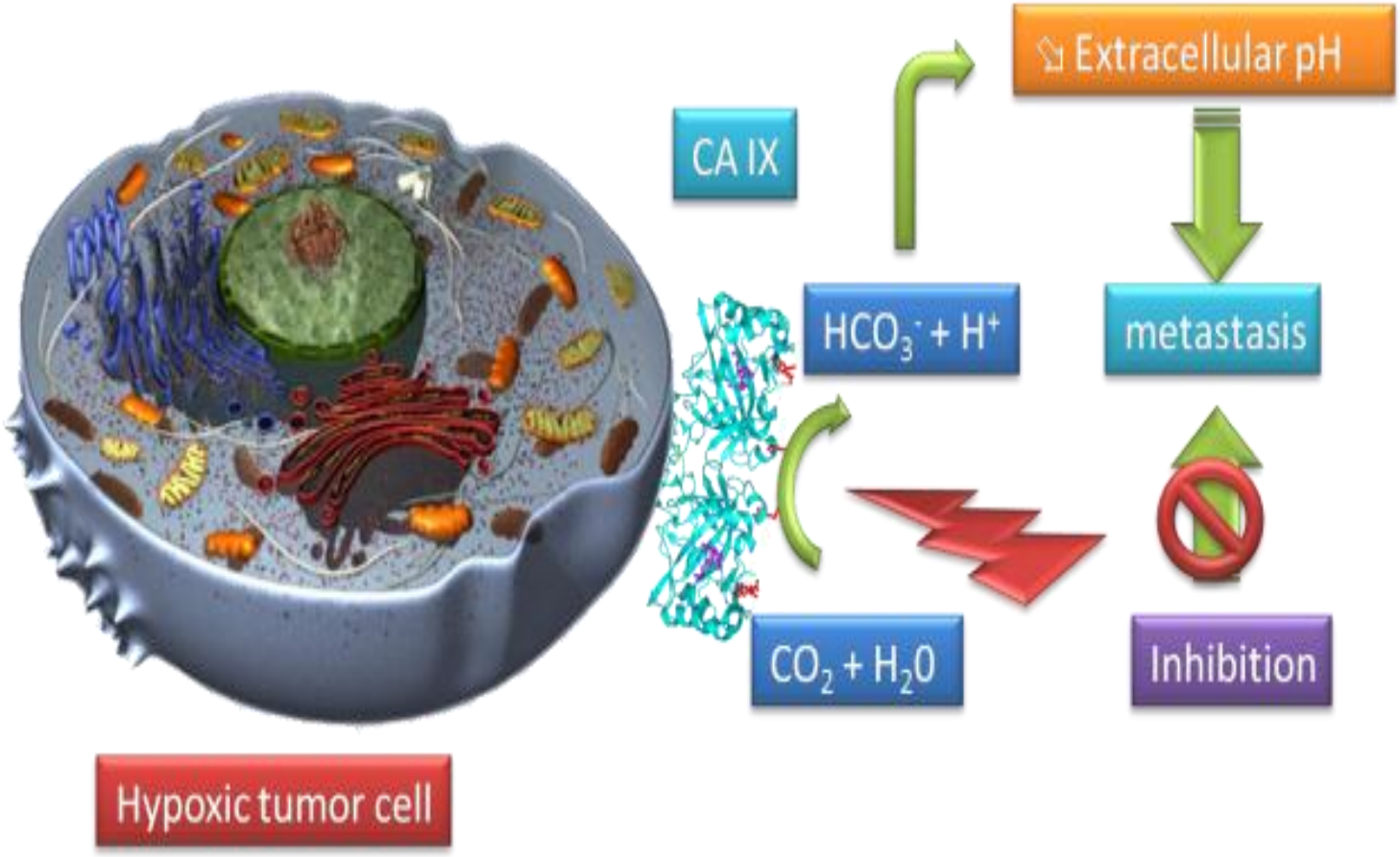
CAIX expression has proven to be an indicator of overall survival

(i.e. the higher the level of expression of CAIX in a tumour, the lower the chances of survival).



CAIX plays a key role in the biology of tumours. Patients with a high CAIX expression have a worse survival rate in most of solid cancers (see Chia et al. 2001).

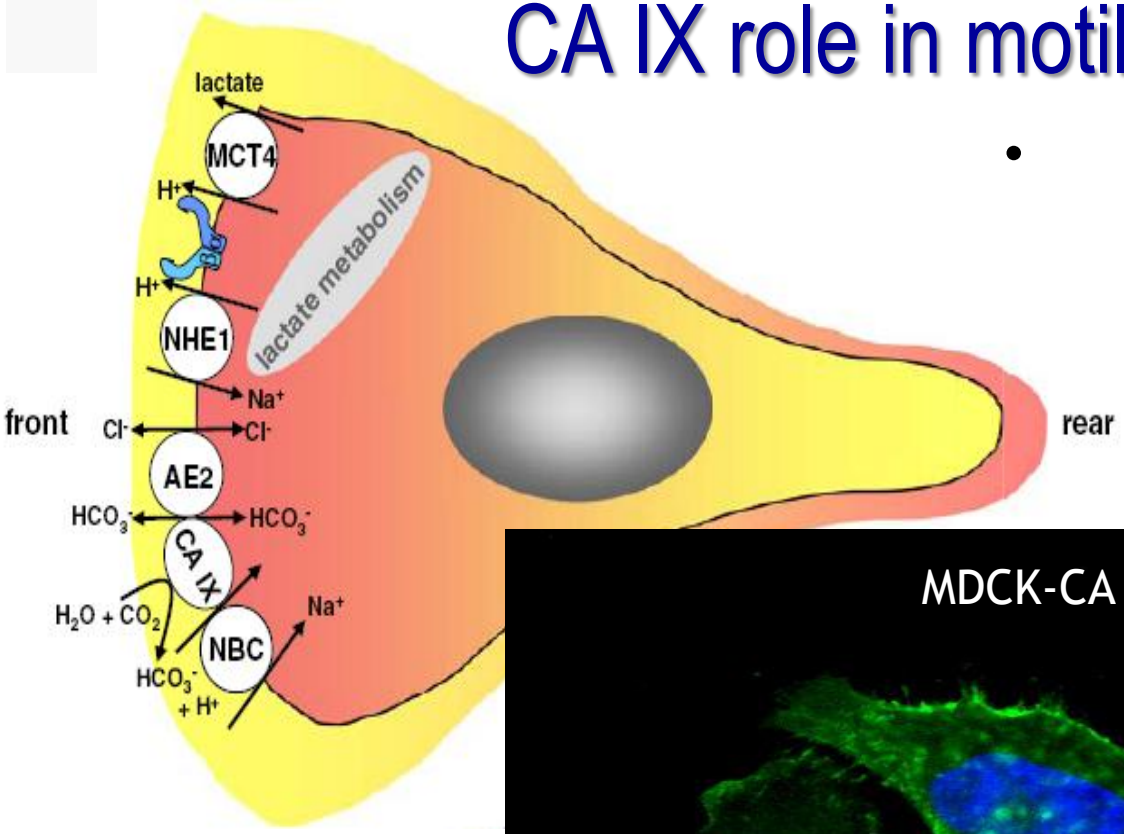
Tumor associated CAIX : Regulate pHi & pHe

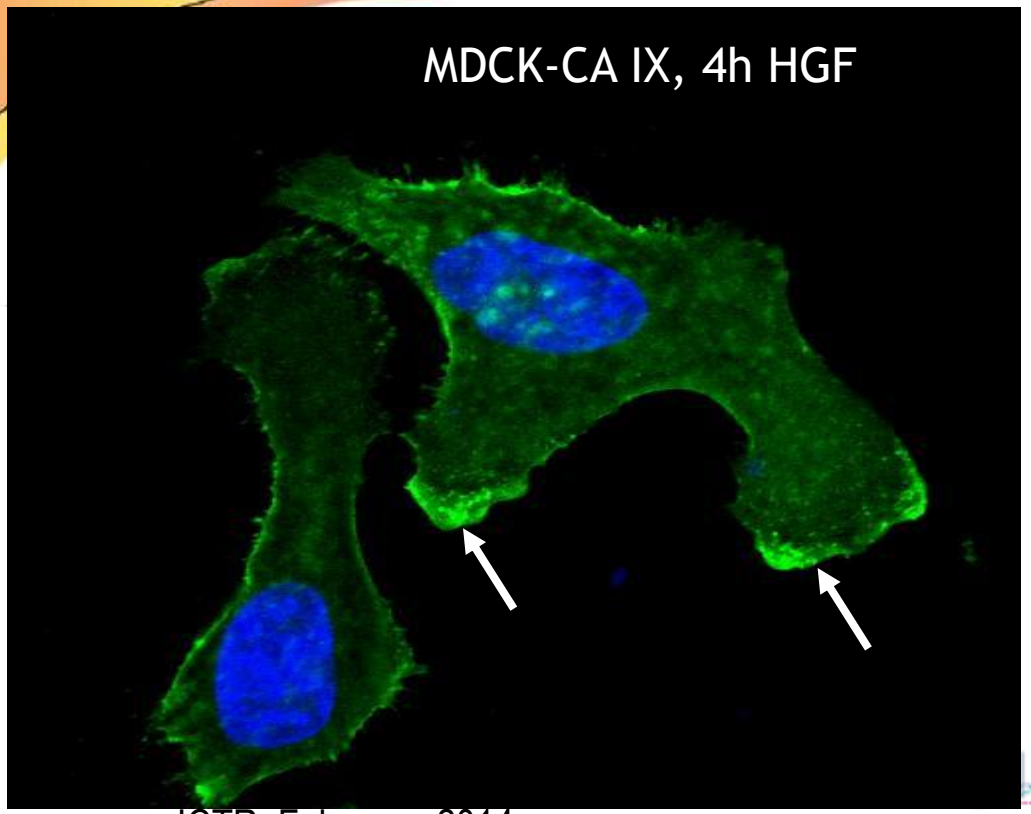


Med Res Rev. **2008**, *28*, 445-63.

Oncotarget. **2012**, *3*, 84-97.

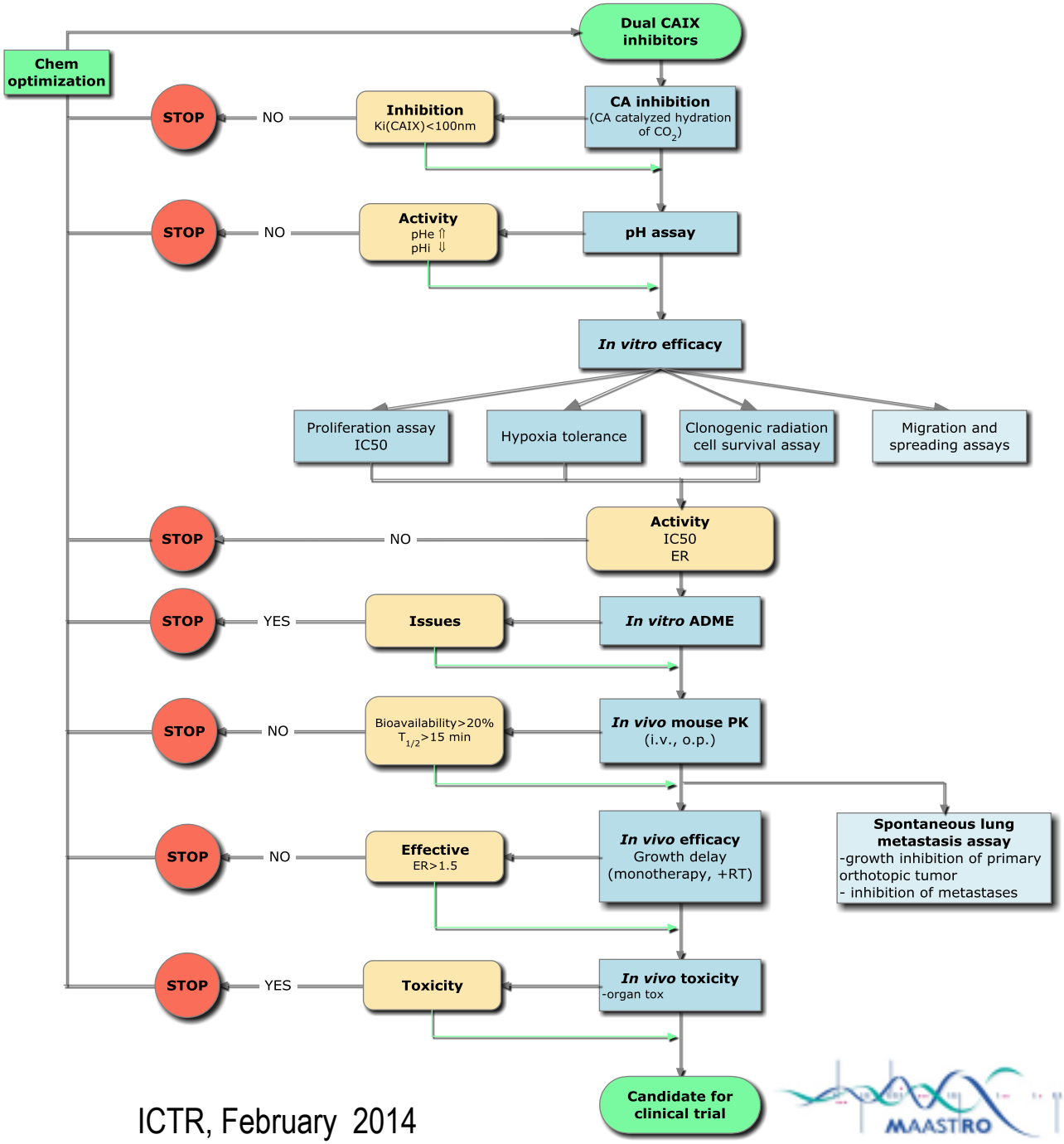
CA IX role in motility & invasion



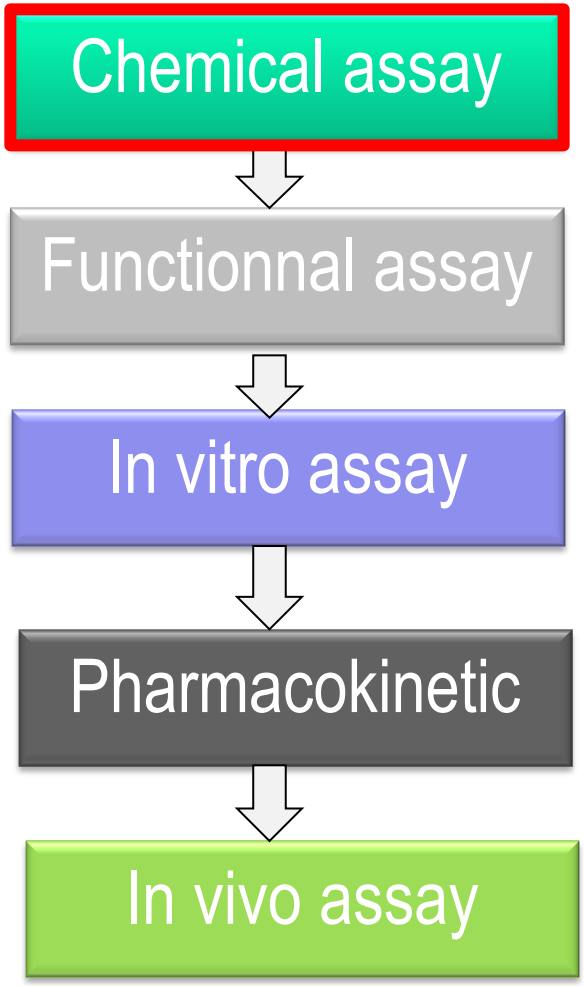


A program to screen compounds and select a development candidate for Phase I clinical evaluation.

For a compound to be considered for clinical evaluation it should be specific, active, effective, safe as well as it should have favorable pharmacokinetic profile. A number of in vitro and in vivo assays are employed at different steps of the screening program to allow prioritization of the compounds according to the balance of their properties.

