Multi Drug Resistance and its Reversal in Acute Myelogenous Leukemia

Jean-Pierre Marie

Centre de Recherche des Cordeliers UMRs872
INSERM/Université Pierre & Marie Curie
And Hôtel-Dieu, Paris

MultiDrug Resistance Phenotype (MDR)

- Biedler & Riehm (Cancer Res 1970; 30:1174)

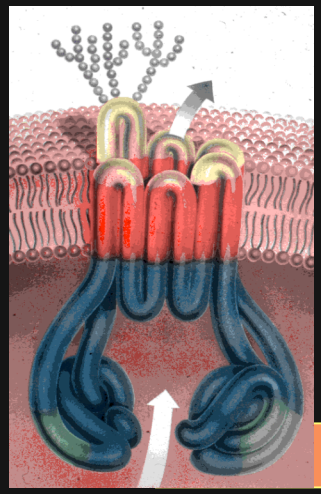
  P388 cell line(rat AML) exposed to increasing doses of actinomycin D became resistant to this drug but is also cross resistantto adriamycin, vinblastine, colchicine...
- This phenomenon is reproducuble on several cells lines (mice, human) resistant to one of these unrelated cytostoxic are also cross résistant to others autres
- This resistance is pharmacologic: the content of the drug decreased compared to the parental cell line

==> this phenotype is the most frequent observed, is inter-species

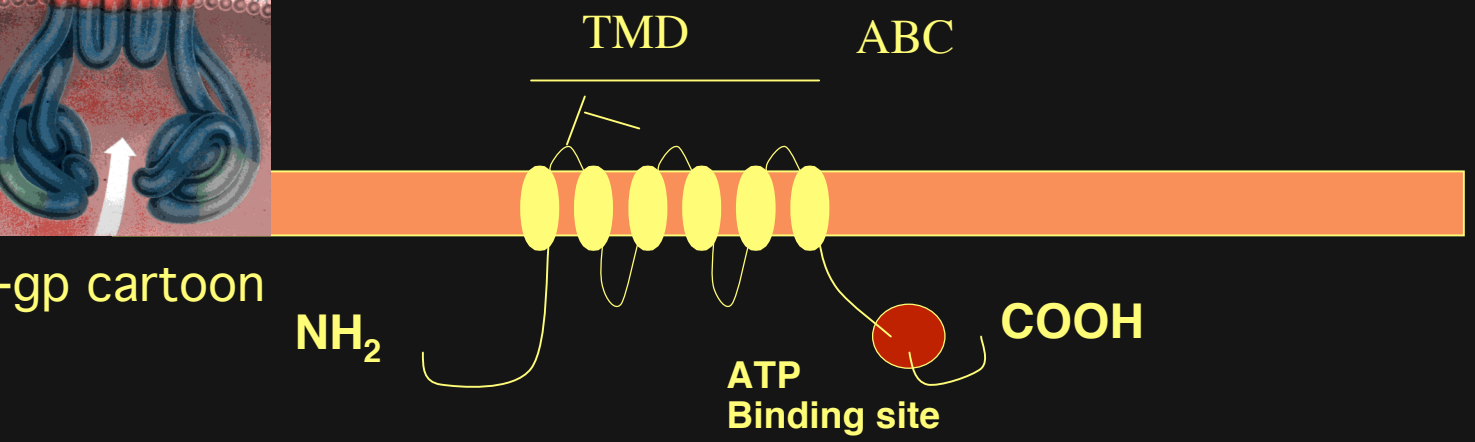
==> the drugs invelved are derived from natural products (xenobiotics)...

- It is energie consuming (ATP)
- It is correlated with the expression of membrane glyco-proteins, called ABC proteins (for ATP Binding Cassette)

Structure of ABC transport proteins



P-gp cartoon

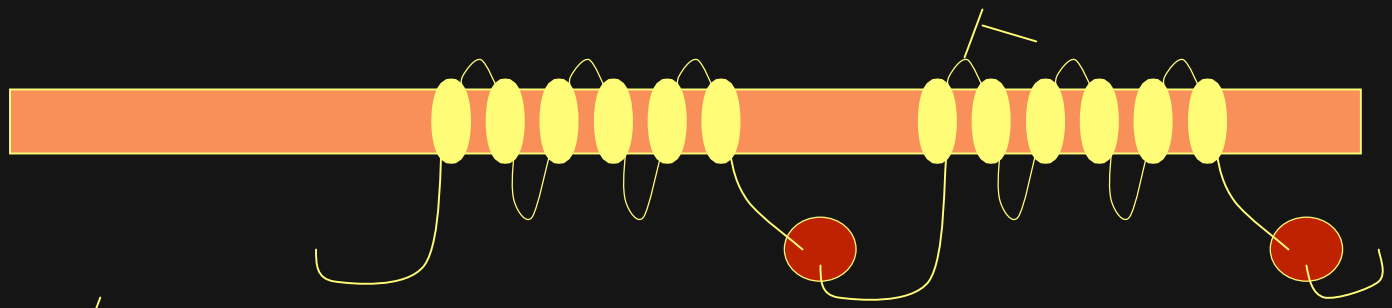


(TMD-ABC)

Minimal structure

Usually intra-cytolasmic

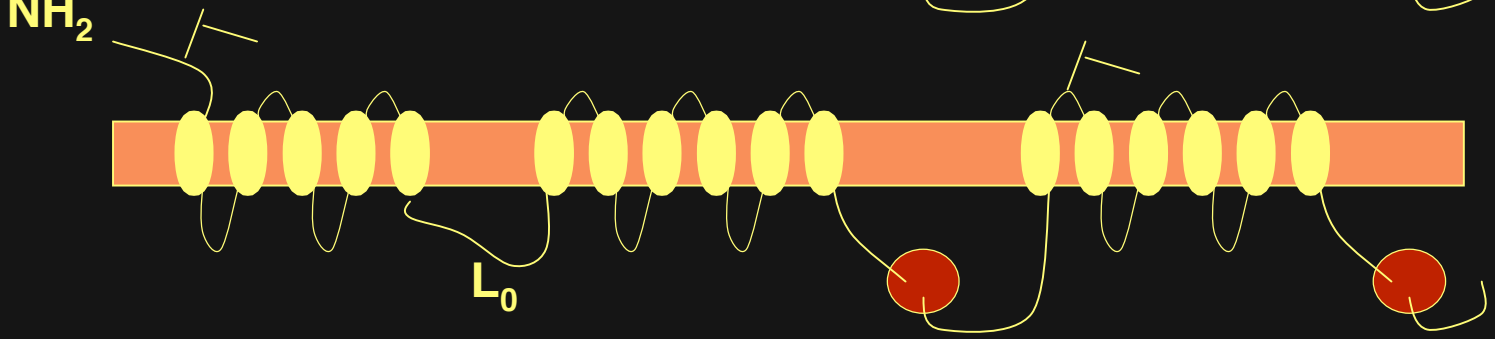
TAP1/2, BCRP



(TMD-ABC)2

Typical structure

P-gp, MRP4-5

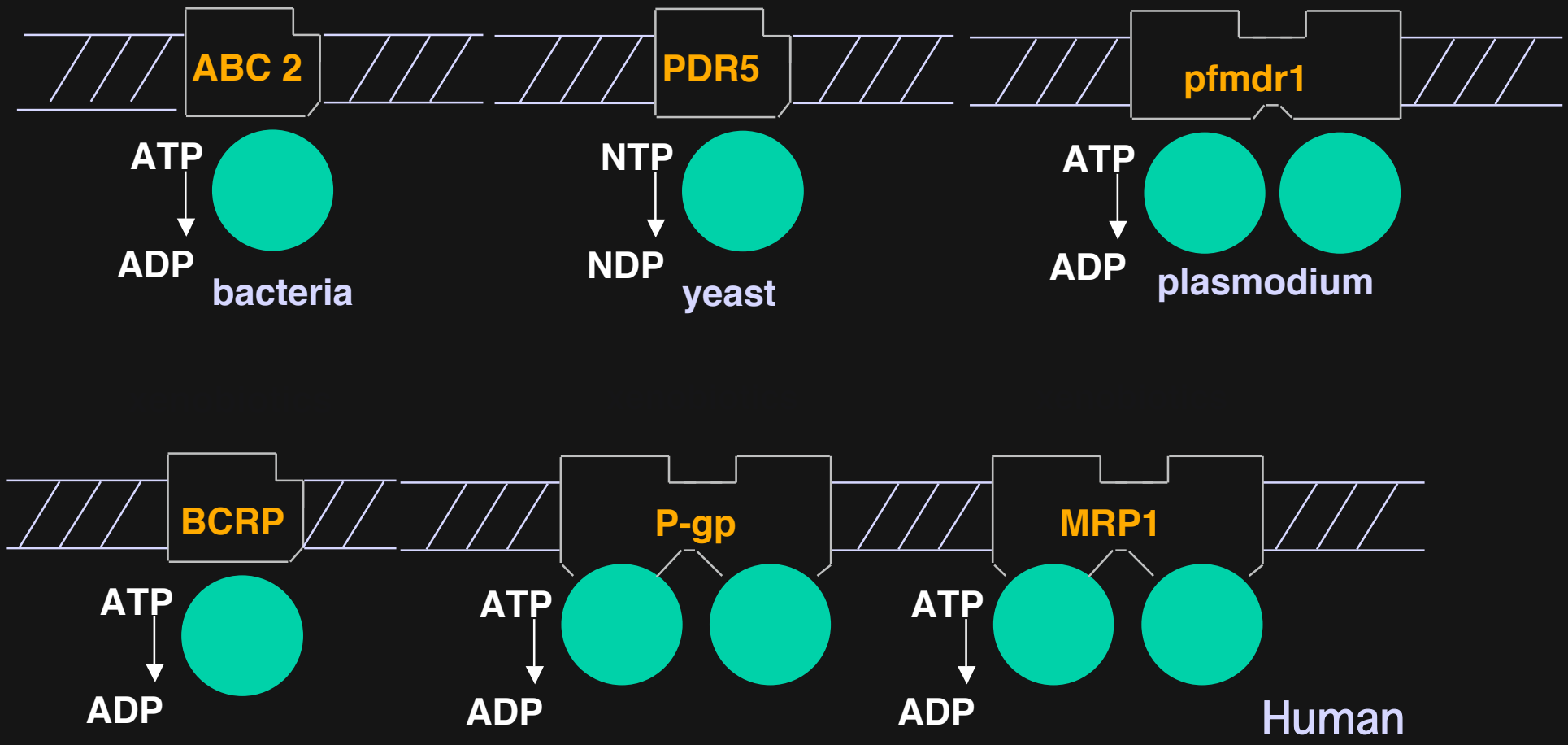


TMD0(TMD-BC)2

Atypical structure

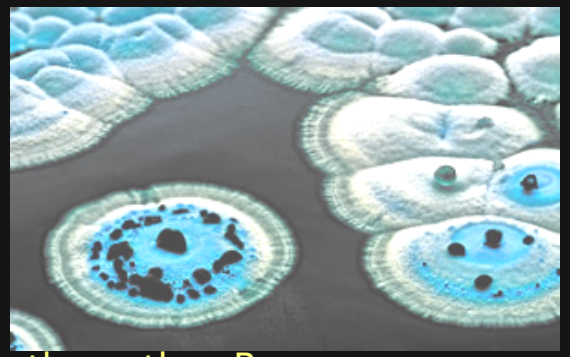
MRP1-2-3-6

"ABC" SuperfamilyTransporter :
highly maintained structures through species



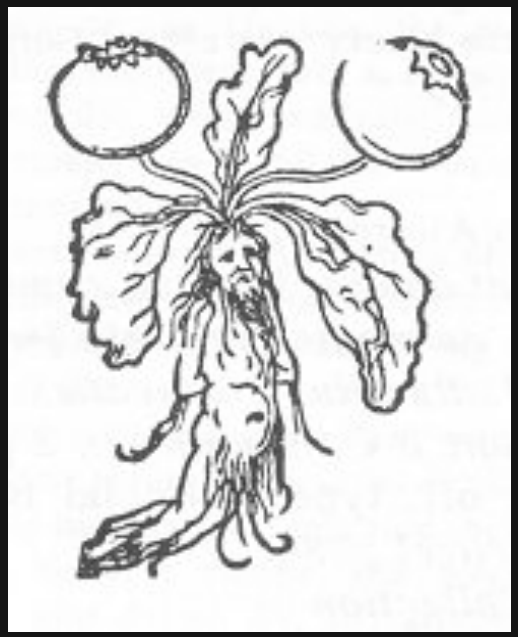
Cytostatiques chassés par la P-gp: **Xénobiotiques**