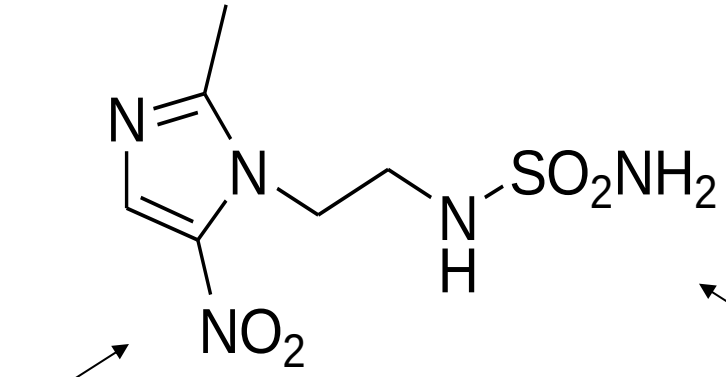


Simplified screening pathway



Rami et al, J Med Chem 2013

Dual drugs: the leading coumpound = DH 348



5-nitroimidazole

- radiosensitizers hypoxia targeting

- nimorazole: clinically relevant doses without toxicity resulting in important benefits

sulfamide

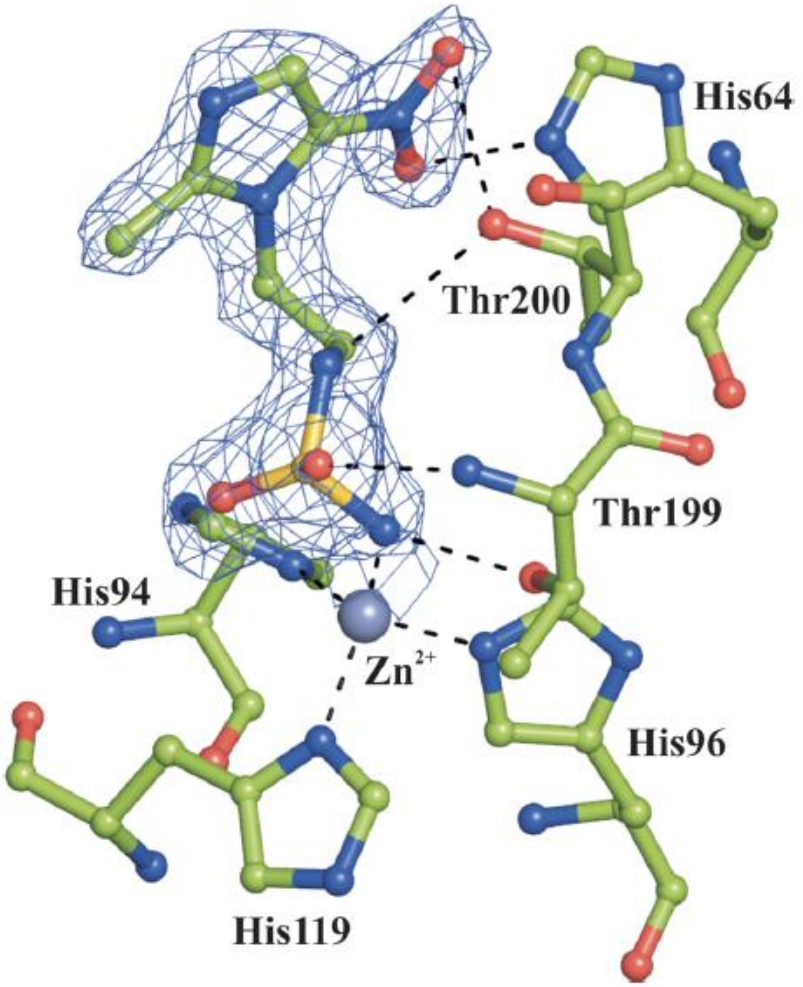
- CAIX targeting

- more targeting tumors compared to normal healthy tissue

- ability to reduce extracellular acidosis

+ negative controle: separate single moieties

Sulfamide blocks the CA active site



Rami et al, J Med Chem 2013

CA inhibition @ chemical assay: low nanomolar efficacy

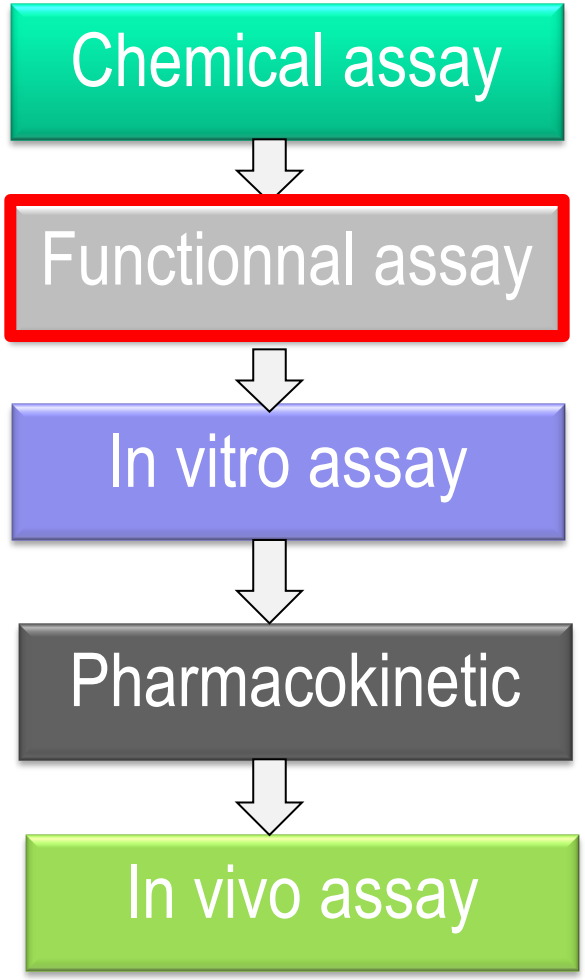
J Med Chem 2013

compd	K_I (nM)[b] hCA I[c]	hCA II[c]	hCA IX[d]	hCA XII[d]	K_I hCA IX	K_I hCA XII
AAZ	250	12	25	5.6		2.14
4a	3203	330	70	64		5.15
4b	107	37	7.9	8		4.56
4c	79	4.8	8.0			0.71
4d	101	3.8	7.3			0.47
6a	105	5.5	7.3			0.68
6b	483	7.4	7		1.02	0.96
6c	79	2.9			0.35	0.34
6d	84	6.6			0.84	0.86
7	9576	10.1			0.49	1.24
9	4435	33.2		8.9	4.07	3.79
11	9120			38	1.41	1.52
12	>20000			>20000		
13	>20000			>20000		
15	8710		21	37	2.47	1.40

[a]Selectivity ratios for [illegible] associated (CA IX and XII) over the cytosolic (CA II) isozyme are also reported. [b]Errors in the range of ±5−10% [illegible] different determinations. [c]Full length, cytosolic isoform. [d]Catalytic domain, recombinant enzyme.