Anthracyclines: Extract from streptomyces



Vinca- Alkaloids Extract from Cantharanthus Roseus

Taxanes Extract from Taxus brevifolia



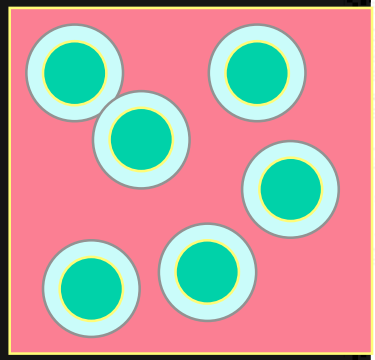
Epipodophyllotoxines
Extract from Mandragore

PROTECTION

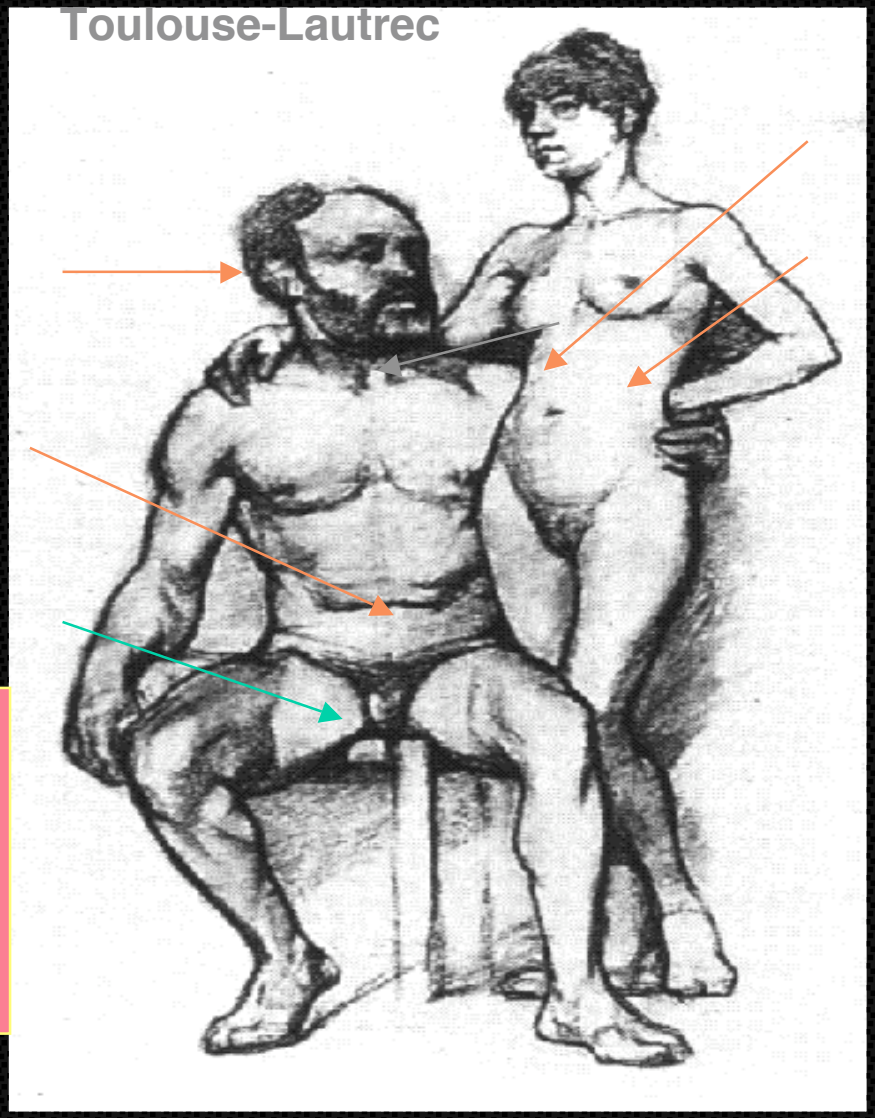
Blood Brain Barrier

Ileon Colon

Blood Testis barrier



Hematopoietic Stem cells

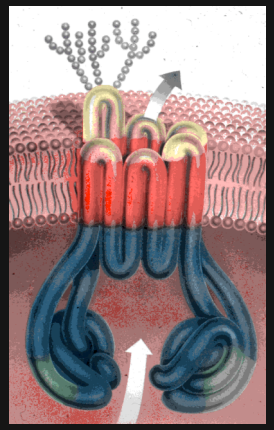


CLEARANCE

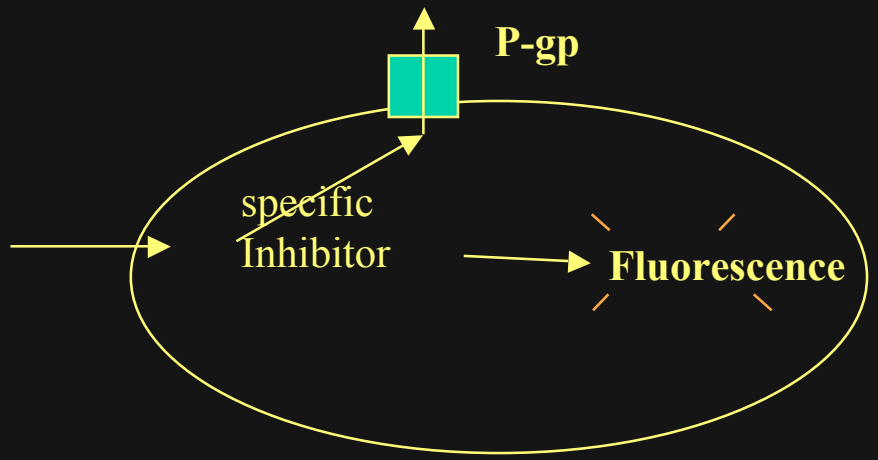
Liver

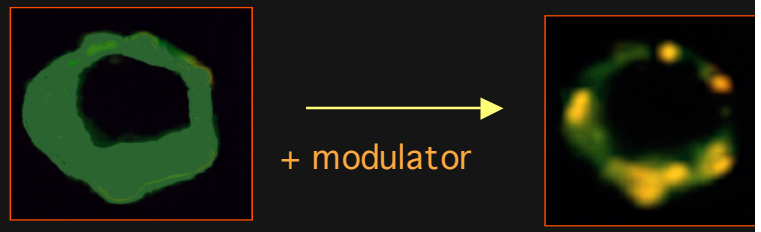
Kidney (proximal tubule)

ABC Proteins in Acute Myelogenous Leukemia : a prognostic factor



P-gp
MRPs
BCRP





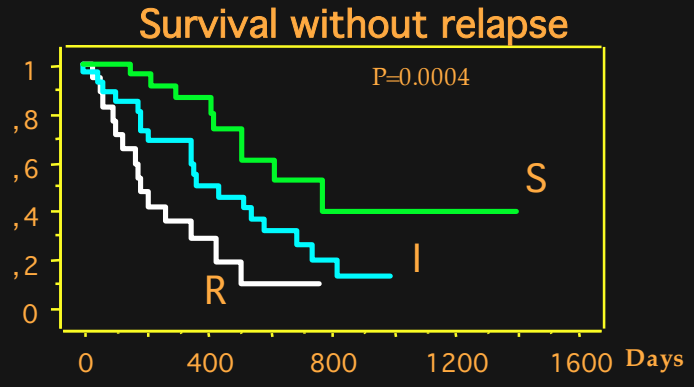
Fluorescence is quantified with flow cyto

The efflux of the fluorescent probe inhibited by a « modulator » of ABC protein allows us to mesure the ABC protein fonction in tumoral cells

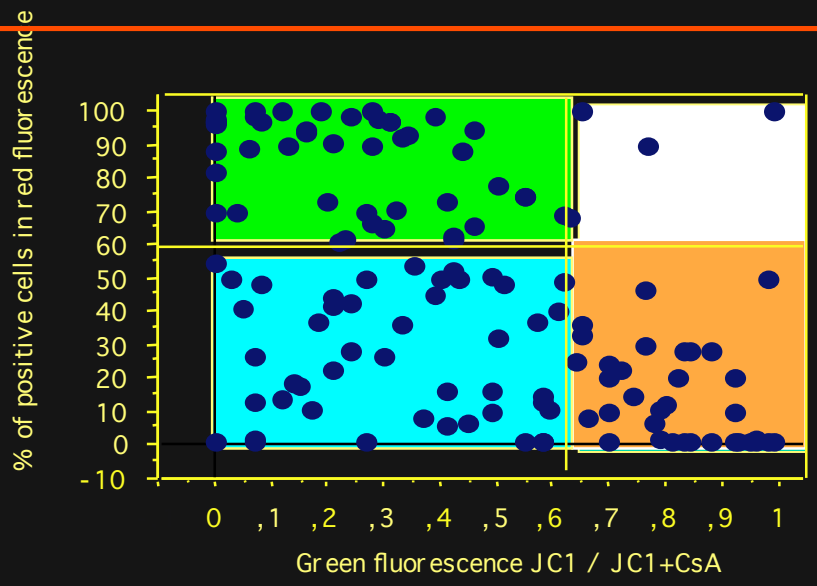
This efflux is HIGHLY predictive for response to chemotherapy in AML

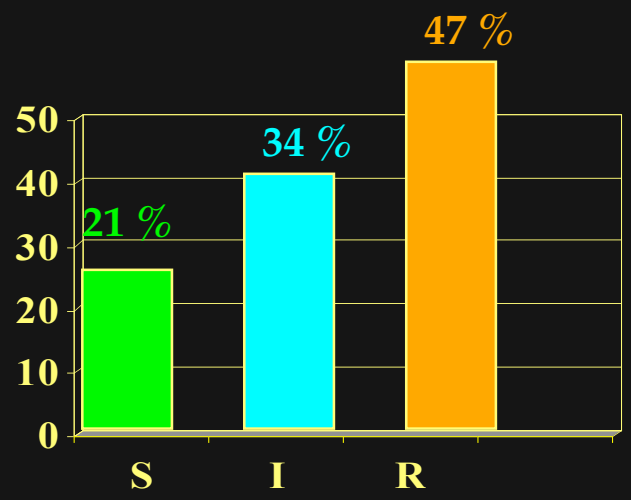
(Legrand, Blood 97 : 502-508, 2001)