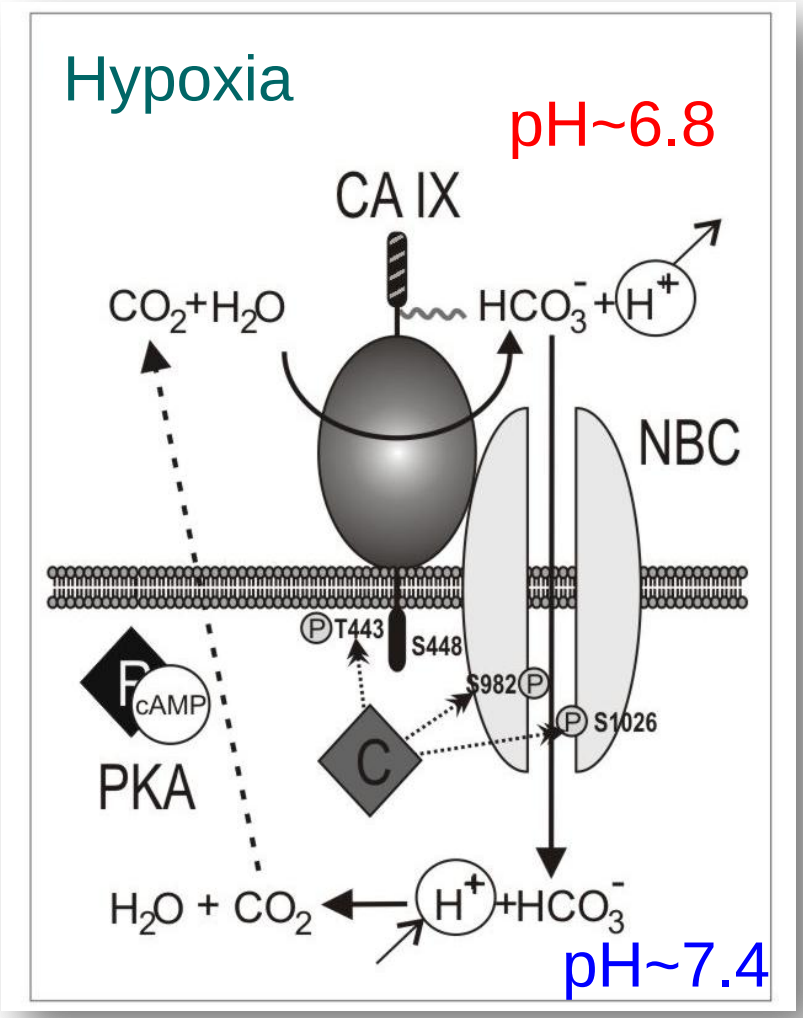
DTP348: Ki 20nM CAIX, 8 nM CAXII

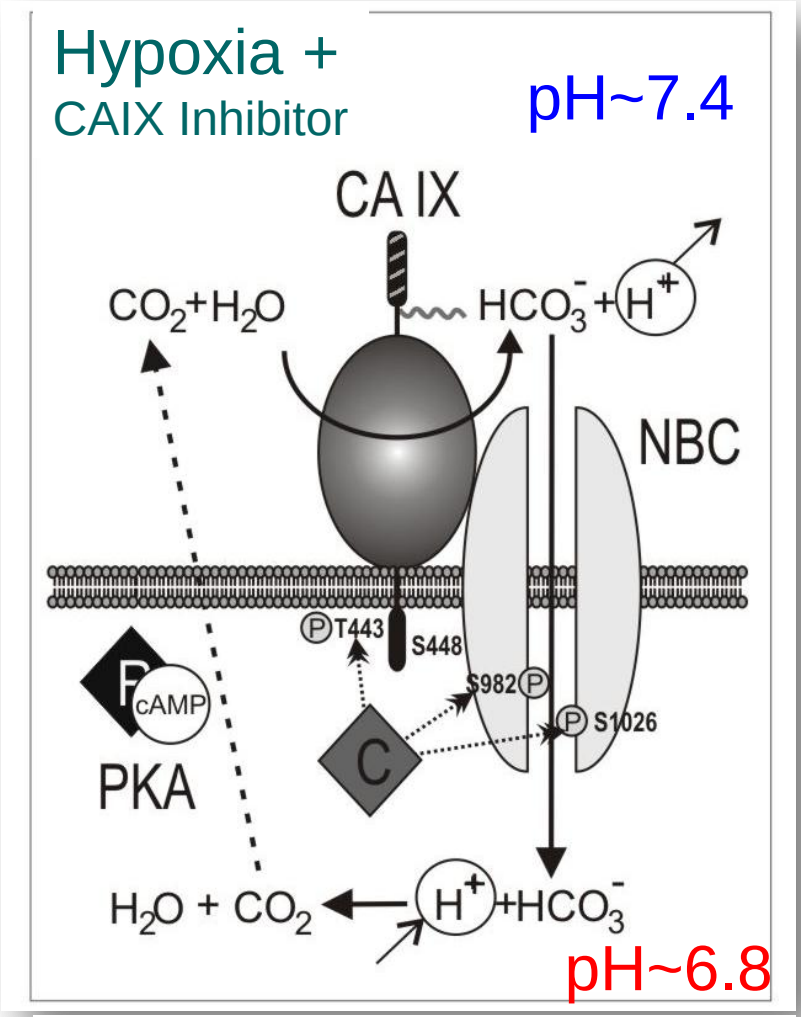
Simplified screening pathway



Rami et al, J Med Chem 2013

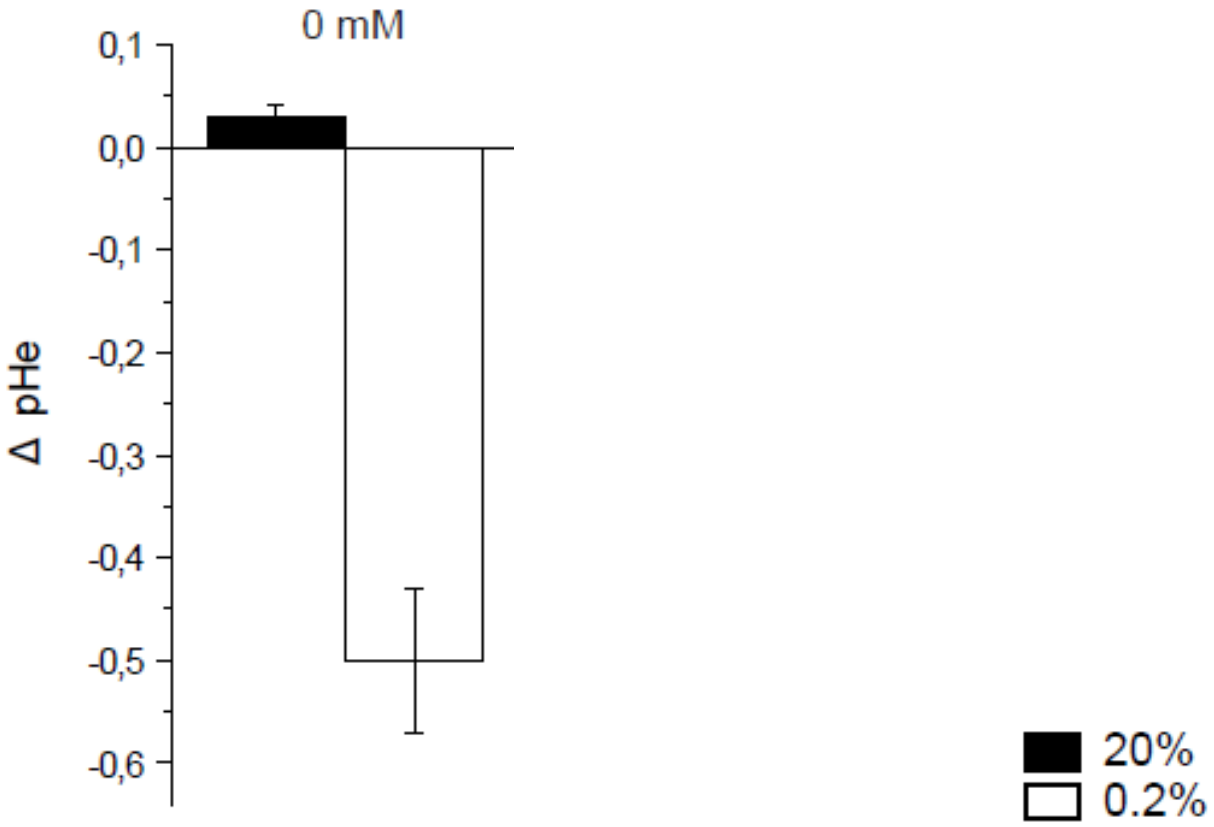
CA IX role in tumour cells



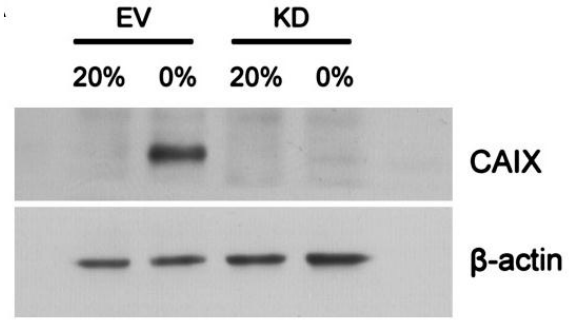


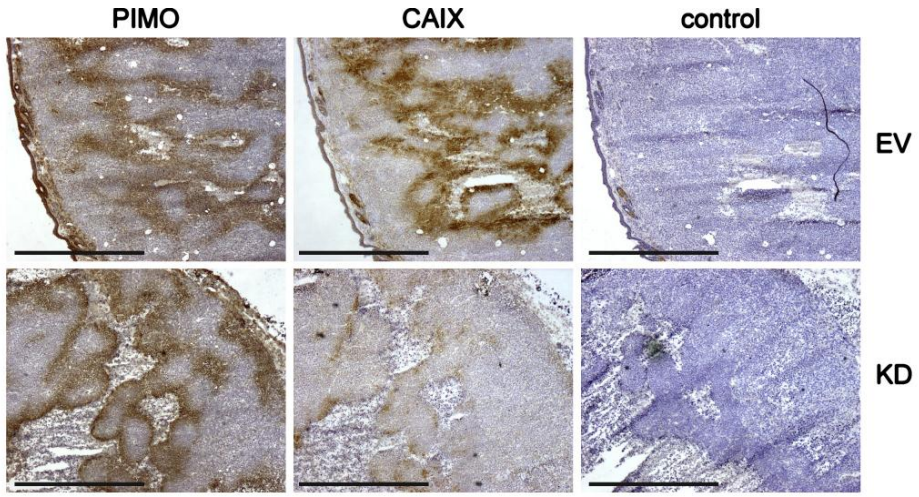
= Toxic for the cancer cell

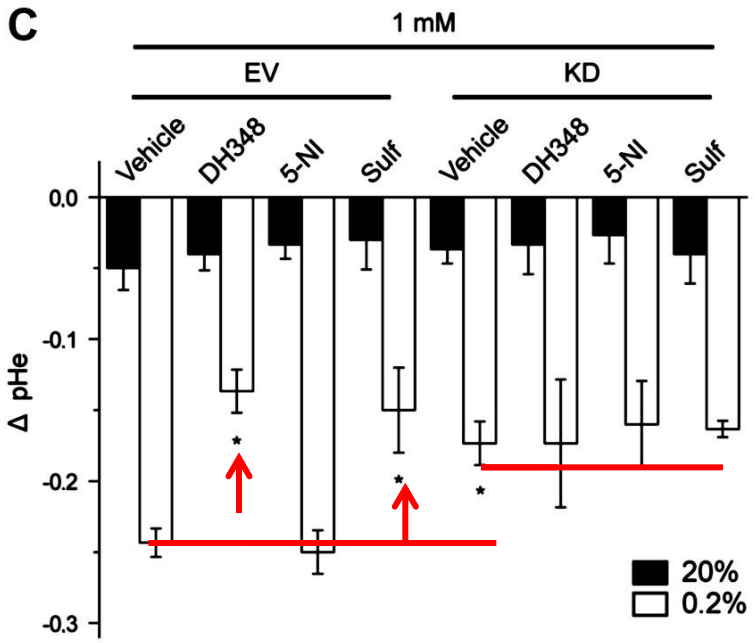
A significant dose-dependent ($P < 0.05$) reduction in hypoxia-induced extracellular acidosis of DH348 was observed, while the effect on cells exposed to ambient air was negligible



… in a CAIX dependent manner

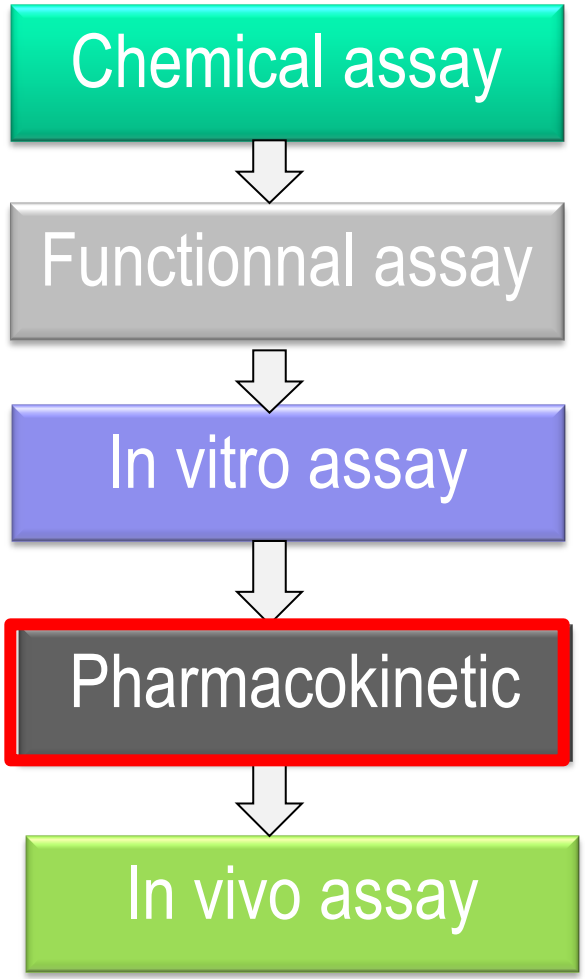






Dubois et al, Radiother Oncol 2013

Simplified screening pathway

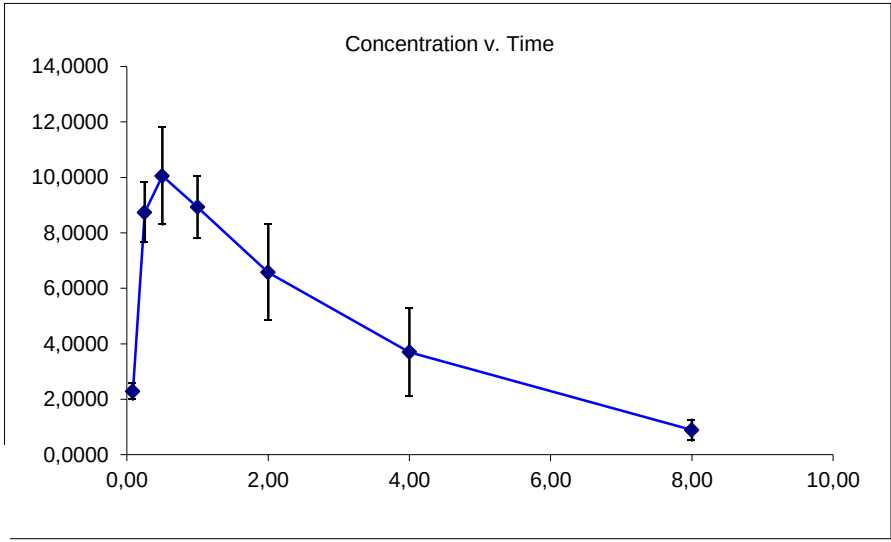


Rami et al, J Med Chem 2013

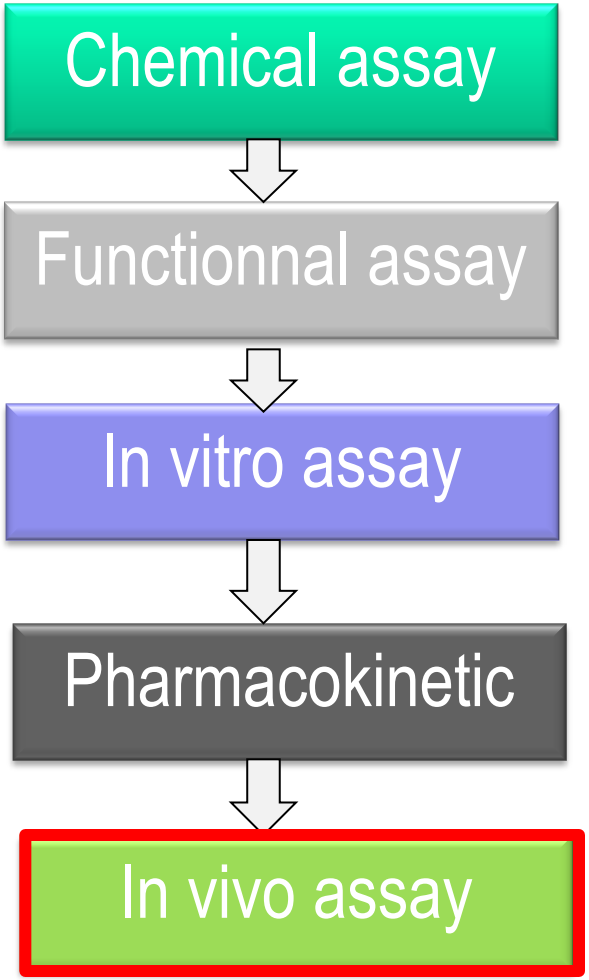
Mouse pharmacokinetics & formulation

Mouse Pharmacokinetic analysis				
Compound	IV Dose (5mg/kg)		PO Dose (50mg/kg)	bioavailability
	Total CLint (mL/min/kg)	AUC to Last (µg-hr/mL)	AUC to Last (µg-hr/mL)	
S4	278	0.306	0.161	3.00%
FC9-403	108	0.752	0.342	4.50%
FC9-398	59.9	1.4	7.85	57.60%
DH307	174.79	0.448	0.356	3.80%
DH338	-1.57	1.497	13.351	90.30%
DH348	44.31	1.829	35.294	178.90%
NKP60	10.47	8.034	78.968	96.00%

+ Oral formulation possible

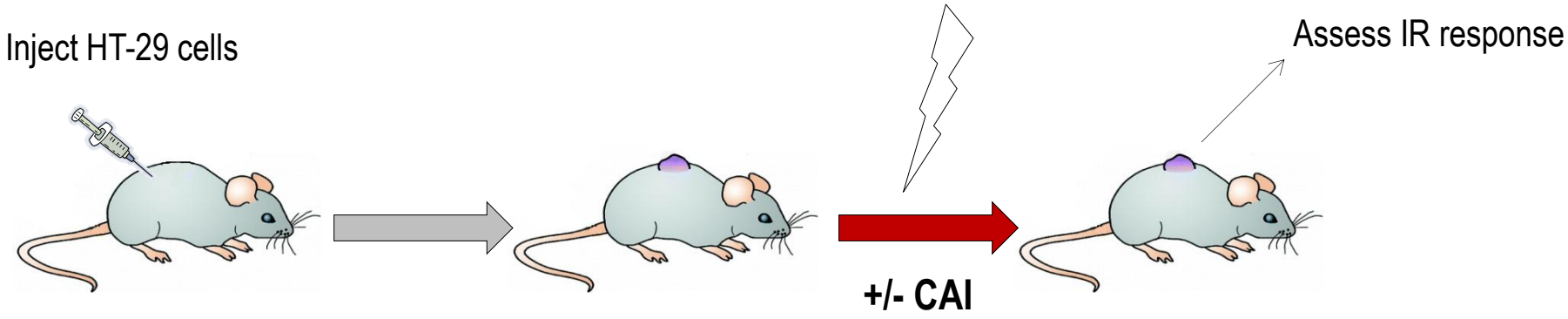


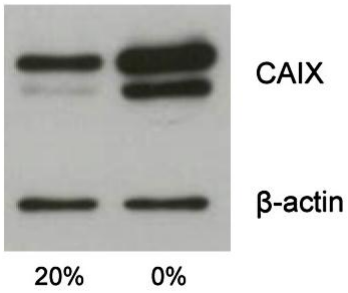
Simplified screening pathway

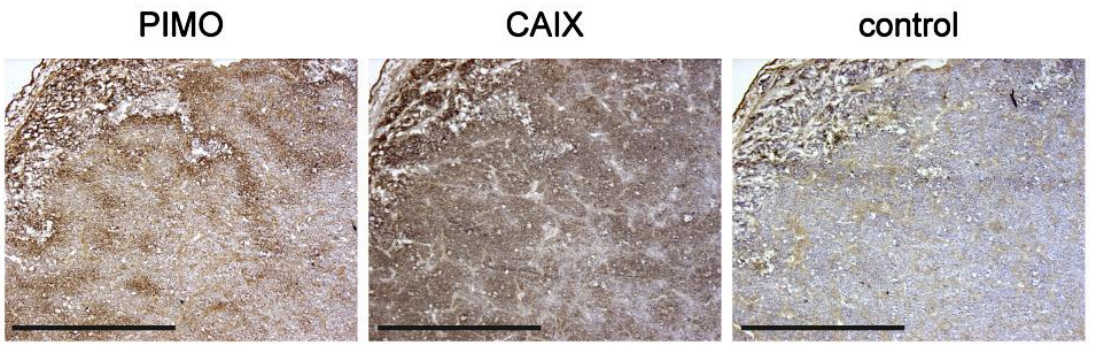


Rami et al, J Med Chem 2013

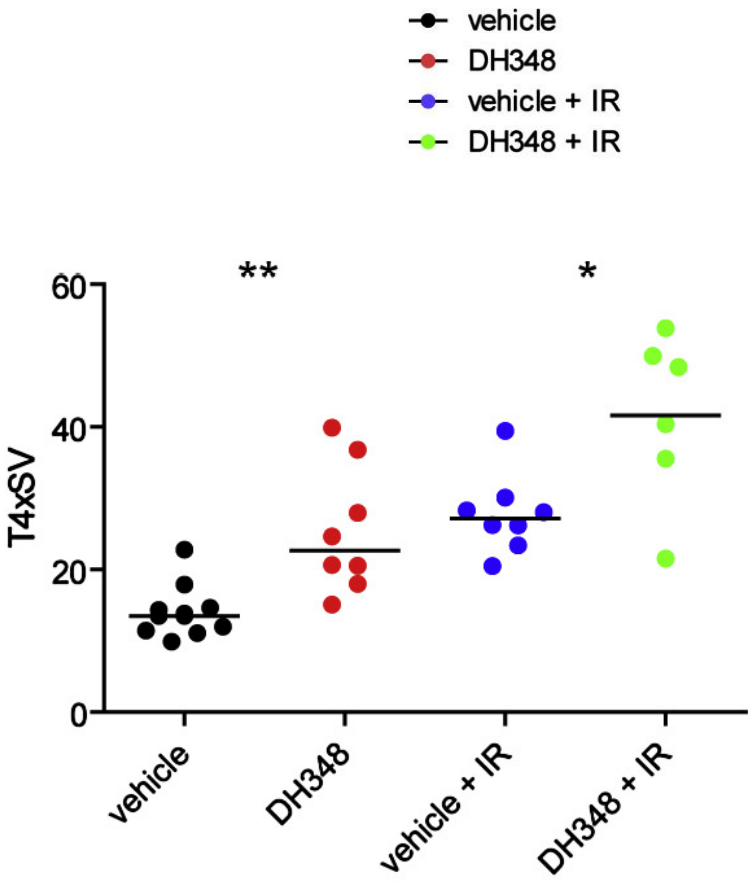
Does DH348 enhances effect of conventional therapies?

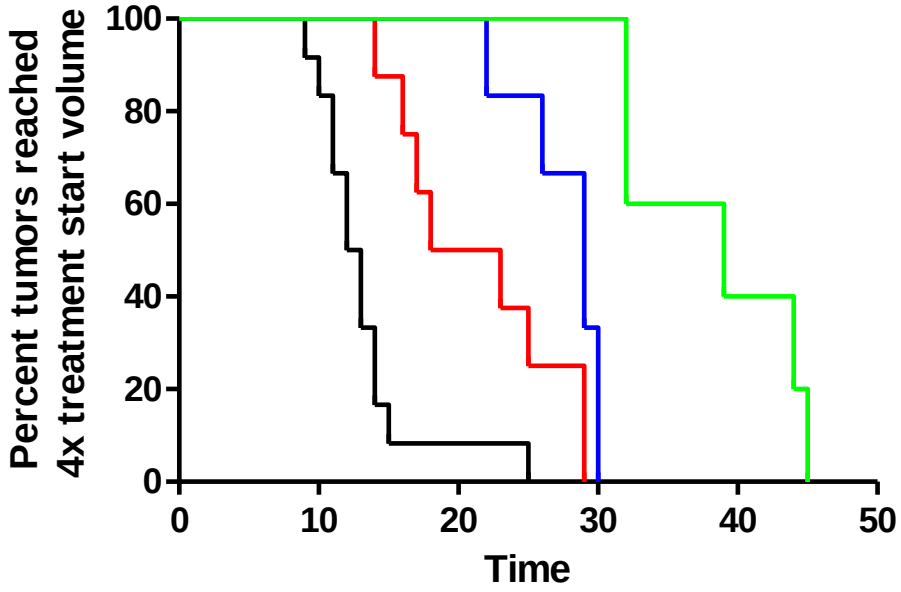






DH348 sensitizes HT-29 tumors to IR (10 Gy SD)…





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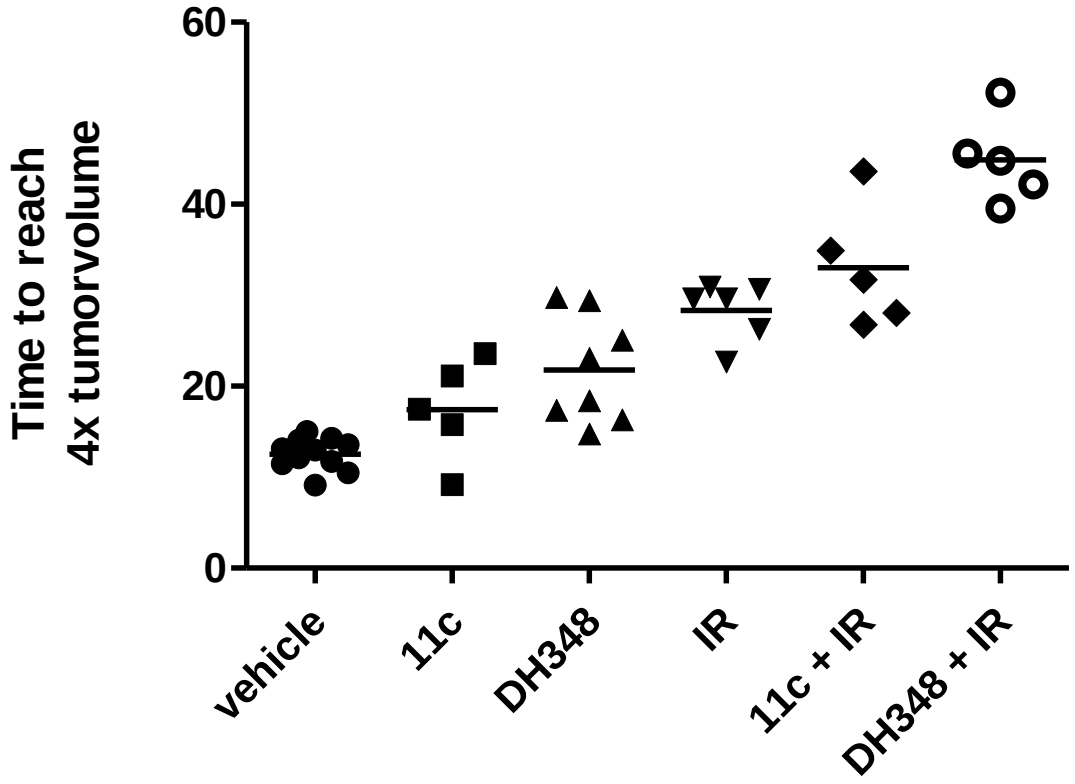
Dubois et al, Radiother Oncol 2013

MAASTRO

… *more* compared to previously published single CAIX targeting (11c)



SER:

DH348 = 1.50

11c = 1.14

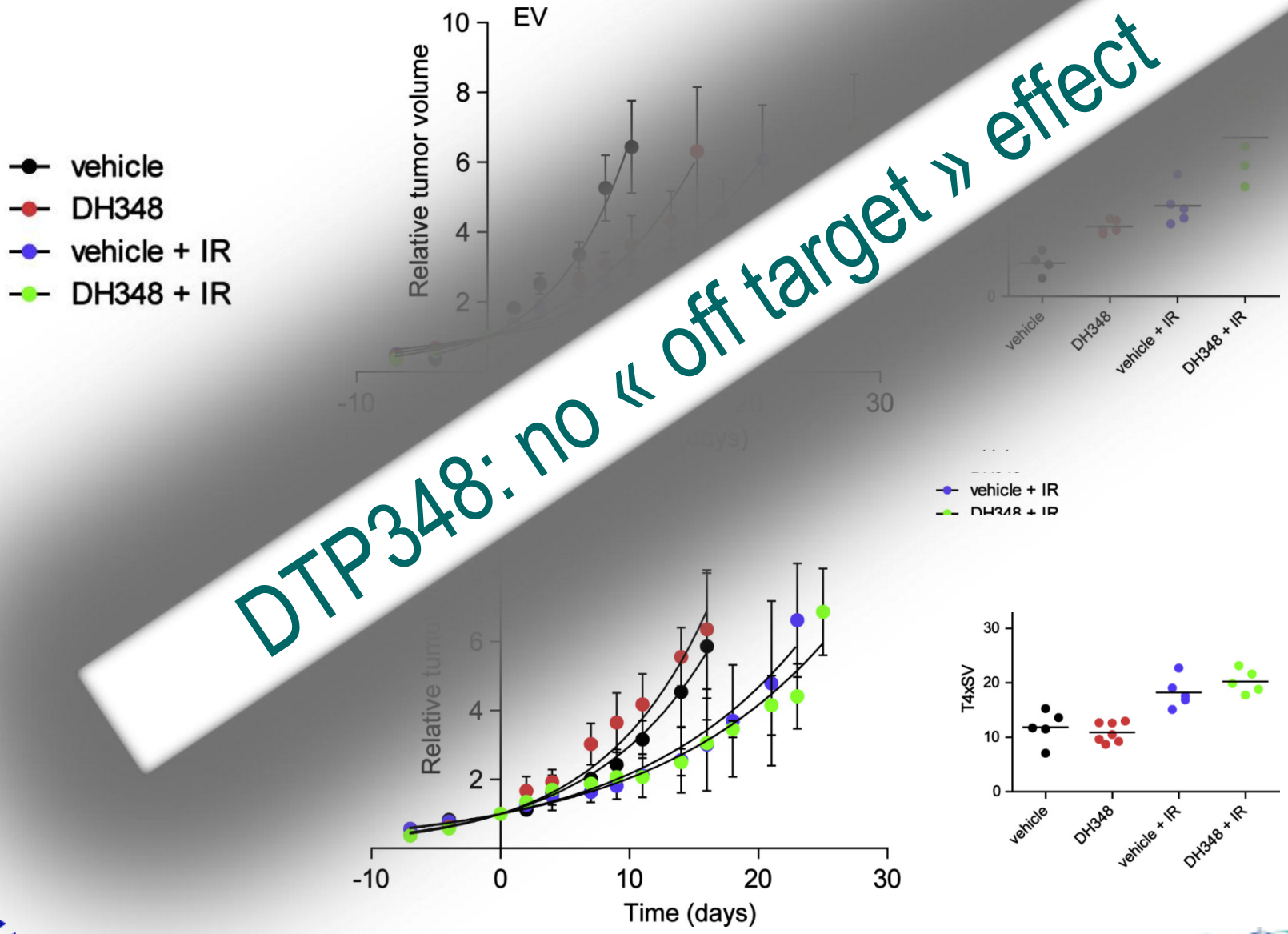
$$SER = \frac{\text{T4xSV Drug + IR}}{\text{T4xSV vehicle + IR}}$$

Universiteit Maastricht

Dubois et al, Radiother Oncol 2011 and 2013

MAASTRO

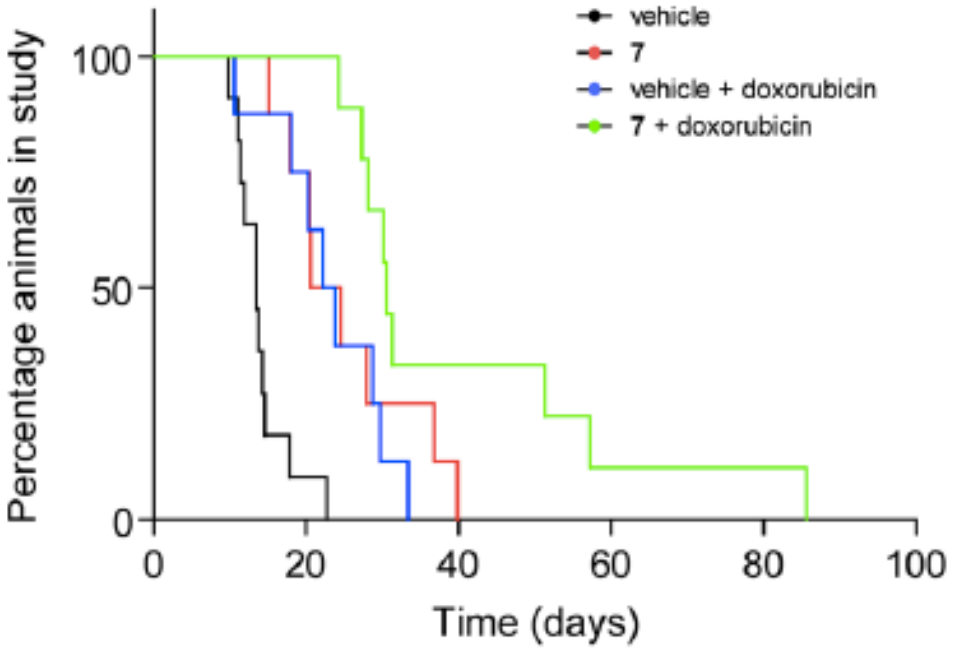
… in a CAIX dependent manner

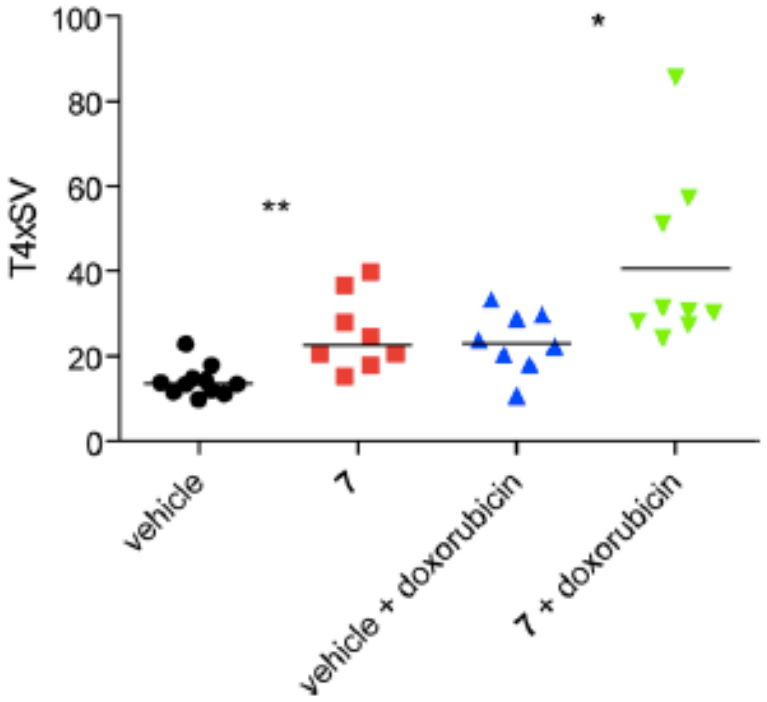


DTP348: no « off target » effect

Dubois et al, Radiother Oncol 2013

… and sensitizes to chemotherapy drugs (such as doxorubicin), having *basic* properties





Rami et al, J Med Chem 2013

Cancer Letters 333 (2013) 76–88

Contents lists available at SciVerse ScienceDirect

Cancer Letters

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

Pharmacological inhibition of carbonic anhydrase XII interferes with cell proliferation and induces cell apoptosis in T-cell lymphomas

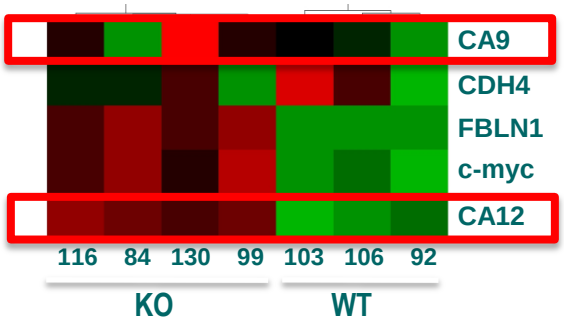
Nadia Lounnas [a,b], Célia Rosilio [a,b], Marielle Nebout [a,b], Didier Mary [a,b], Emmanuel Griessinger [a,b], Zouhour Neffati [a,b], Johanna Chiche [b,c], Hergen Spits [d], Thijs J. Hagenbeek [e], Vahid Asnafi [f], Sally-Ann Poulsen [g], Claudiu T. Supuran [h], Jean-François Peyron [a,b,i,j,*,1], Véronique Imbert [a,b,*,1]

✔ CA XII expression is upregulated in mouse T lymphoma cells, in a human T lymphoma cell line and in human T-ALL samples.

✔ CA XII participates to cell survival of T lymphoma cells by maintaining an alkaline intracellular pH.

✔ CA XII may represent a new therapeutic target for T ALL/LL.
- alone
- as an adjuvant therapy (L-asparaginase, dexamethasone)

Transcriptomic analysis
Affymetrix-MoGene-1_0-st-v1



Courtesy of V. Imbert

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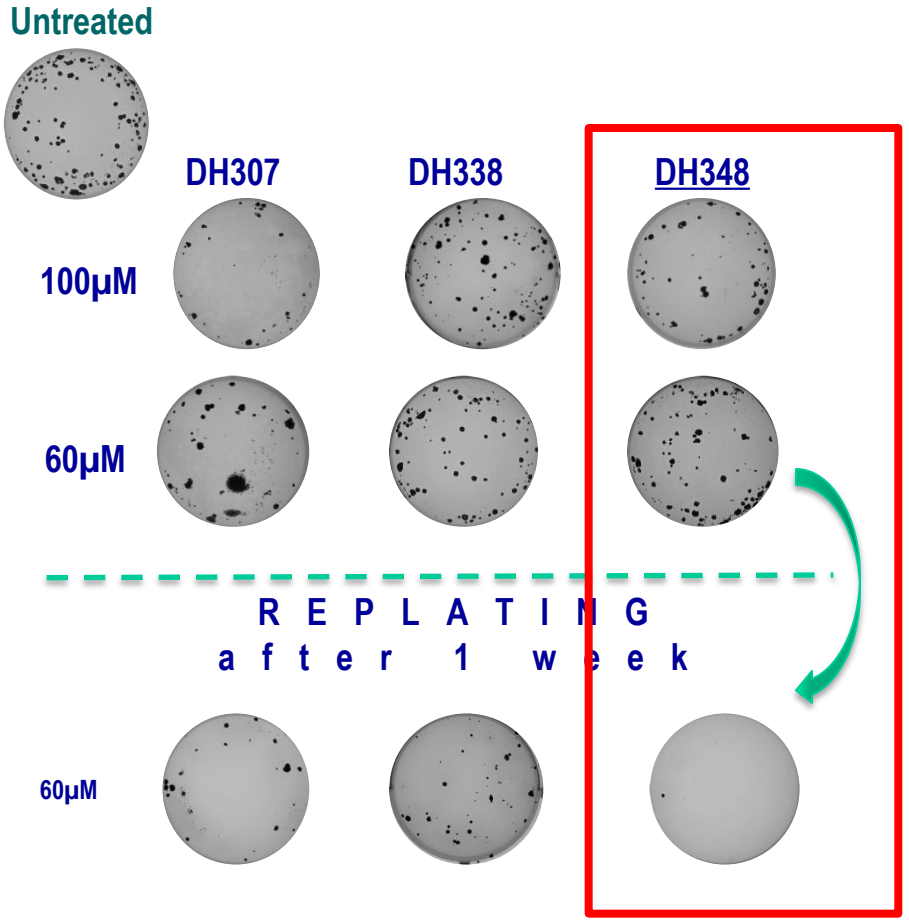
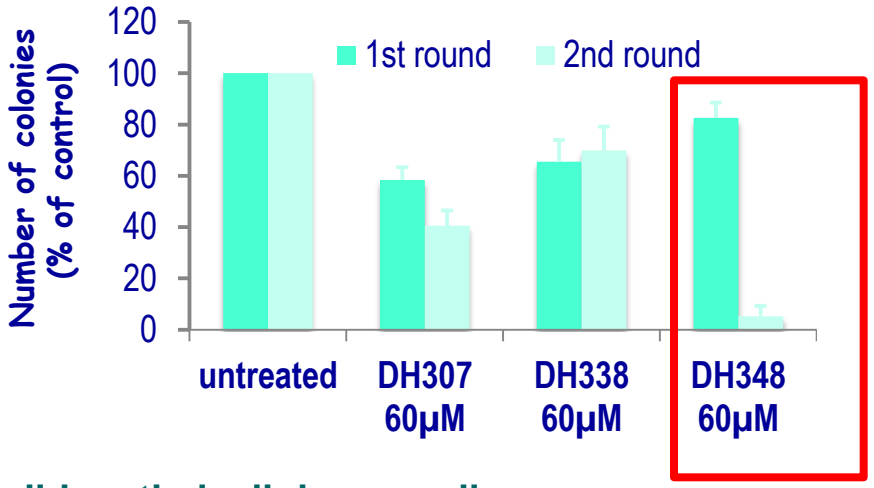
Therapeutic effect of DH348 on lymphoma cells CAXII





Mouse Lymphoma tPTEN-/- Cells cultured in solid methylcellulose medium

Courtesy of V. Imbert

Conclusions

- Bifunctional 5-nitroimidazole CAIX targeting drugs are promising compounds with good PK to combine with IR and/or chemotherapy

- Ongoing:
  - Test dual compound oral formulation (50 mg/kg) in different models (lung, glioma, H&N, colon)
  - Test separate single moieties in vivo
  - Assess normal tissue toxicity on intestinal (short-term toxicity) and lung (long-term toxicity) epithelium combined with irradiation
  - Large-scale toxicity studies
  - GMP production
  - First in human clinical trial

What are the requiremenst of the « perfect drug »?