The other pronostic factors in AML are
- Caryotype (clonal abnormatities)
- AML secondary to myelodysplasia



in vitro/in vivo Resistance in AML tested with JC1: 3 prognostic groups

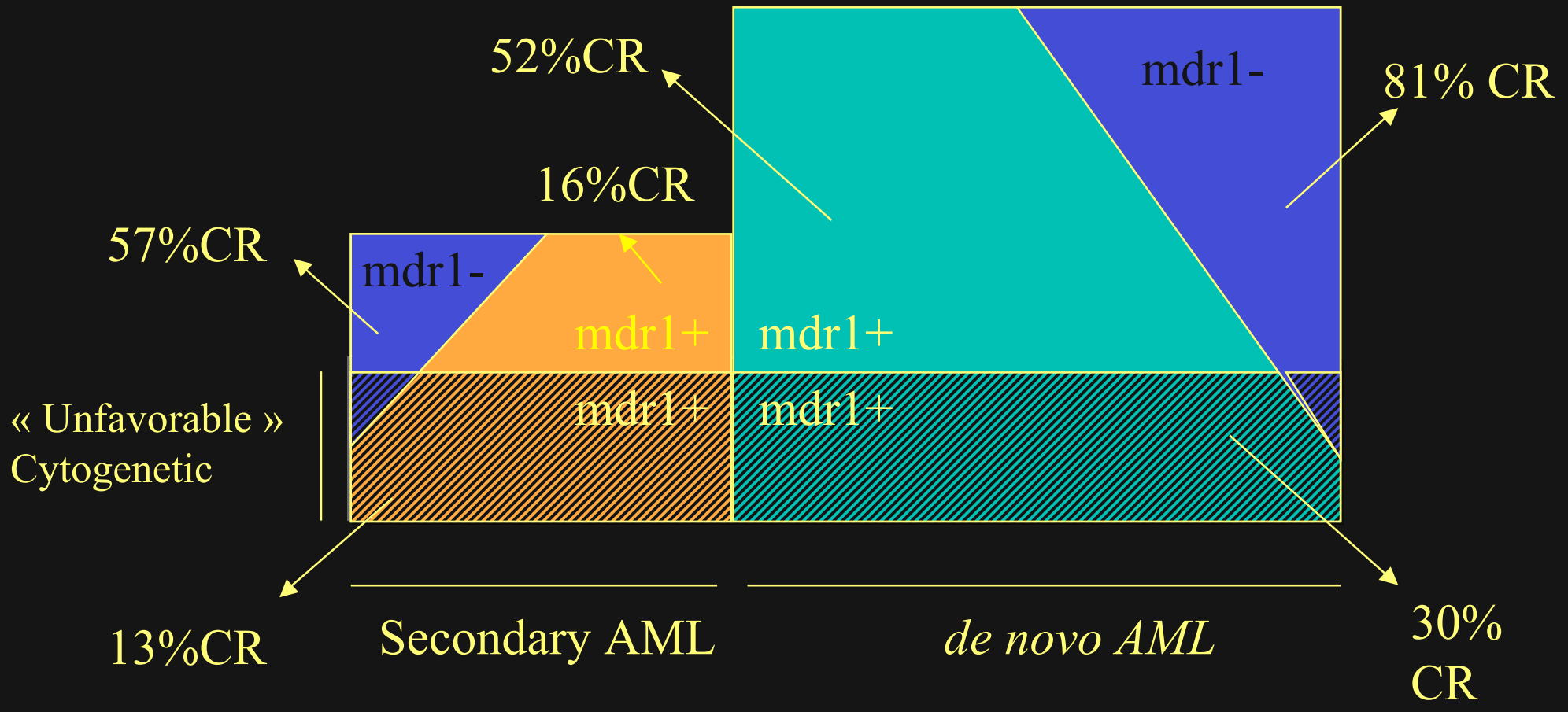




Resistant disease after AML10 treatment

Pgp (UIC2)	IC50 DNR	IC50 etoposide	CD34
0.19	12 µM	839µM	43%
0.30	20µM	1731µM	66%
0.40	94µM	2131 µM	91%

MDR1/Cytogenetic and secondary AML in 146 elderly AML (SWOG study)



Leith et al, Blood 1997

Cytotoxic potentiation of daunorubicin by verapamil and cyclosporin A in a P-gp+ cell line (Ross, Blood 1995)

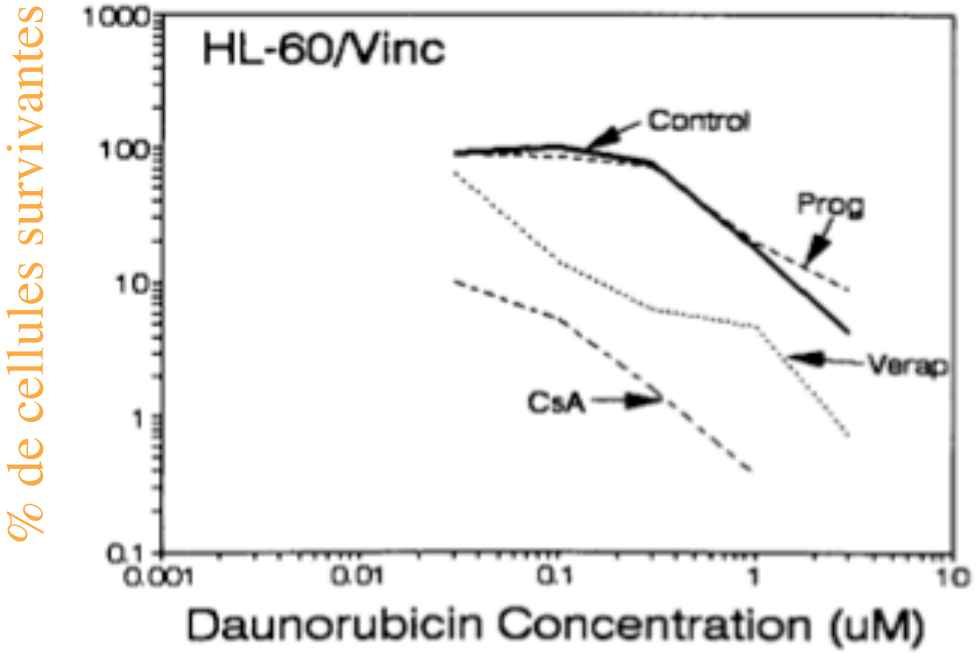


Fig 2. Effects of the MDR modulators verapamil (6.6 μmol/L), cyclosporin-A (5 μmol/L) or progesterone (10 μmol/L) on the cytotoxicity of DNR to HL-60 cells. Cells were exposed to DNR and/or MDR modulator for 72 hours, then surviving cell number was determined by flow cytometry. The coefficient of variation for each experimental point in the figure is less than 10% of the mean value.

- Use of « modulators » With cytotoxic to reverse MDR in tumor

Reversion of P-gp efflux :
from bench to clinic

- Since 1981 (Tsuruo: verapamil), dozen of compounds were described as P-gp « modulators ». Mechanism of action is mainly competition with drug for P-gp.
- Many phases I and phases II of cytostatics + modulator were published in leukemia and solid tumors.
- During the last 10 years, several compounds with high reversal potency were developed specifically for clinical MDR reversal by pharmaceutical companies.
- Randomized phase III (cytostatics ± modulators) in AML are now completed and published

Pharmacological Drug Resistance Modulation : why AML is a good candidate for clinical trials

- MDR phenotype is easy to measure in circulating leukemic cells (flow cytopmetry)
- Intracellular pharmacokinetics of cytostatics (± modulator) could be checked in circulating leukemic cells during treatment
- Anthracyclines and VP16 are major drugs in the treatment of AML
- Results could be stratified according to MDR status before treatment

Trials with Modulators

Originally developed for other therapeutic indications：

Quinine	phase III (AML)
Cyclosporin A	phase III (AML)

Compounds specifically developped for modulation (derived from)

PSC833	(Cyclosporine D)	phases III (AML...)
LY335979	(Quinoline (MS-073))	Phases III (AML)
GF 120918	(Acridocarboxamide)	phase I (solid T)
VX-170	(Pipecolinat)	Phases II (solid T)

Randomized trials in AML/MDS with Chemotherapy ± Quinine

- QUININE : 3 French trials in AML and MDS

  - In relapsing/refractory/secondary AL (E Solary, Blood 1996;88:1198) :

  ID Ara-C+ Mitox±Q: 315 pts, Clinical Resistance : 40% vs 28% (p=0.03)

  - In high risk MDS (E Wattel, Br J Haematol 1998;102:1015):

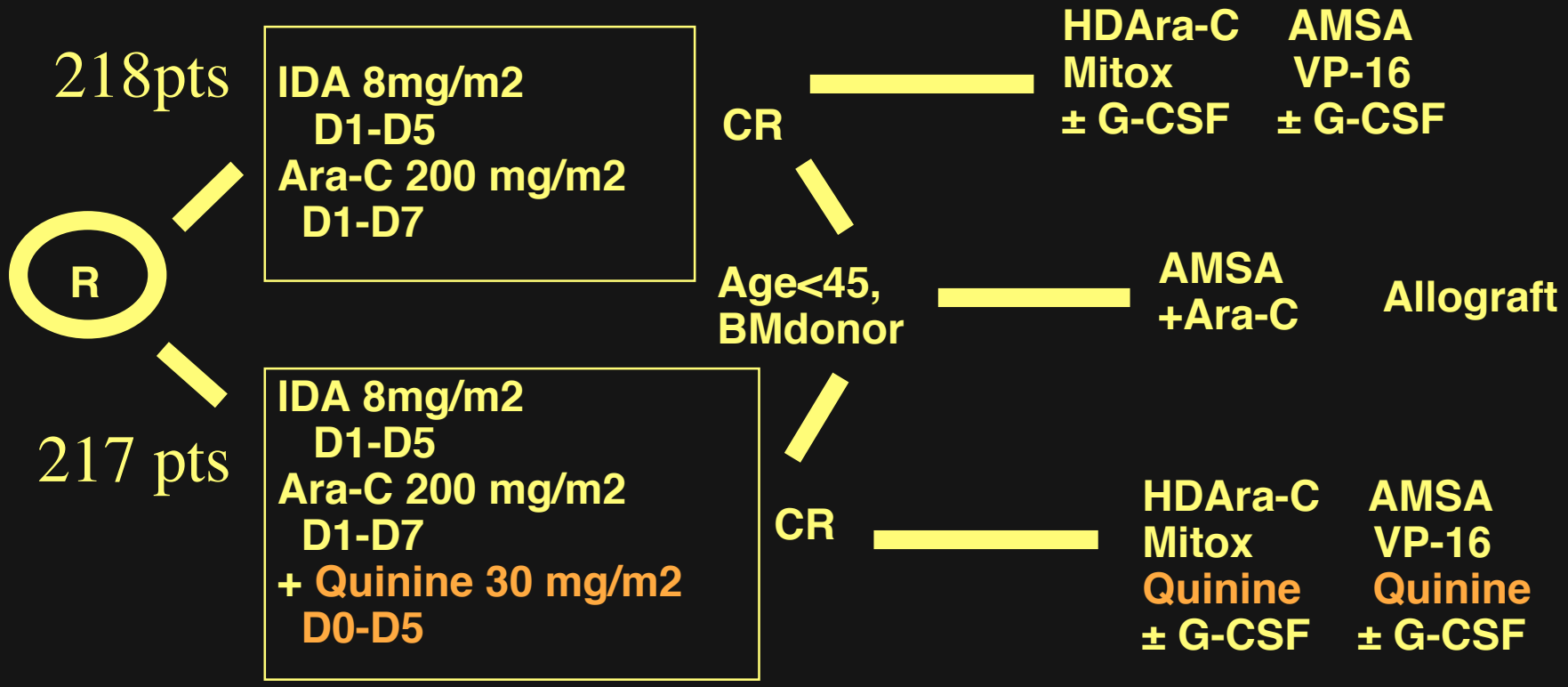
  IDAra-C+Mitox±Q: 131pts: CR:41% vs 47% (NS),
  but 18% CR without Q vs 52% Cr with Q in P-gp (+) (p=0.02)

  - In de novo AML (E Solary, Blood 2003, 102:1202-10) : Ara-C+Ida±Q:435 pts,

  CR: 81% vs 82% (NS),
  but 46% CR without Q vs 80% CR whith Q in pts with functional P-gp (p=0.02)

Ara-C+ Ida ± Quinine in *de novo* AML (GOELAM2)