- 1. Supra-additive effect with radiation
- 2. Low toxicity, tumour specific
- 3. Oral administration
- *Not shown* 4. Biomarkers (tissues, blood, imaging…)
- 5. Known target
- 6. *Multifunctional*, known mechanisms of actions
- 7. Antitumoral effect alone («cure» rather then « response »)
- 8. Chemosensitizing effect
- 9. Effect on µmetastasis
- 10. Cheap to manufacture

CAXII:
T-ALL & T lymphoma

Acknowledgements

Maastro CAIX group

- Ludwig Dubois
- Sarah Peeters
- Simon van Kuijk
- Ala Yaromina
- Natasja Lieuwes

Florence

- Claudiu Supuran
- Andrea Scozzafava
- Fabrizio Carta

Oxford

- Adrian Harris
- Simon Wigfield
- Alan McIntyre

Nice

- Véronique Imbert
- Nadia Lounnas

Montpellier

- Jean-Yves Winum
- Marrouan Rami
- Nanda Kumar Parvathaneni

EUROXY

METOXIA

KWF KANKER BESTRIJDING

Pre-Seed Grant

DUALTPHARMA

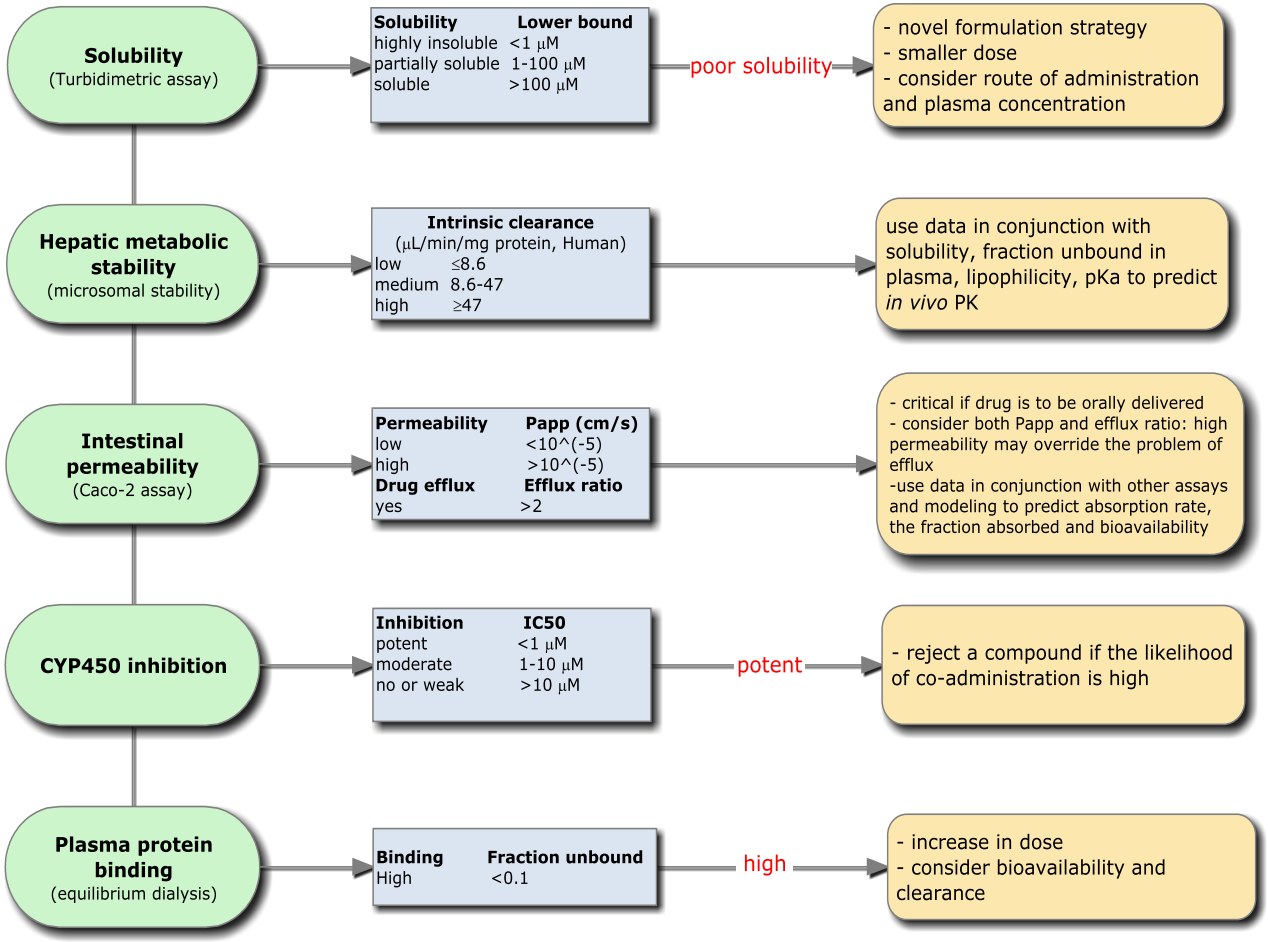
www.dualtpharma.com

GCS Cross Border Cluster Stimulation

Universiteit Maastricht

ICTR, February 2014

ADME in vitro screening



ADME *in vitro* screening.

Absorption, distribution, metabolism and excretion (ADME) properties contribute to the drug success rates. Early detection and eradication of poor ADME properties in vitro eliminates unfavourable compounds and improves the quality of compounds in the pipeline. A number of important ADME properties that contribute to pharmacokinetic profile are listed. ADME properties act interdependently underlying the necessity to consider a specific property in conjunction with other assays.

ADME in vitro screening

Cyprotex 2013

compound	Solubility bound (µM)	Permeability efflux ratio	Stability clearance	Metabolism cytochrome inhibition (µM)	Plasma protein binding mean fraction unbound
DH307	>100	2X9	1X2	>25	0.432
DH338	>100	1.11	6.67	>25	0.422
DH348	>100	0.638	4.88	>25	1.000
NKP60	>100	1.11	2.27	>25	0.308

- Solubility: compound considered soluble > 100 µM
- Caco-2 permeability: efflux ratio < 2 indicates (1) reasonable oral bioavailability and (2) low efflux possibility by P-glycoprotein transporters
- Hepatic stability: low intrinsic clearance if CL < 8.8 µl/min/mg protein
- CYP450 metabolism: no or weak inhibition when IC50 > 10 µM
- Plasma protein binding: how higher unbound fraction, how more free drug available

PK simulation on based on ADME data

Cyprotex 2013

Mouse: Oral route 50 mg/kg dosing (mouse weight 0.03 kg)

All values are given as Median (10 – 90 percentile)

Com-pound	C_{max} (µg/ml)	t_{max} (h)	AUC (µg·h/ml)	$t_{1/2}$ (h)	MRT (h)*	Bioavaila-bility	Elimination rate con-stant (h^{-1})	Volume of distri-bution** V_d(l/kg)	Volume of distribuition V_{ss} (l/kg)	Clearance (ml/min/kg)
DH307	1.4 (0.23-2.3)	3 (2.9-3.3)	13 (3.4-21)	7 (5.6-11)	12 (11-18)	0.27 (0.06-0.35)	0.099 (0.064-0.12)	10 (7.1-16)	12 (9.1-18)	16 (13-21)
DH338	12 (2.7-20)	2.5 (2.2-3.9)	69 (31-130)	6.9 (5-10)	6.5 (5.1-15)	0.77 (0.43-0.87)	0.1 (0.066-0.14)	5.1 (3.2-8.9)	3.5 (2.4-7.6)	8.7 (5.2-14)
DH348	11 (1-22)	3.7 (3-5.5)	110 (21-310)	6.1 (5-10)	11 (8.1-23)	0.69 (0.16-0.81)	0.11 (0.07-0.14)	2.5 (1.3-5.4)	3.2 (1.7-7.6)	4.6 (1.9-10)
NKP60	47 (13-70)	2.8 (2-5.2)	420 (130-1700)	6.5 (4.7-18)	11 (5-28)	0.95 (0.61-0.99)	0.11 (0.039-0.15)	0.95 (0.67-2)	0.95 (0.61-0.99)	1.8 (0.46-4.7)

*Mean residence time

**V_d: Proportionality factor to calculate amount remaining in the body from measured blood plasma level.

Info needed on
- Solubility
- Caco-2 permeability
- Hepatic stability
- CYP450 metabolism
- Plasma protein binding
- LogP and PKa

CA IX role in tumor cells

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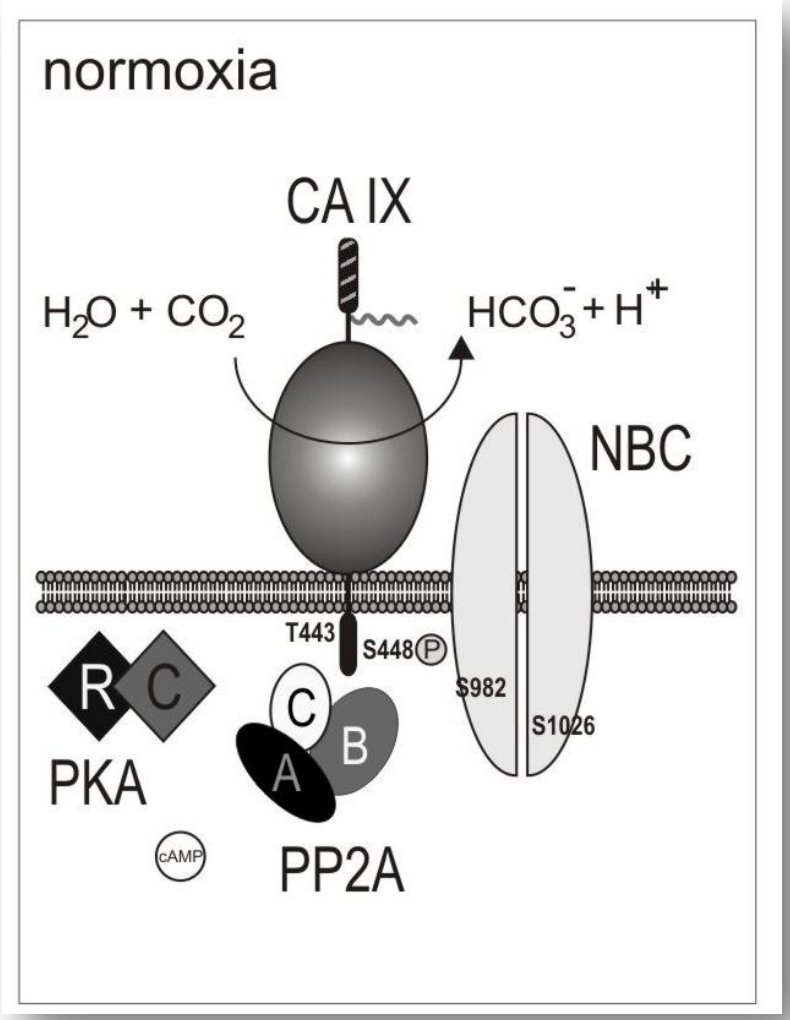
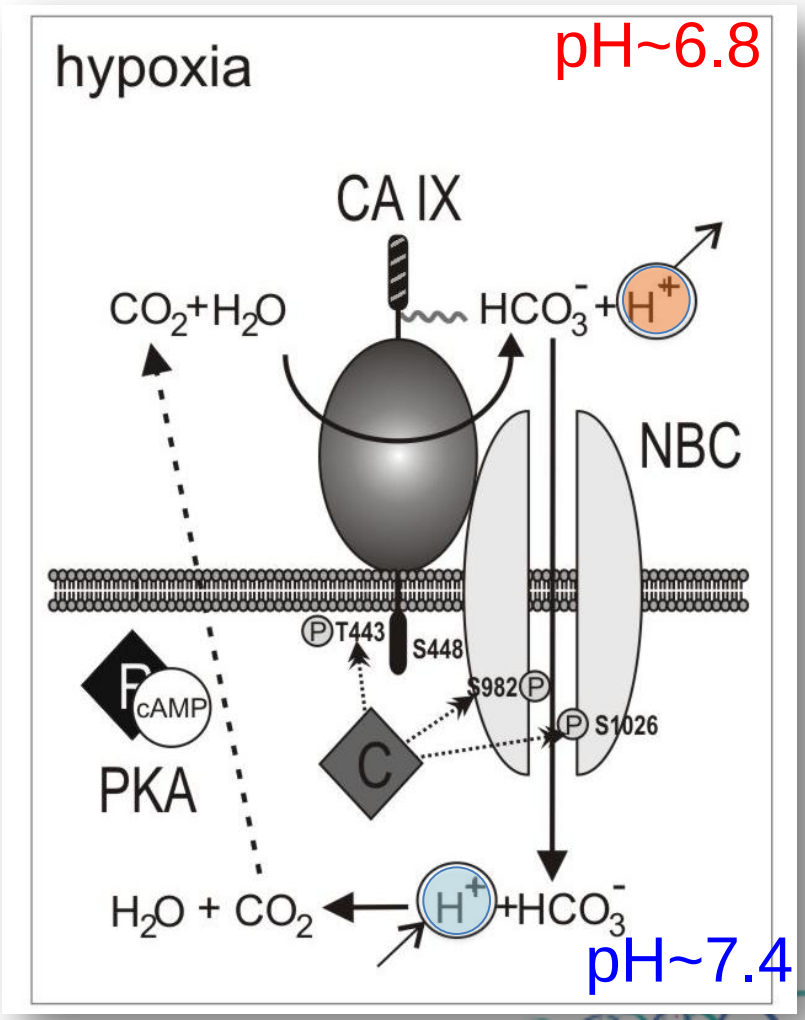
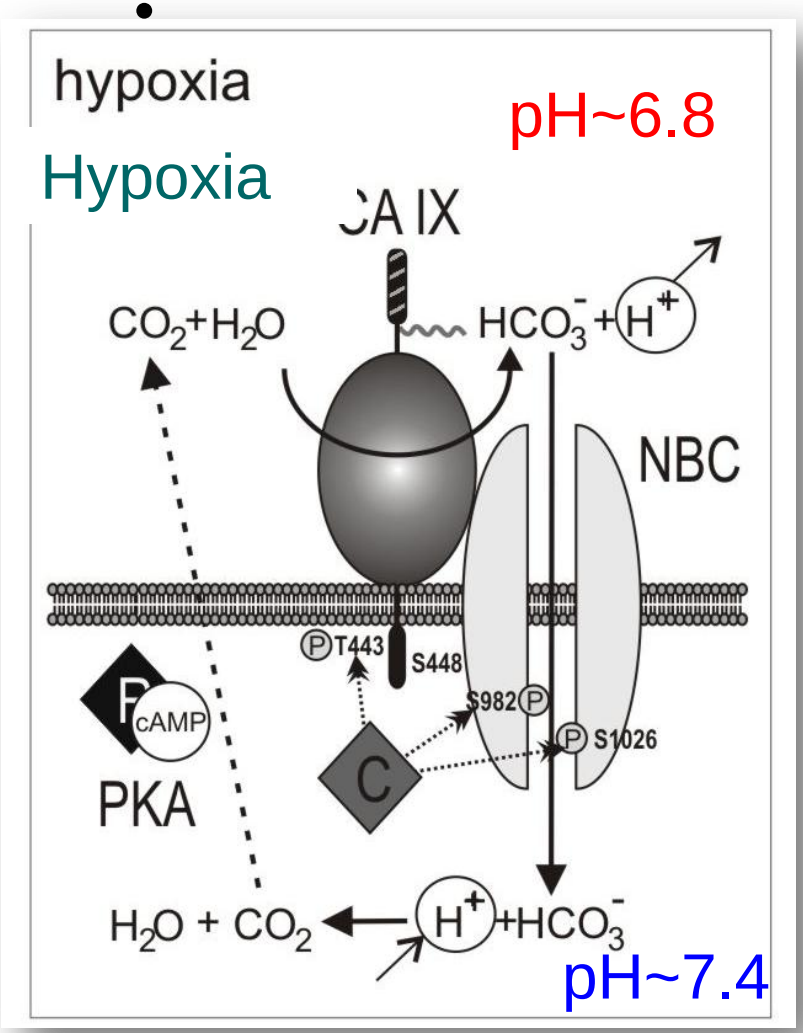
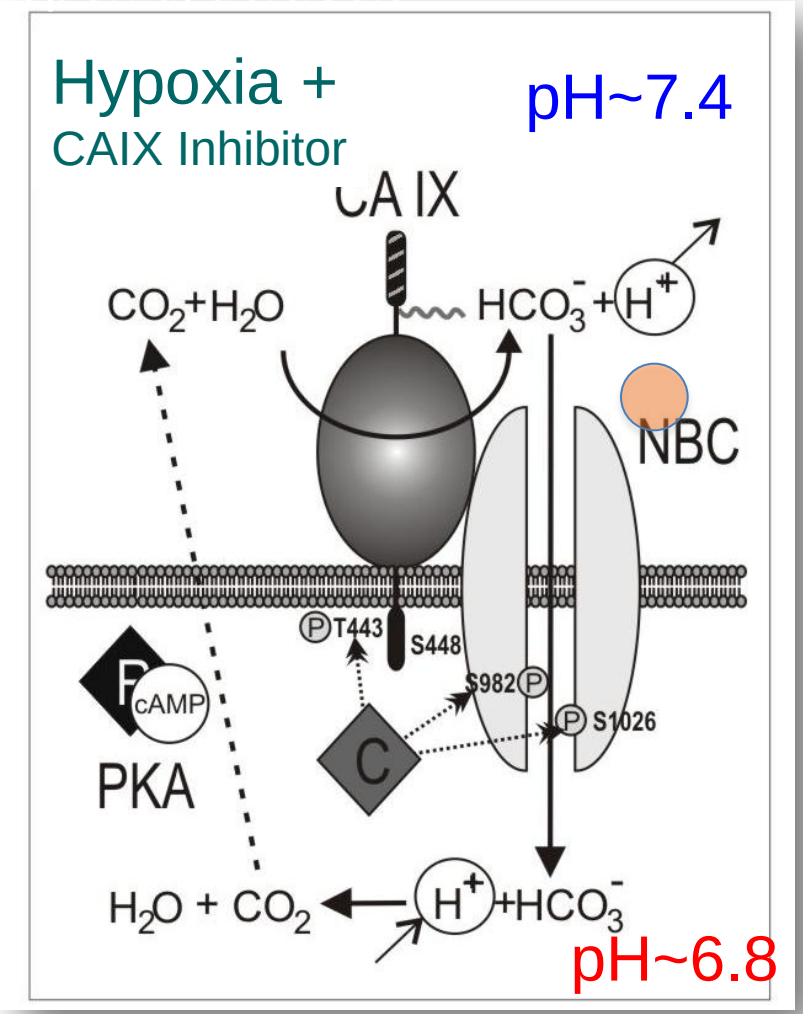




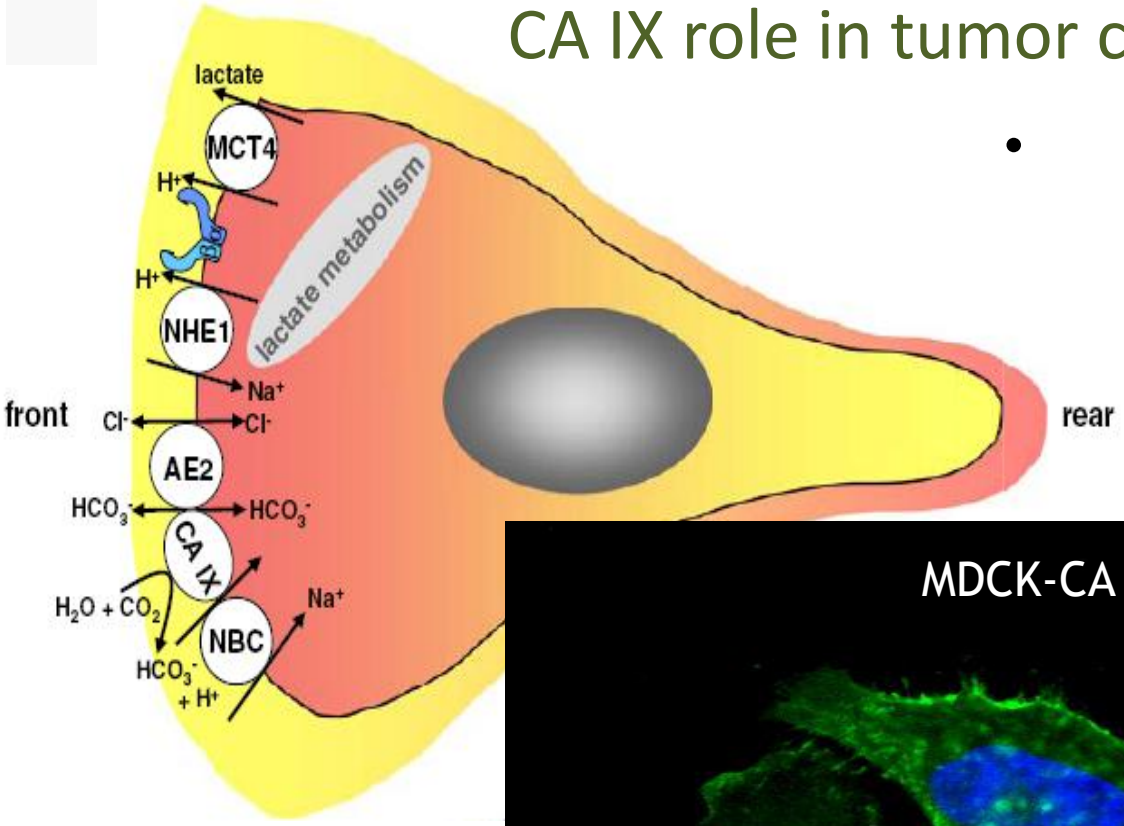
CA IX role in tumour cells

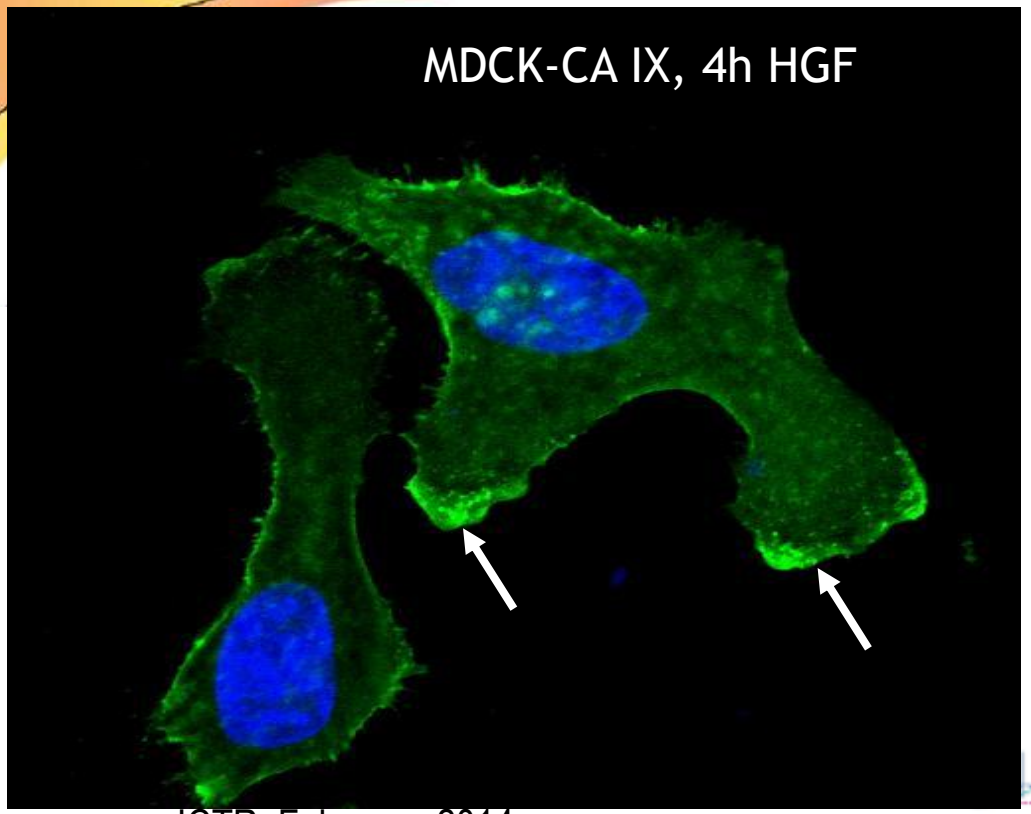




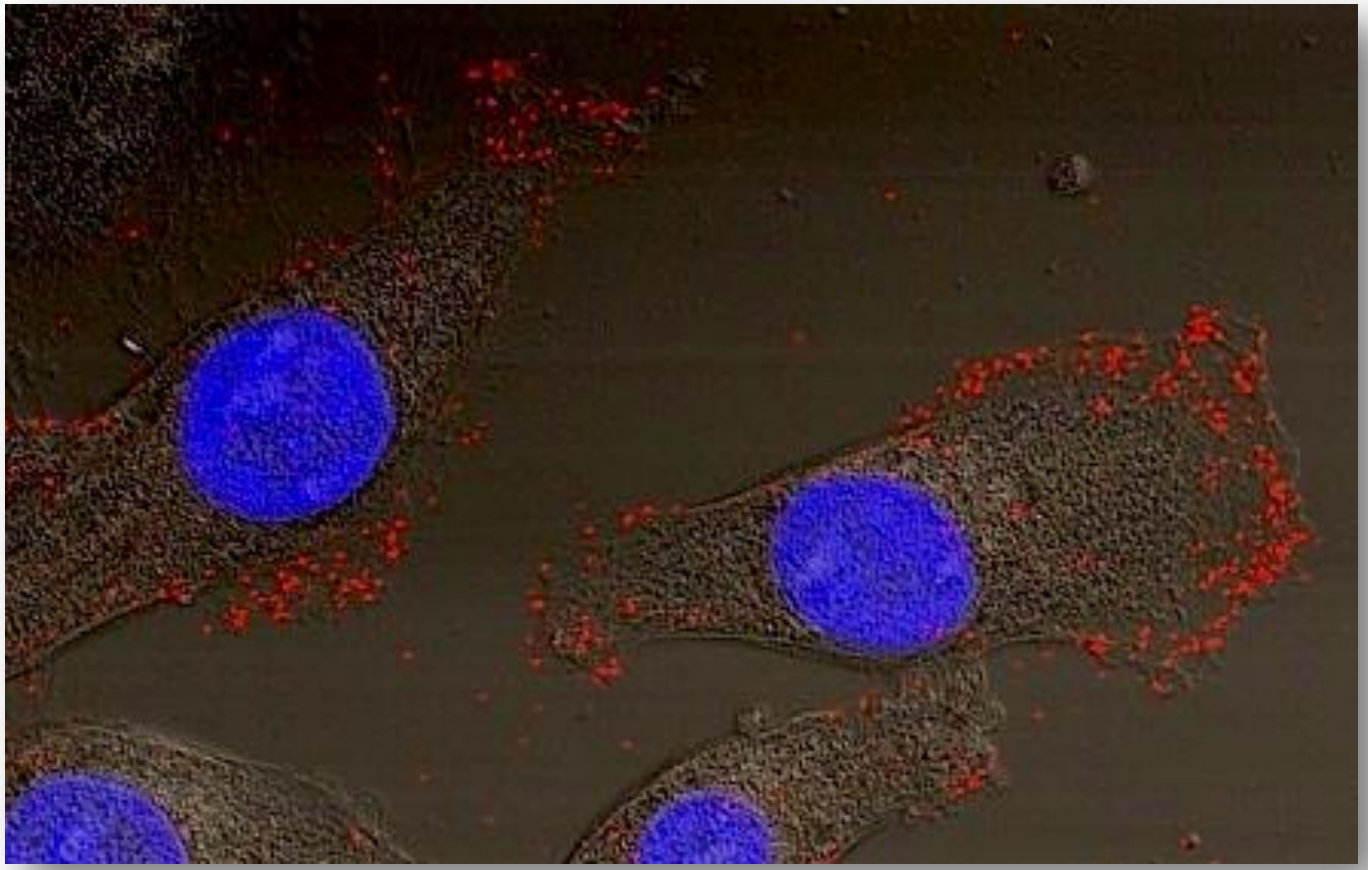
= Toxic for the cancer cell

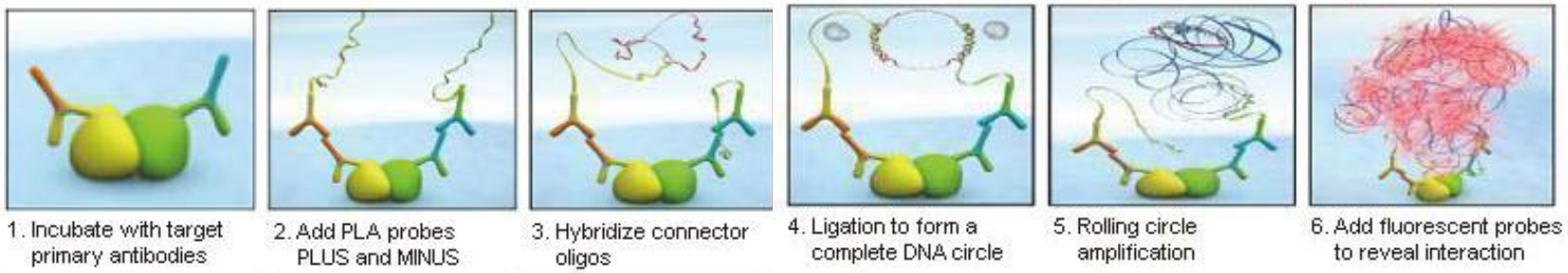
CA IX role in tumor cells



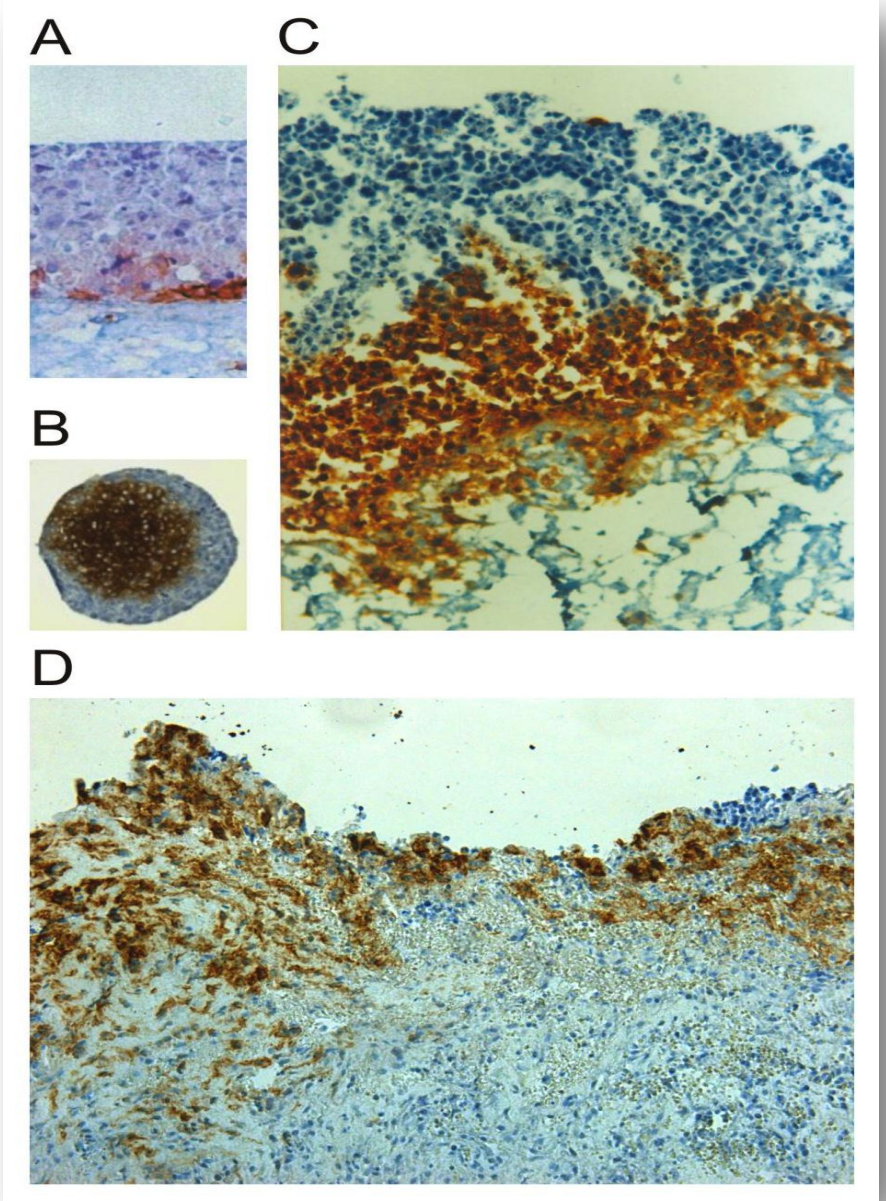


CA IX interacts with bicarbonate transporters

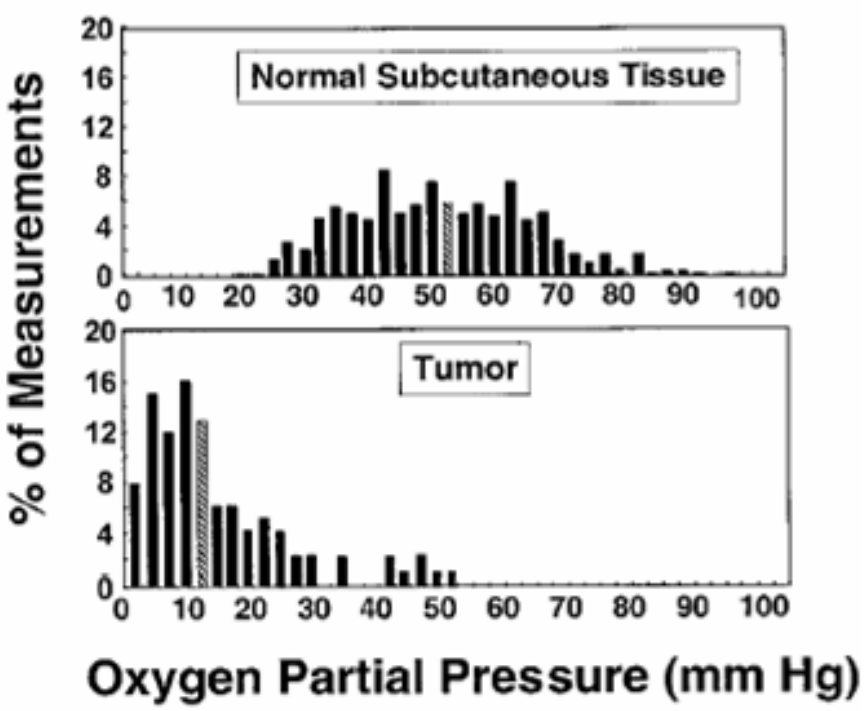




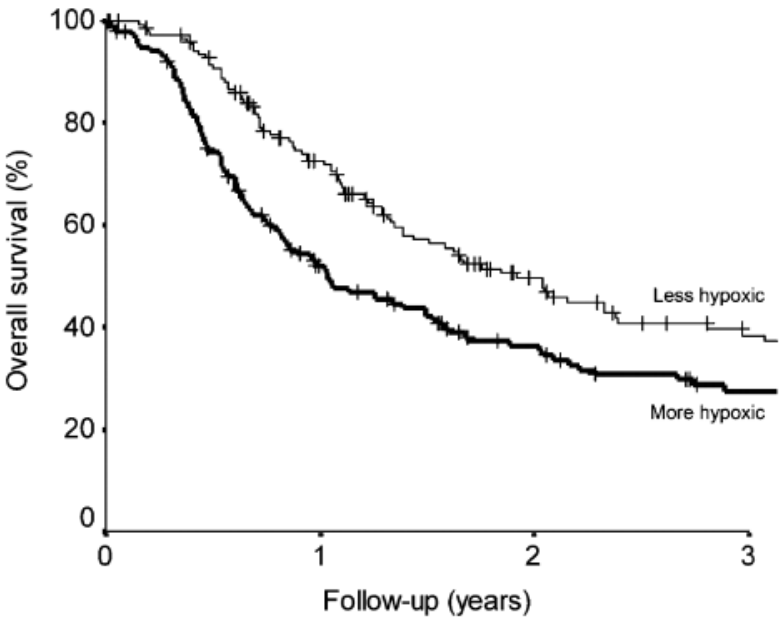
CA IX is present in invading tumor cells



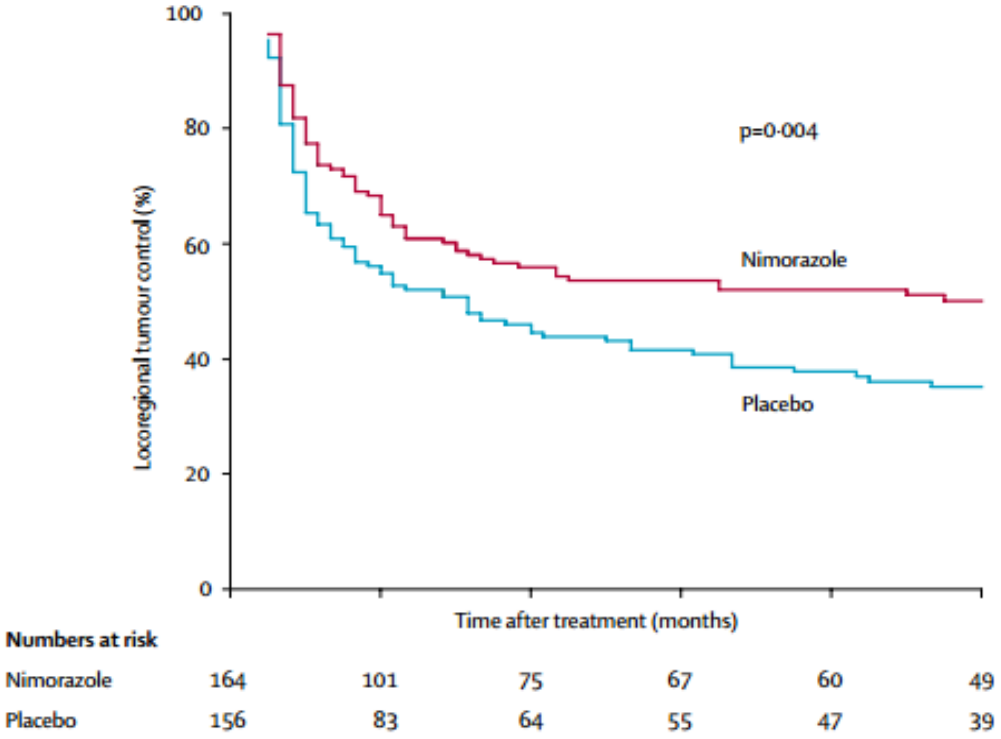
Clinical relevance of hypoxia on irradiation