E SOLARY et al, ASH2000 #2172

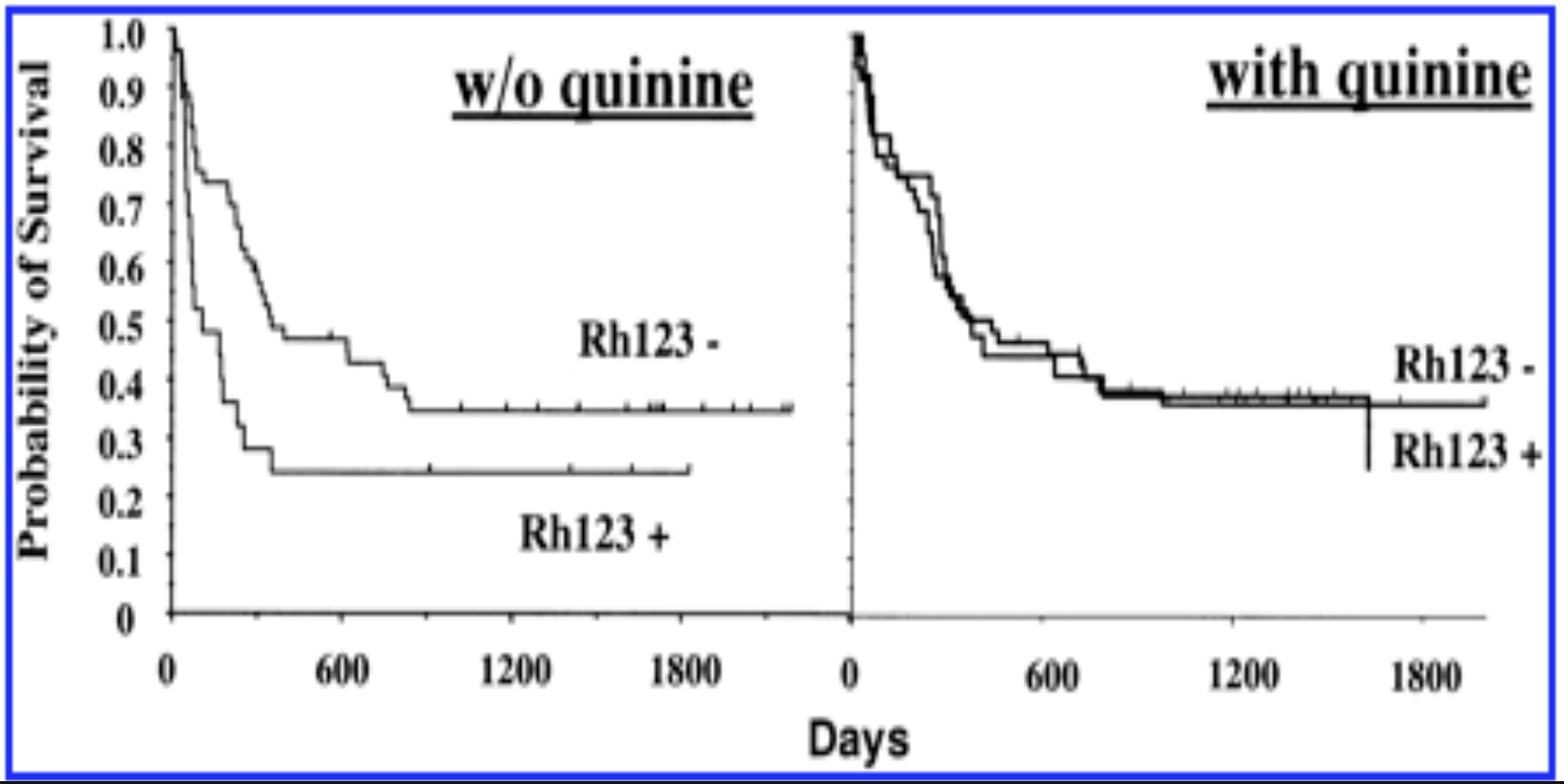


GOELAM 02 - Initial response

	w/o quinine	with quinine	*P*
All patients	169/206 (81%)	169/208 (82%)	NS
Function positive	11/24 (46%)	24/30 (80%)	0.02
negative	46/53 (87%)	41/51 (80%)	0.54
Gene positive	35/40 (87%)	28/35 (80%)	0.57
negative	78/88 (89%)	72/89 (81%)	0.70
Protein positive	16/20 (80%)	29/33 (88%)	0.70
negative	79/92 (86%)	66/83 (80%)	0.36

Quinine increases the CR rate in MDR+ patients when defined by Rh123 exclusion

GOELAMII de novo AML: Quinine rescued the Event Free Survuval in P-gp(+) AML



EFS according to
Arm (+/-quinine)
and P-gp efflux

Addition of CsA to HDAra-C + DNR in poor risk AML

226 pts randomized (SWOG Study, Blood 2001, 98:3212)

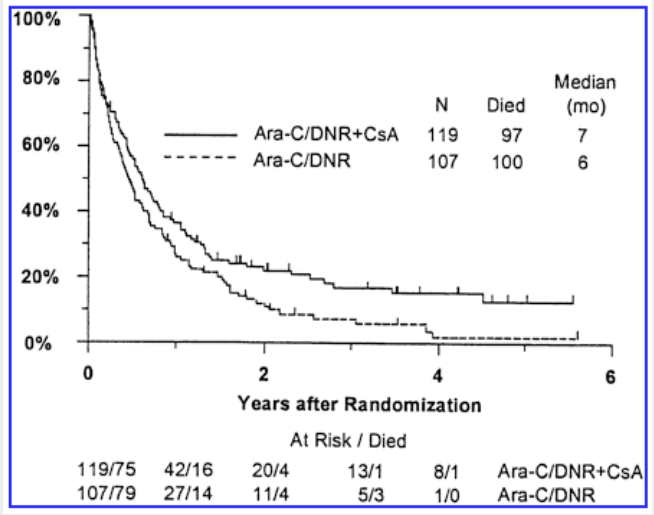
Ara-C 3g/m2/d x 5

DNR 45mg/m2/d CI x 3d

± CsA 16mg/kg/d CI x 3d

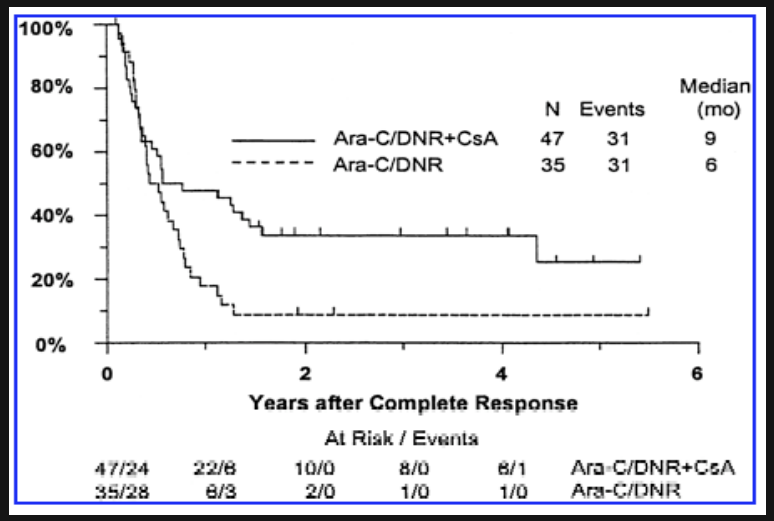
P-gp « Neg » (129pts)			P-gp « Pos » (68pts)		All	
HDA+Dnr	-	+ CsA	-	+ CsA	-	+ CsA
CR	34%	39%	26%	46%	33%	39%
Res Dis	49%	36%	45%	30%	47%	31%
DNR(ng/ml) median					11.8	23.1

Overall Survival



P=0.046

Disease Free Survival

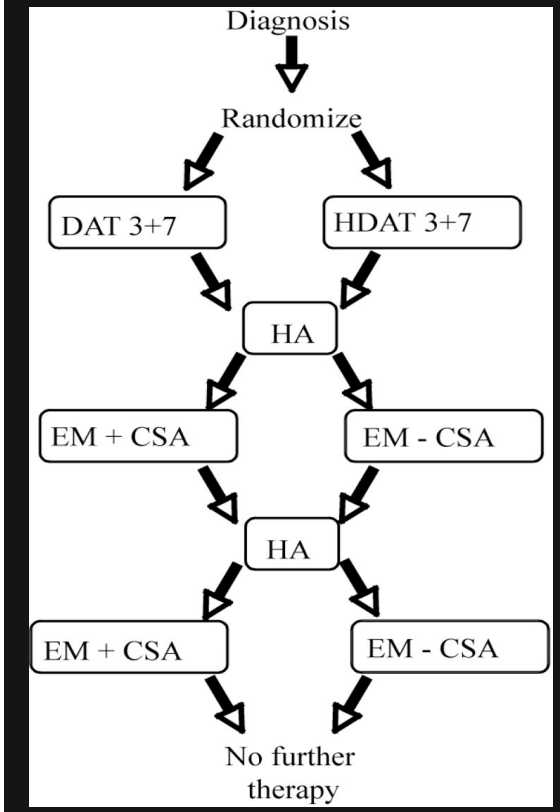


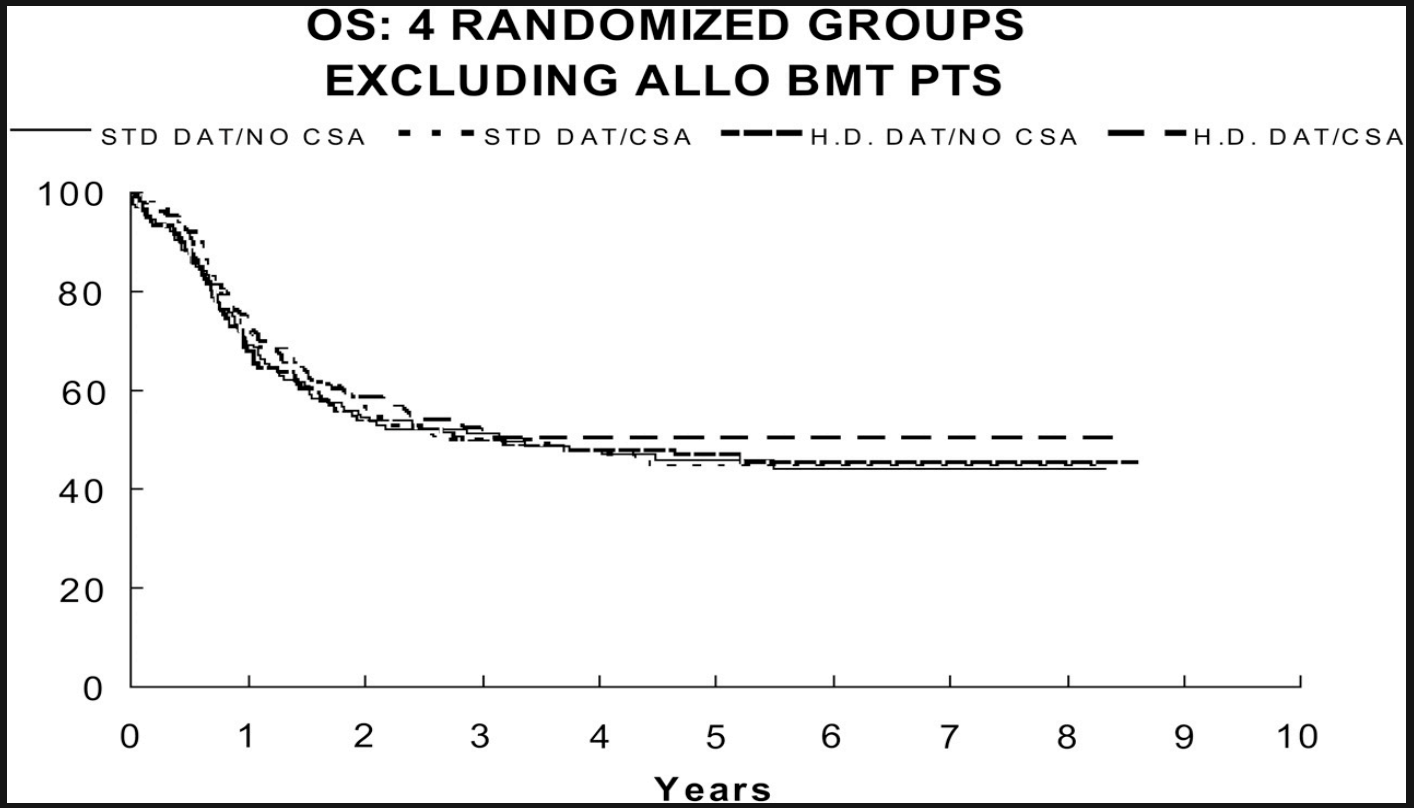
P=0.037

Randomized use of cyclosporin A (CsA) to modulate P-glycoprotein in children with AML in remission: Pediatric Oncology Group Study 9421; (Becton, D. et al. Blood 2006;107:1315-1324)