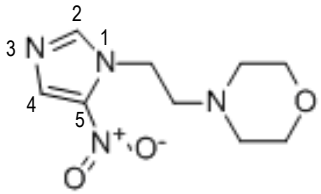


Head & Neck cancer: 397 patients



5-nitroimidazoles improve tumor control (DAHANCA 5)





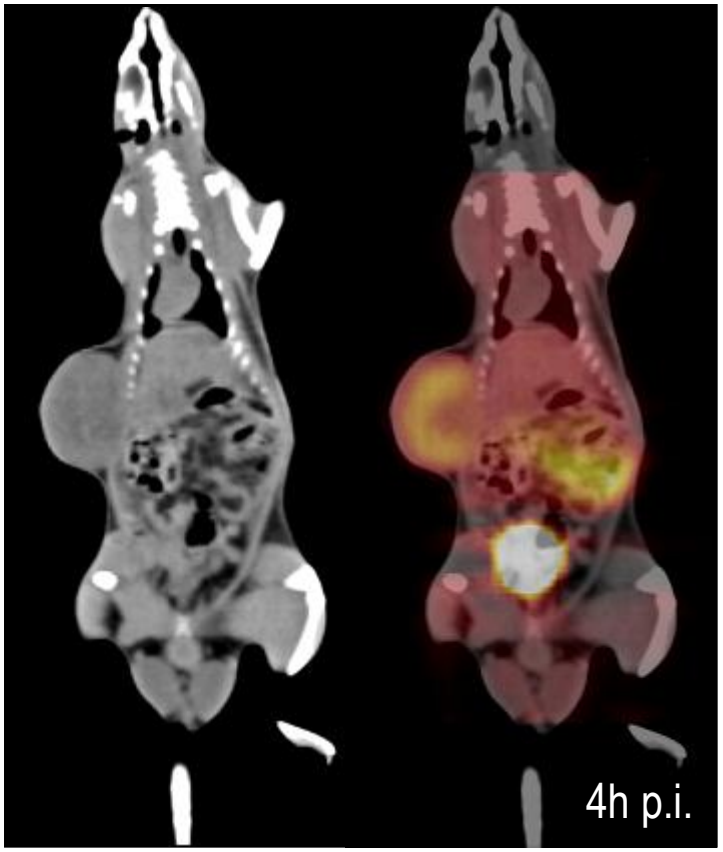
Nimorazole

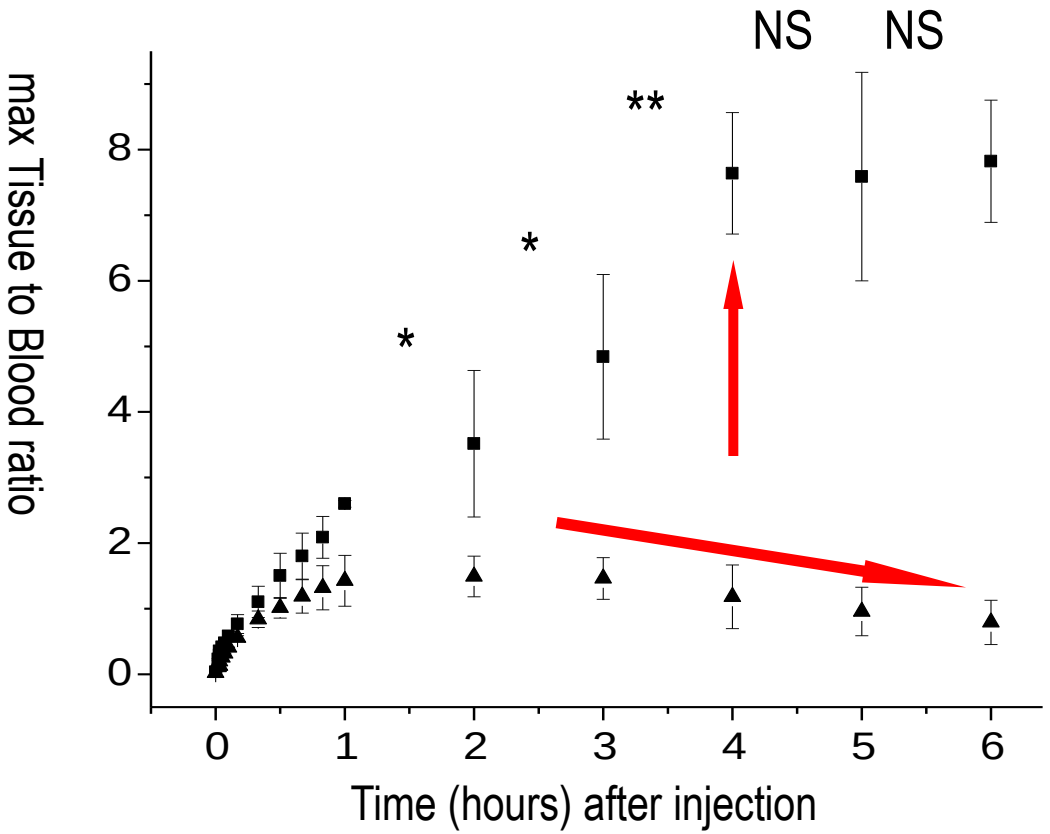
Herceptin:

Δ « Companion biomarker »

Ti[illegible]min:

Rhabdomyosarcoma: optimizing imaging conditions

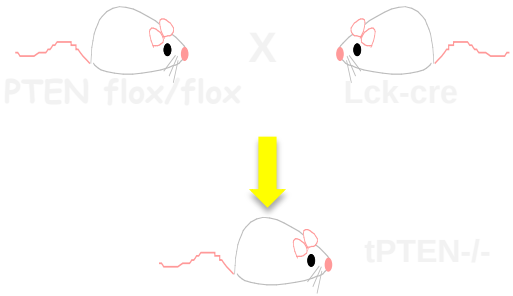


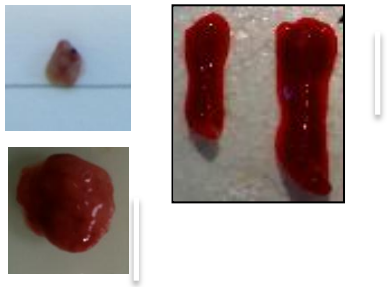


Dubois, Lambin et al. PNAS 2011

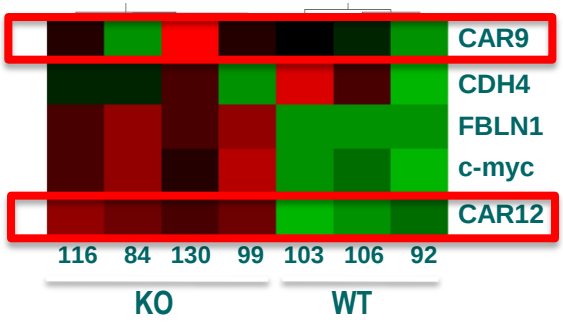
CARBONIC ANHYDRASE XII : A POTENTIAL THERAPEUTIC TARGET FOR T CELL ACUTE LEUKEMIA/LYMPHOMA.

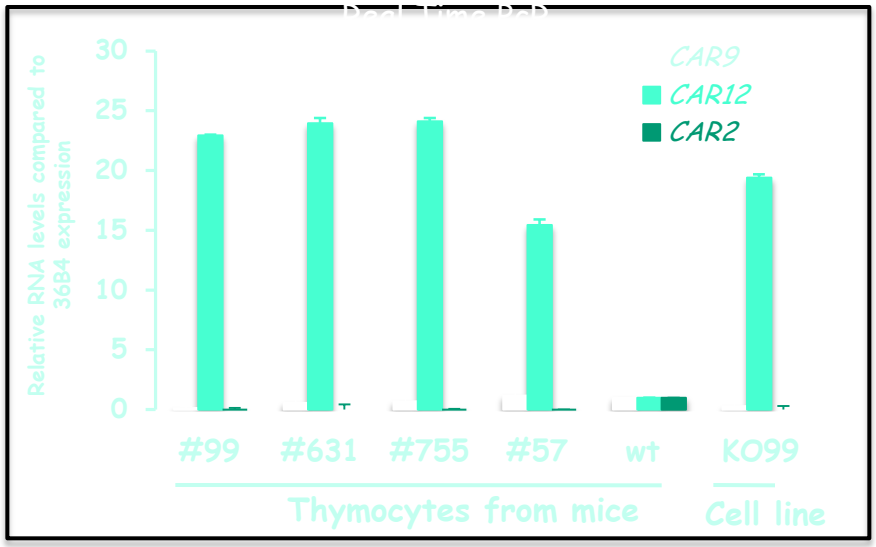
Dr Véronique IMBERT INSERM U1065 Mediterranean Centre for Molecular Medicine (C3M) - Nice – France





Transcriptomic analysis
Affymetrix-MoGene-1_0-st-v1





Perspectives

✔ Xenograft tumor model (3D+hypoxia)

tPTEN-/- KO99 cell line with **luciferase** reporter gene (plenti-Fire Luciferase)

In vivo treatments

DH307
DH348

Follow up of tumor progression by Imaging

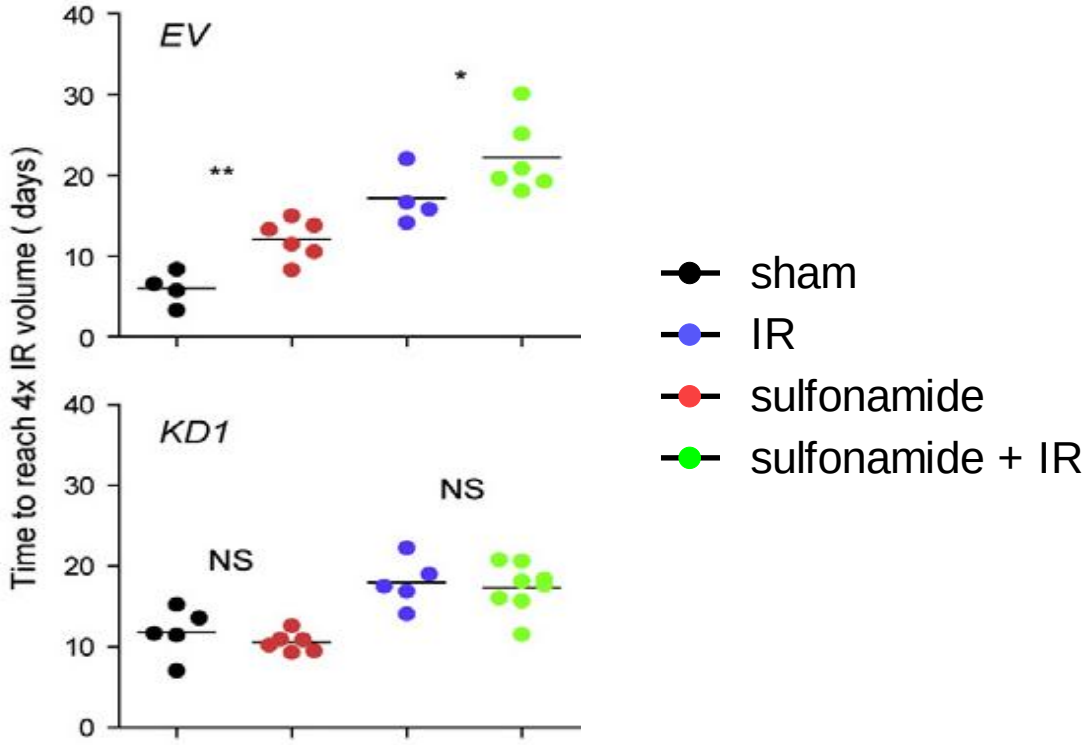
vehicle drug

✔ Study the capacity of DH307 and DH348 to enhance the effects of conventional therapies used in T-ALL/LL (dexamethasone/L-asparaginase) *in vitro (mouse tPTEN-/- cells and human samples)* and *in vivo*

Inhibition of CAIX activity, an attractive therapeutic avenue?

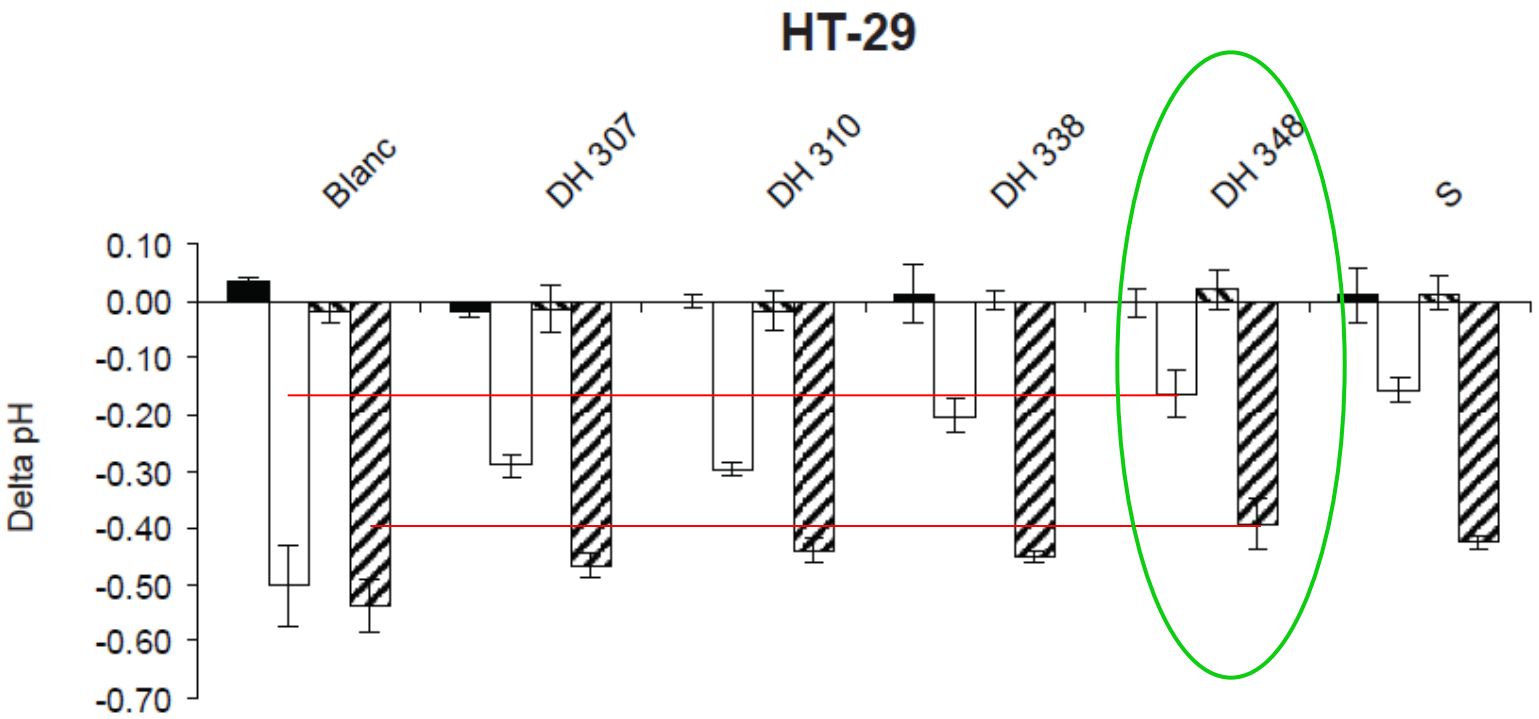
What have we done so far?

1. Indanesulfonamides are able to inhibit extracellular rate of acidification and tumor cell proliferation under hypoxia in a CAIX dependent manner
2. Indanesulfonamide treatment sensitizes HT-29 tumors to irradiation in a CAIX dependent manner



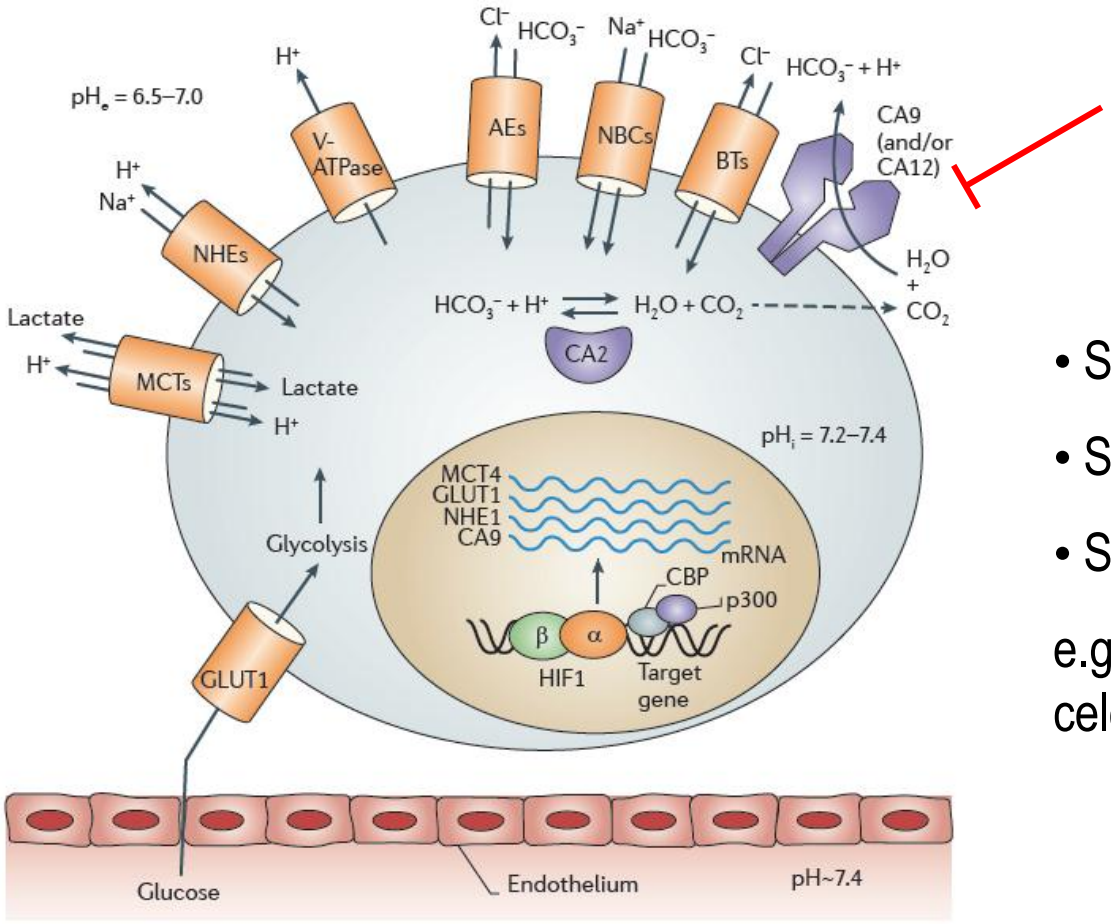
Dubois et al, Radiother Oncol 2011

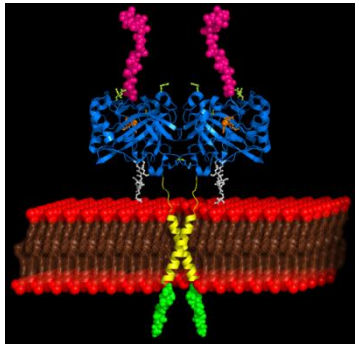
Reduction of extracellular acidification upon hypoxia



Rami et al, J Med Chem 2013

CAIX: ensure Extracellular acidification and CAIX

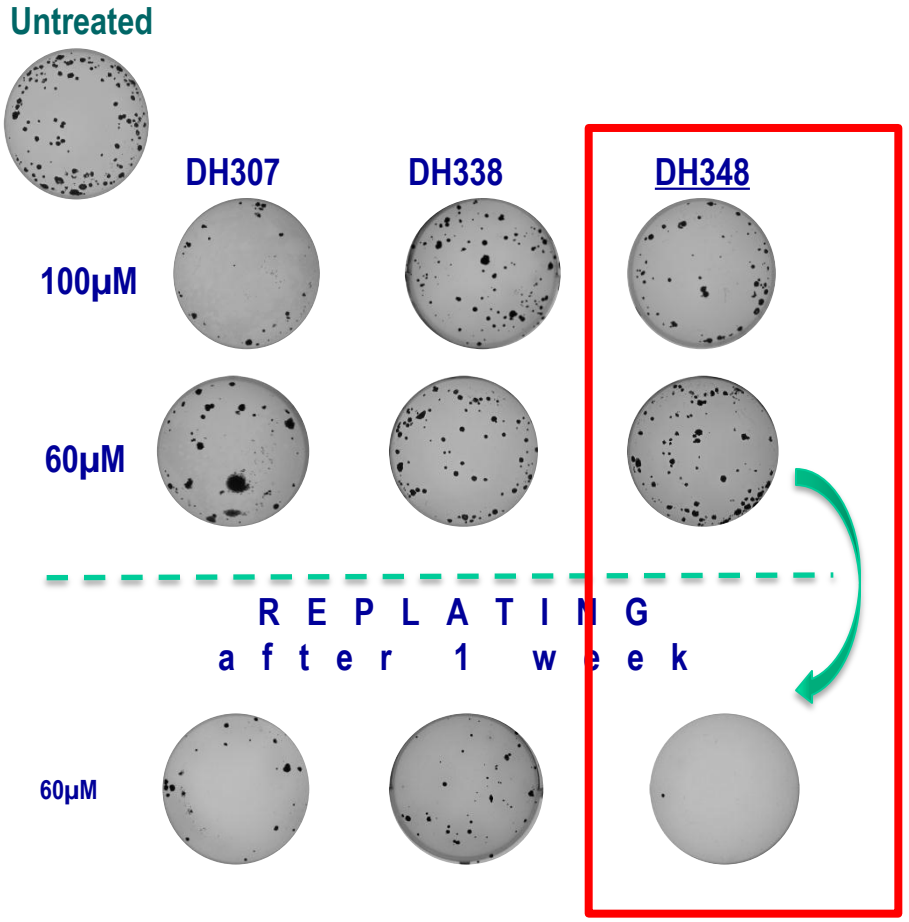


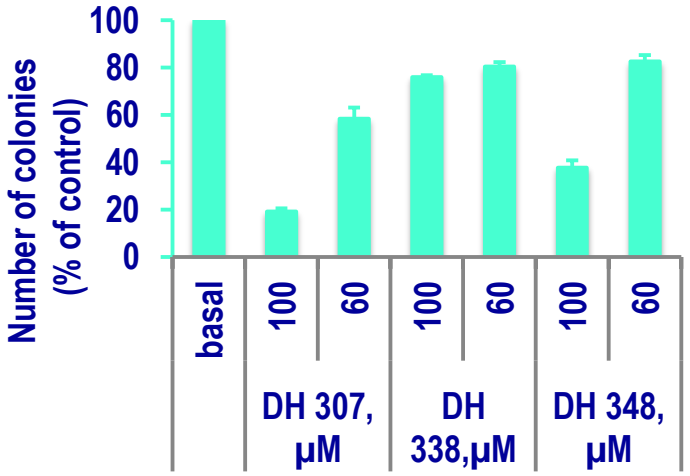


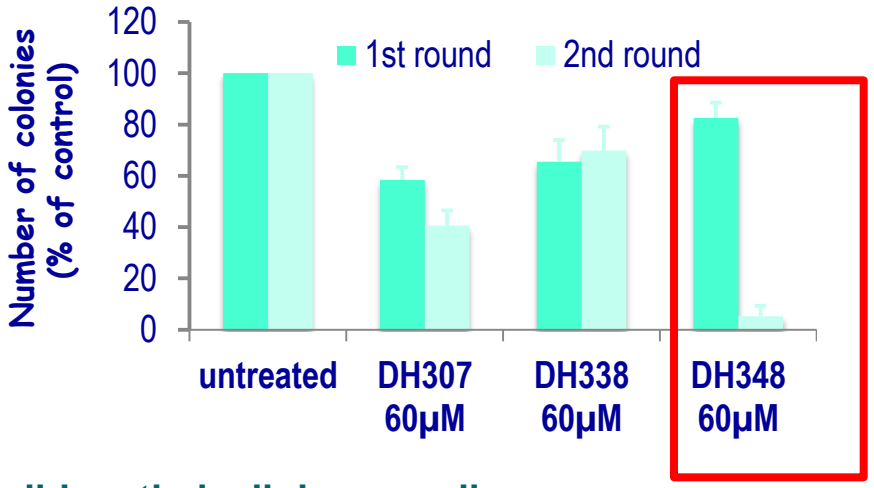
- Sulfonamides
- Sulfamates
- Sulfamides

e.g. acetazolamide, indisulam, celecoxib

Therapeutic effect of DH348 on lymphoma cells CAXII







Mouse Lymphoma tPTEN-/- Cells cultured in solid methylcellulose medium

Universiteit Maastricht

Courtesy of V. Imbert

MAASTRO