OS for 4 randomized groups excluding BMT patients: No difference

But : 1. only 14% of the patients were MDR1+ in children AML
2. CsA used only after complete CR





Modulator development: PSC833/ Valspodar (Novartis)

Cyclosporin A analog

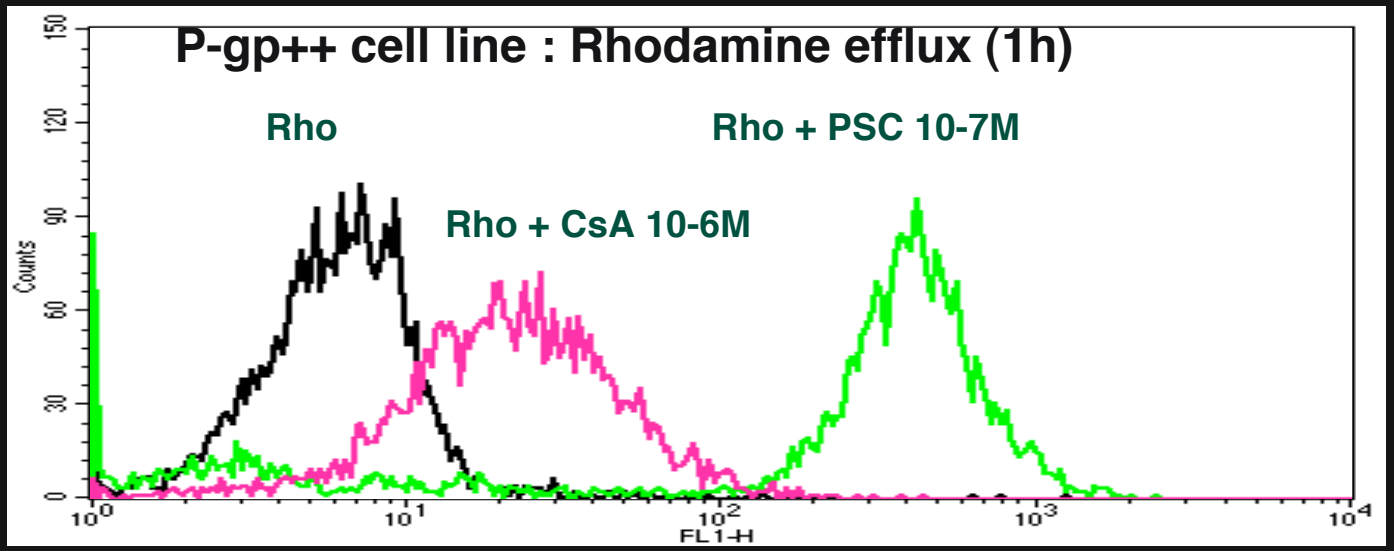
- less toxique (nephrotoxicity, immuno-suppressive)
- better P-gp inhibition

Þ Screening of CsA analogs : PSC833

No nephrotoxicity

No immunosuppressive effect

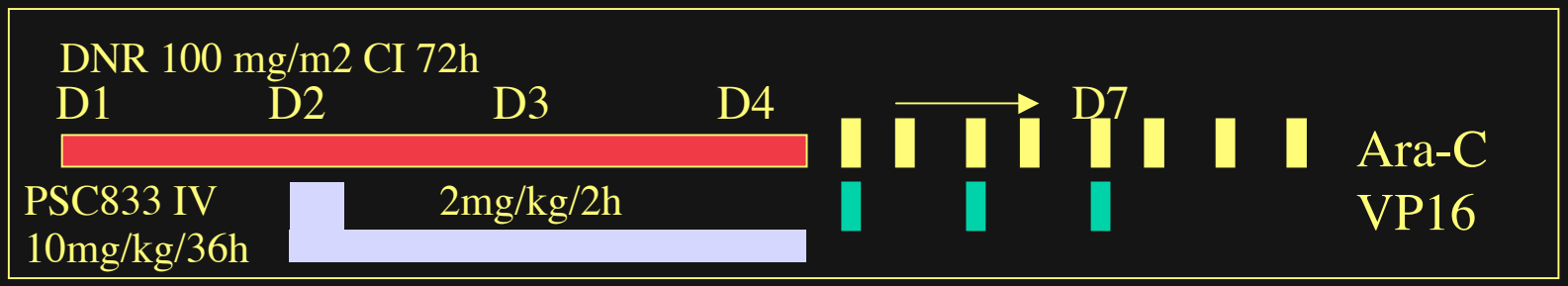
x10 to x100 times more potent as P-gp modulator than CsA

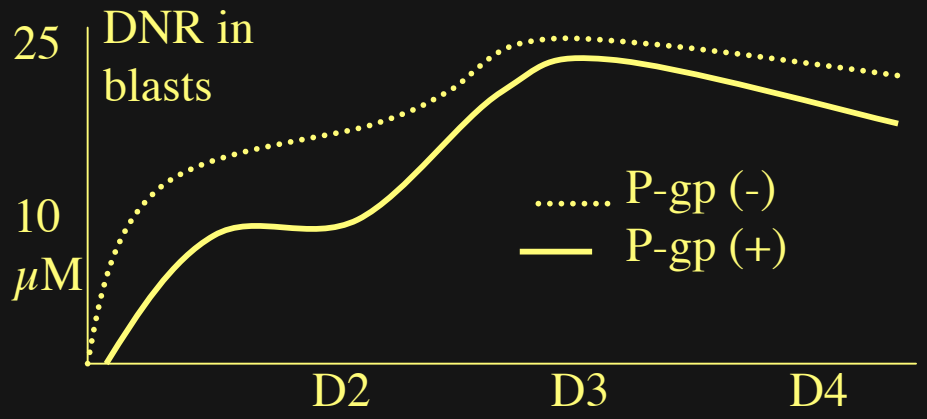


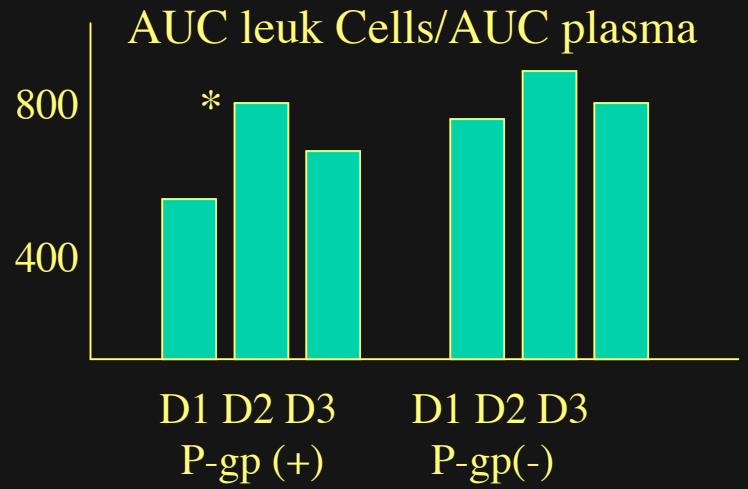
PSC833 increases the intracellular concentration of DNR in AML cells in vivo

Tidefelt et al, JCO 2000, 18:1837 (Karolinska Inst.)

- 10 pts with AML studied (7P-gp+, 3 P-gp-)



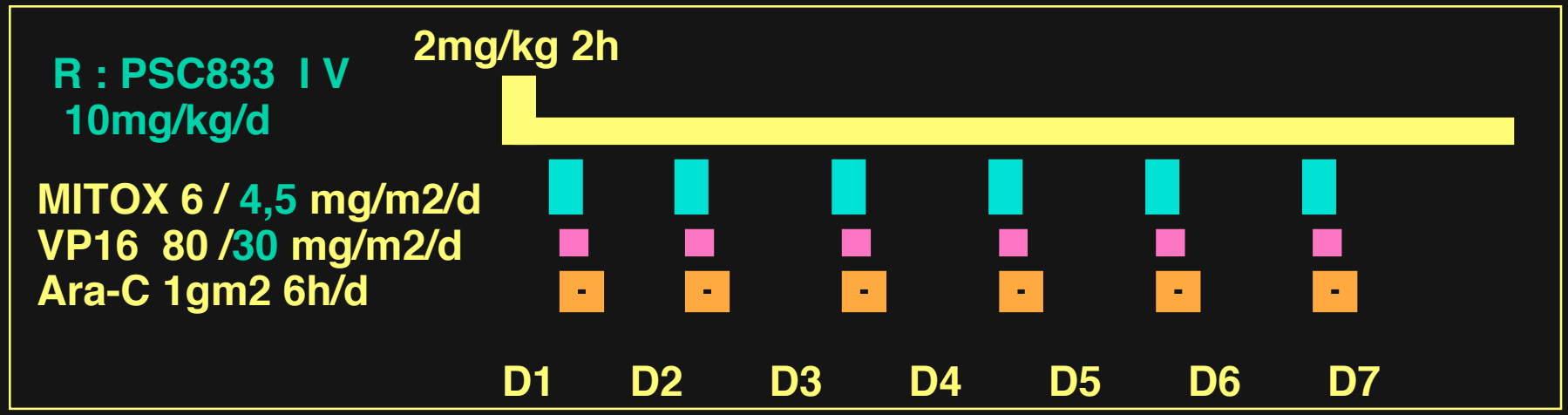




PSC 833 (Valspodar) : Phases III in AML

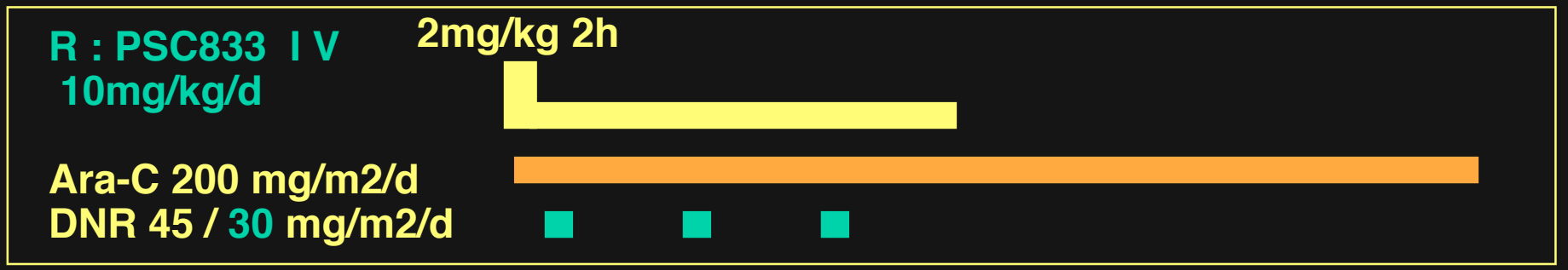
Novartis trial C301 : MEC (Mitox/Ara-C/VP16) ± PSC 833

Patients refractory to tt or in early relapse (<1year): 102 centres in 17 countries, 250 inclusions. 97-99.



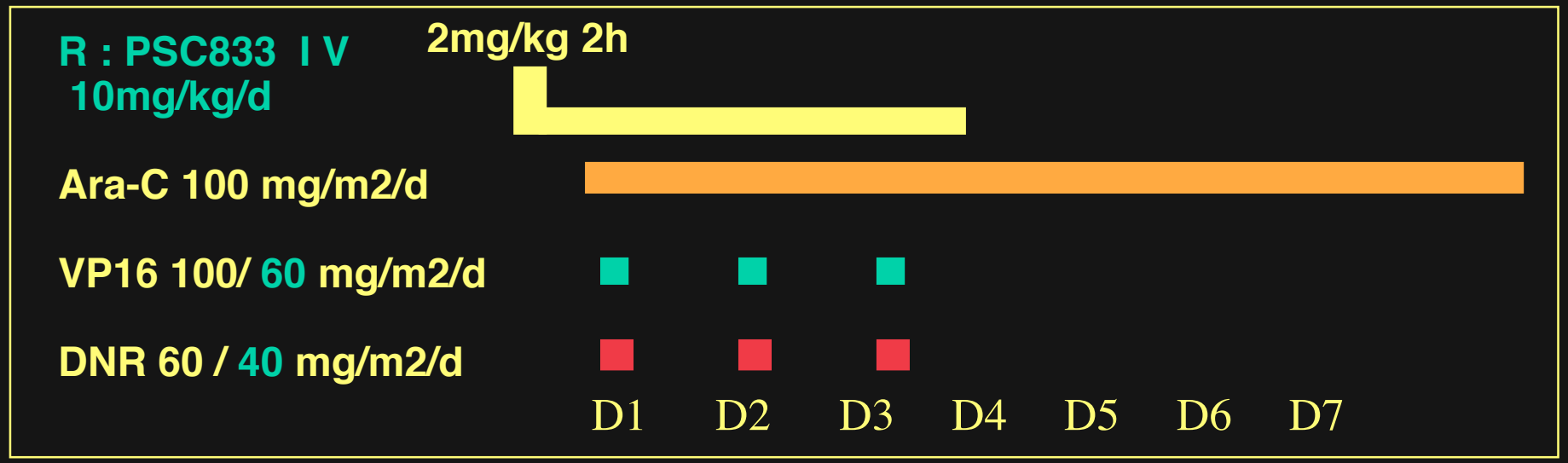
Novartis trial C302 : DNR+AraC ± PSC833 in AML≥65years

Induction treatment : 97 centres, 466 inclusions.



PSC833 in elderly AML : the CALGB trial (ASH 1999, abstract 1704)

Pts >60yo with de novo AML (87) and post MDS AML (33) were included. The trial was hold in March 99 to assess toxicity.



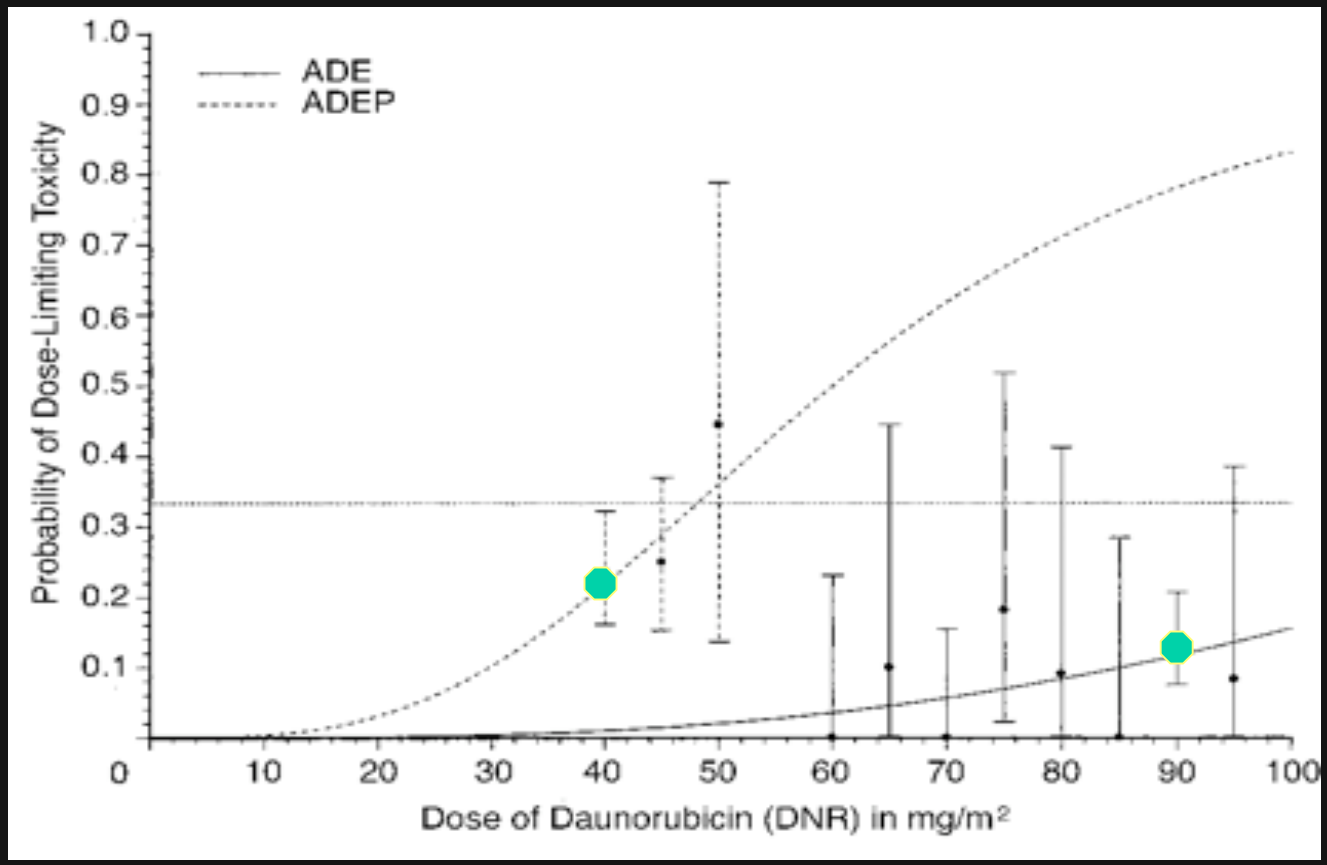
120 pts analyzed: ADE: CR: 45%; Deaths: 27%

PSC833+ADE CR: 31%; Deaths: 54%

Trial was stopped due to excessive toxicity (infection++)

CALG B 9621: Dose escalation of DNR and VP16 ± PSC833 and Ara-C in young untreated AML: 410 pts included

Estimation of maximum-tolerated dose of DNR



40 mg/m2 (+PSC833) and 90 mg/m2 were choosen for the phase III

Kolitz JE et al, JCO 2004, 21:4290

de novo « young » AML:Ara-C+ DNR+VP16±PSC833

CALGB9621
(Kolitz, JCO 2004; 22:4290)

Tt	Nb	CR in 1 course	ESF	EFS <45a (220)
ADE	394	85%	1 y	0,8 y
ADE-P	192	94% (p=0.02)	1,7 y	2,4 y (p=0.007)

R : PSC833 I V
10mg/kg/dx3d (during DNR and VP16)

Ara-C 100 mg/m2/dx7d

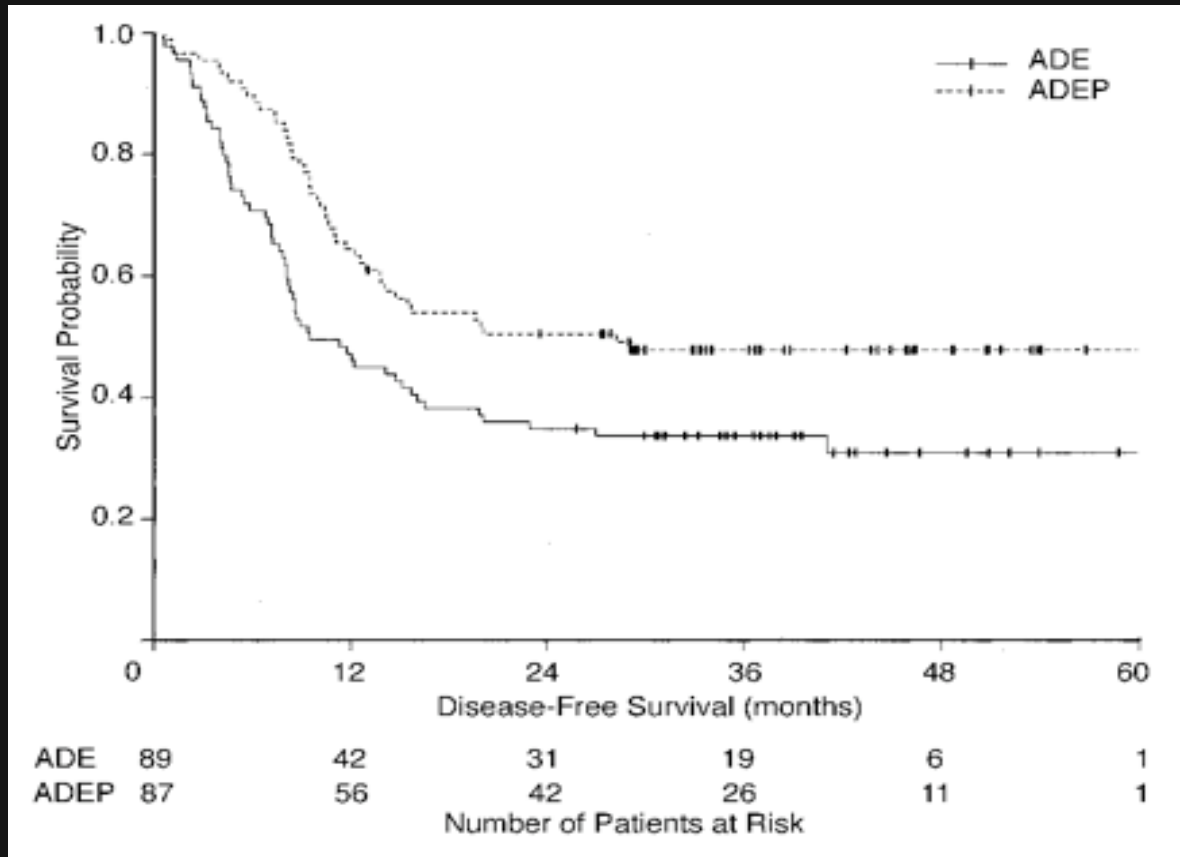
VP16 100->150/ 40->60 mg/m2/dx3d

DNR 60->95 / 40->50 mg/m2/dx3d

LTD finding :

without PSC833: 90 DNR + 100 VP16
with PSC833: 40 DNR + 40 VP16

Phase III stopped prematurely due to the decision to stop PSC833 development

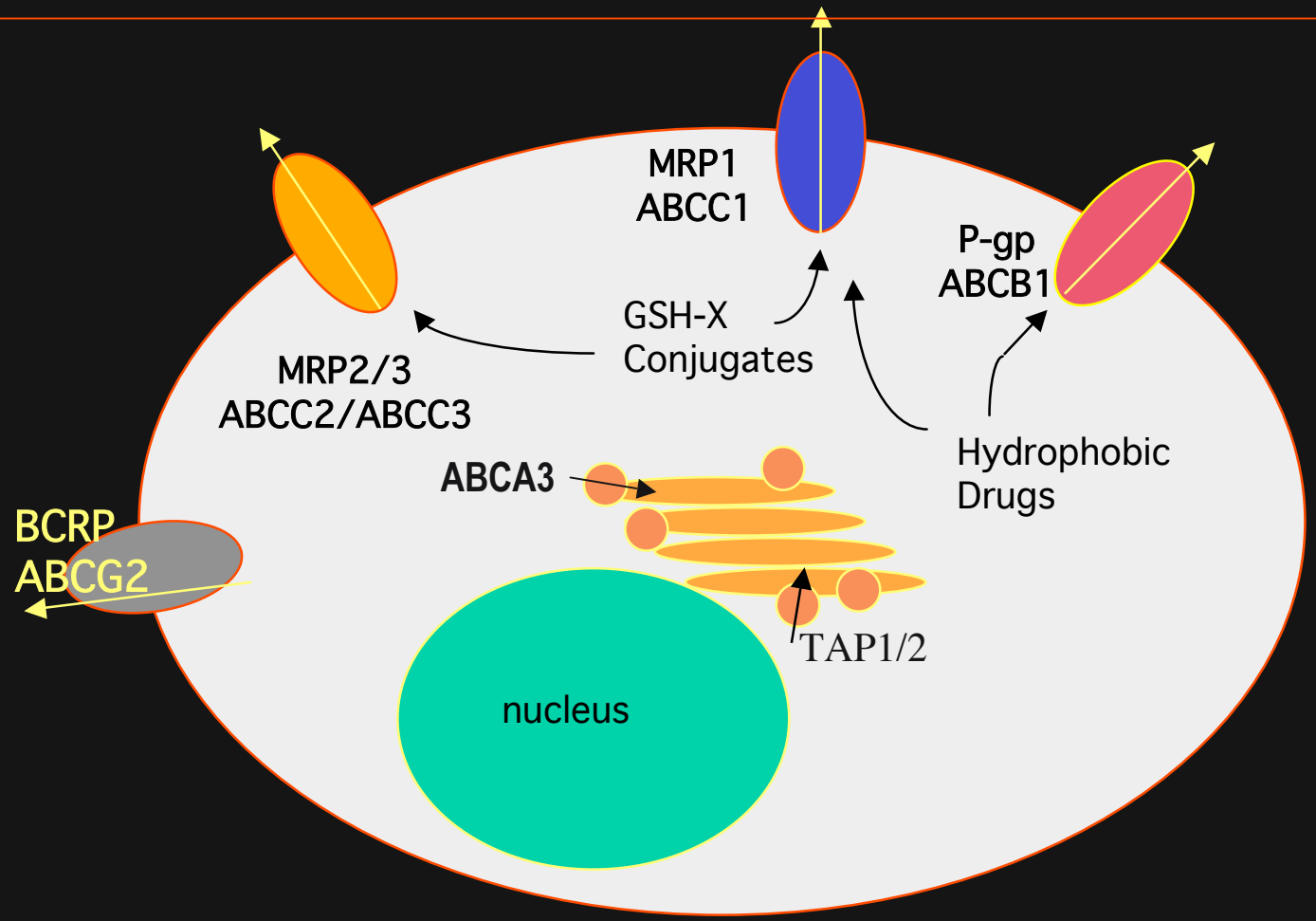


Evaluation of ABC transporter - Modulators Trials

- Very efficient modulators (like PSC-833) decrease the clearance of cytostatic drug(s) co-administred : to be able to evaluate the response and toxicity, the AUC of these drugs have to be the same in both arms (with and without modulator).
- Addition of modulator will benefit only to patients with functional P-gp : results have to be stratified according to functional tests.
- Addition of intermediate/ high doses of Ara-C to anthracycline/VP16 ± modulator could mask the effect of P-gp modulation

Transport Mechanisms involved in Drug Resistance

P-gp
MRP1
MRP2
MRP3
BCRP
TAP1/2
ABCA3



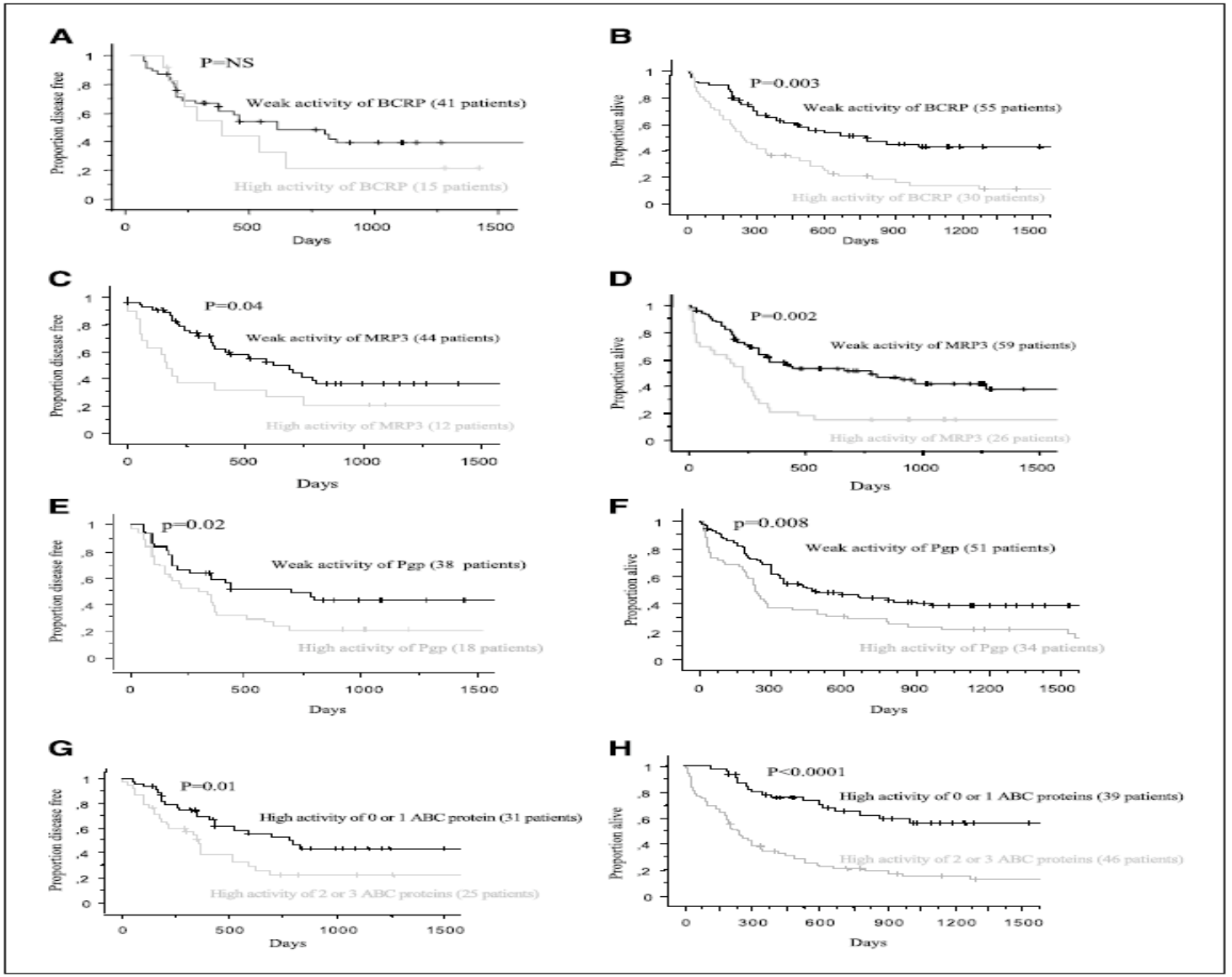
cDNA transfection of de ABCB1, ABCC1, ABCG2, in sensitive cell lines gives a MDR phenotype

Cell line Transfection

Chimio Résistant (R) Sensible (S)	P-gp (ABCB1)	MRP1 (ABCC1) (ifGSH)	MRP2 (ABCC2) (if GSH)	BCRP (ABCG2)
Anthracyclines	R	R	R	R
Mitoxantrone	R	S	S	R
Vinca-alkaloids	R	R	R	S
Taxanes	R	S	S	S
VP16	R	R	R	R
Methotrexate	S	S	R	NT

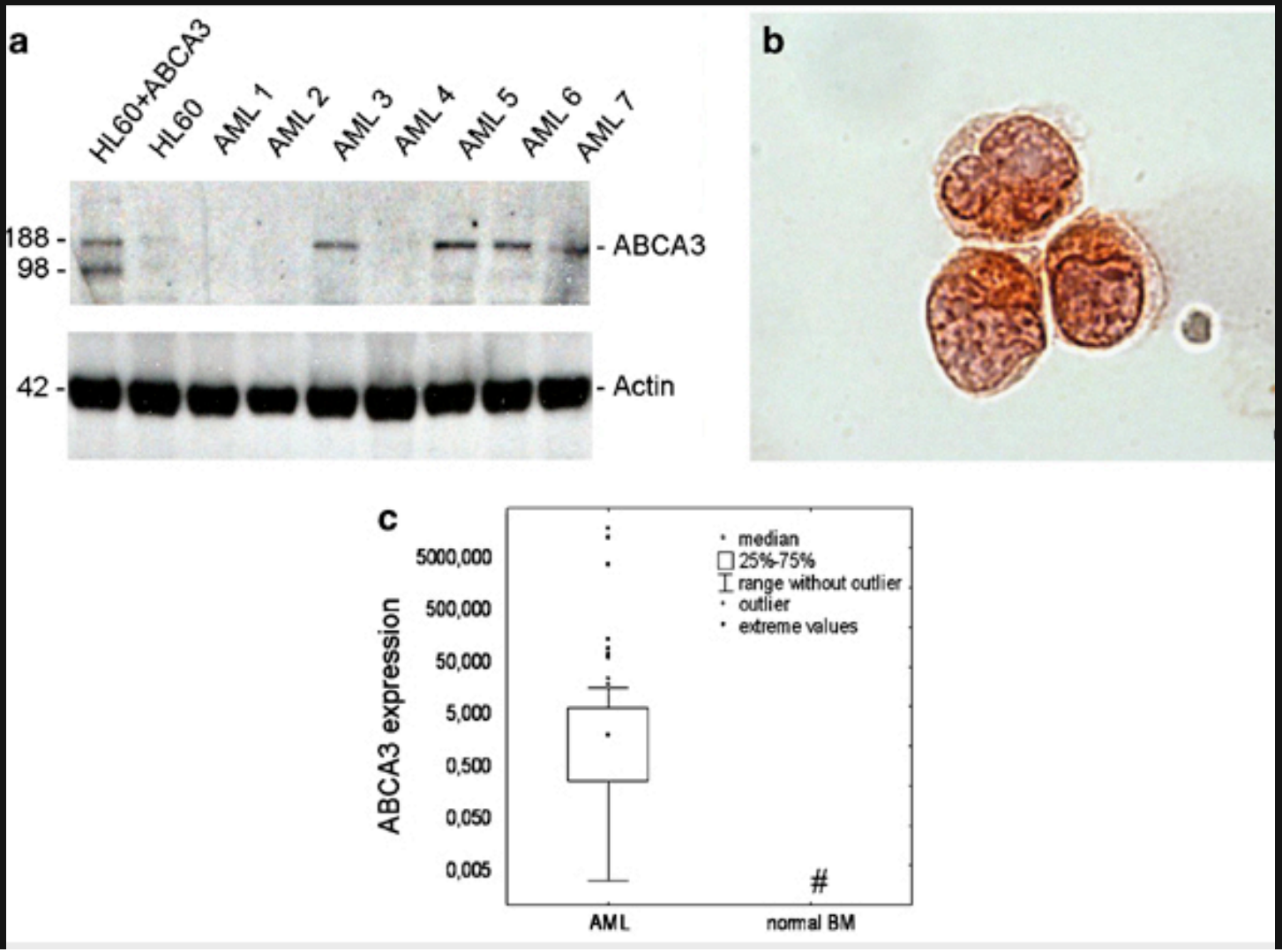
ABC Protein expression and pronostic value in AML

(C Marzac et al, Clin Cancer Research, 2005)



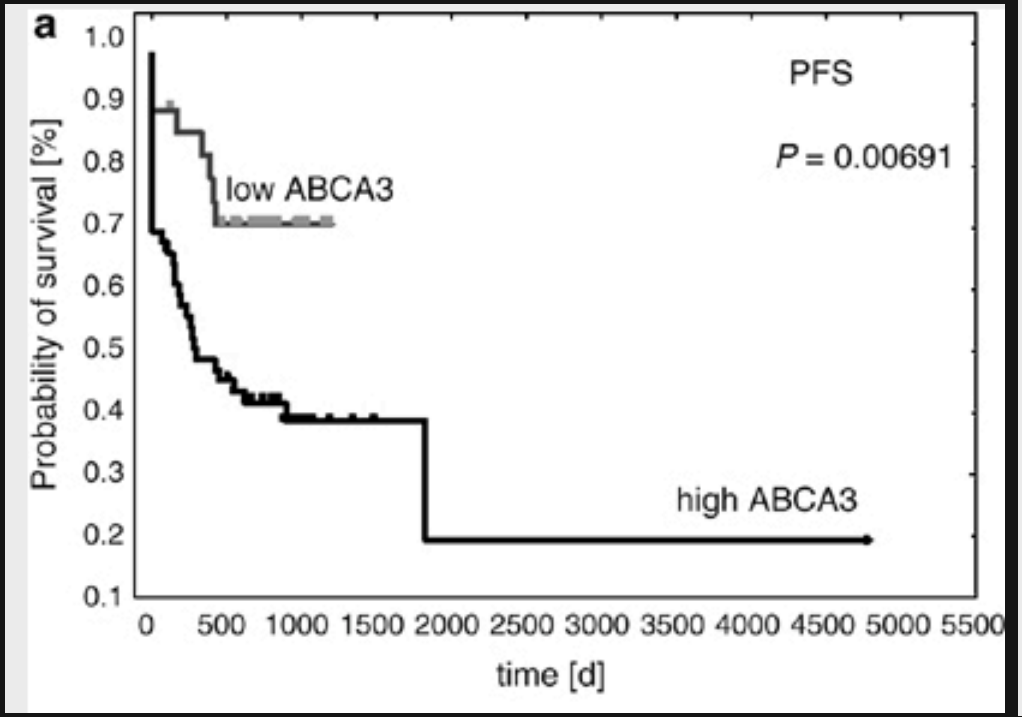
ABCA3 expression in AML:

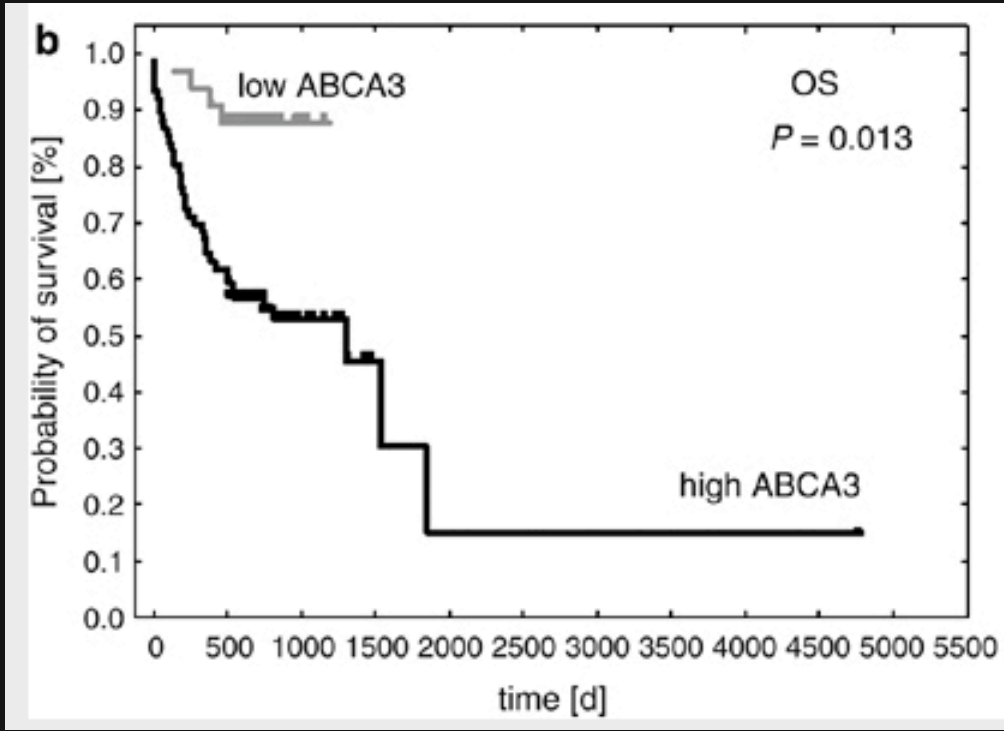
Chapuy et al, Leukemia 2008; 22:1576



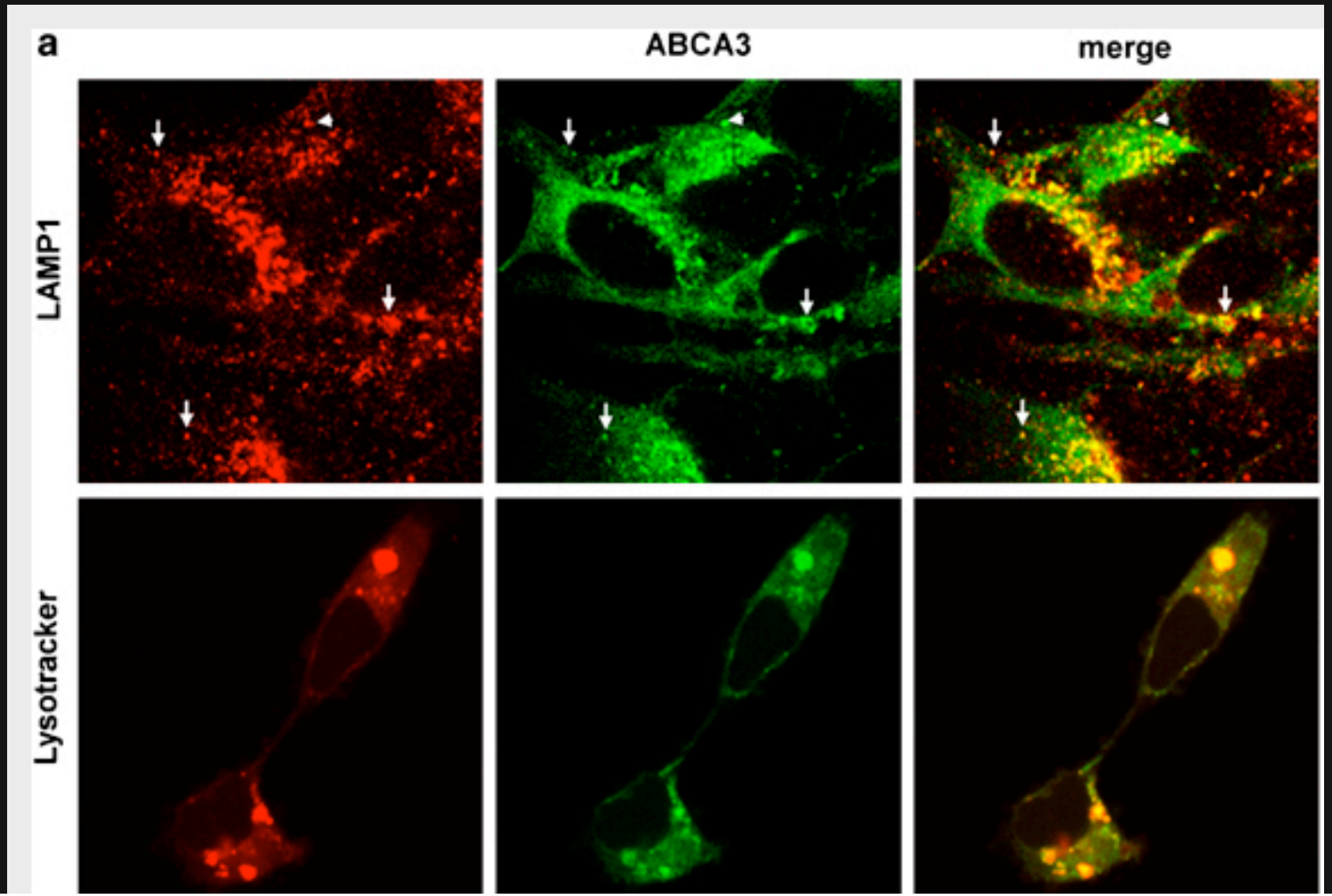
ABCA3 expression in AML: pronostic value on 86 AML

Chapuy et al, Leukemia 2008; 22:1576





ABCA3 is localized in the endosomal system



mRNA expression of 49 huABC proteins in « extreme » cohorts of AML

- « sensitive » AML to one standard treatment (CR>3y)
- « resistant » to such treatment (failures and CR<3months)

Patients features

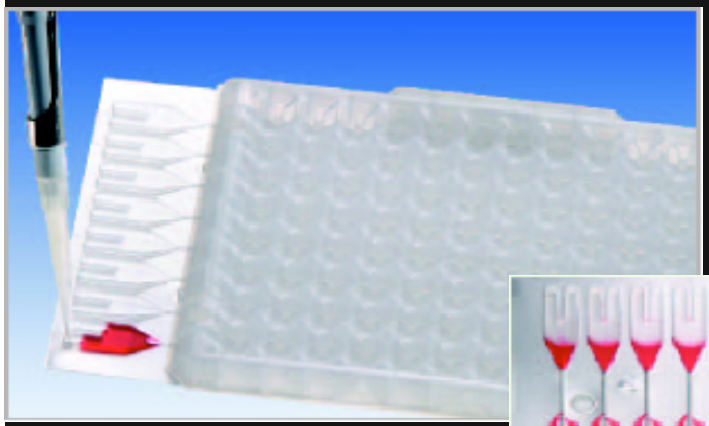
		Sensibles	Résistants
Age médian au diagnostic	50 (17-78)	48 (17-78)	52 (19-73)
Sexe			
Femme	30	20	10
Homme	21	11	10
Sous-types FAB			
M0	0	0	0
M1	20	10	10
M2	11	8	3
M3	1	1	0
M4	7	6	1
M5	8	5	3
M6	4	1	3
M7	0	0	0
Cytogénétique			
Favorable	11 (22%)	11	0
Intermédiaire	26 (50%)	14	12
Défavorable	14 (28%)	6	8

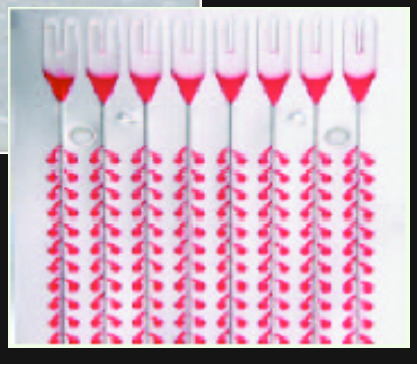
C Marzac, O Legrand, E18 UMRs872 and E Garrido, CNRS UPR 2301

Taqman Low Density Array

7900Ht Fast real-time PCR system (Applied biosystems)

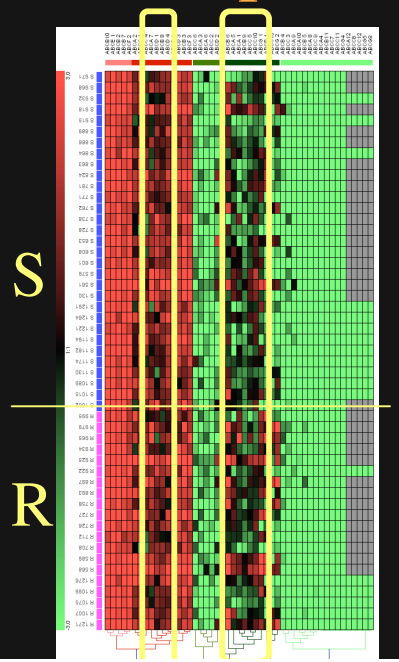
Principle: simultaneous quantitative PCR, in **1 µl microwells**



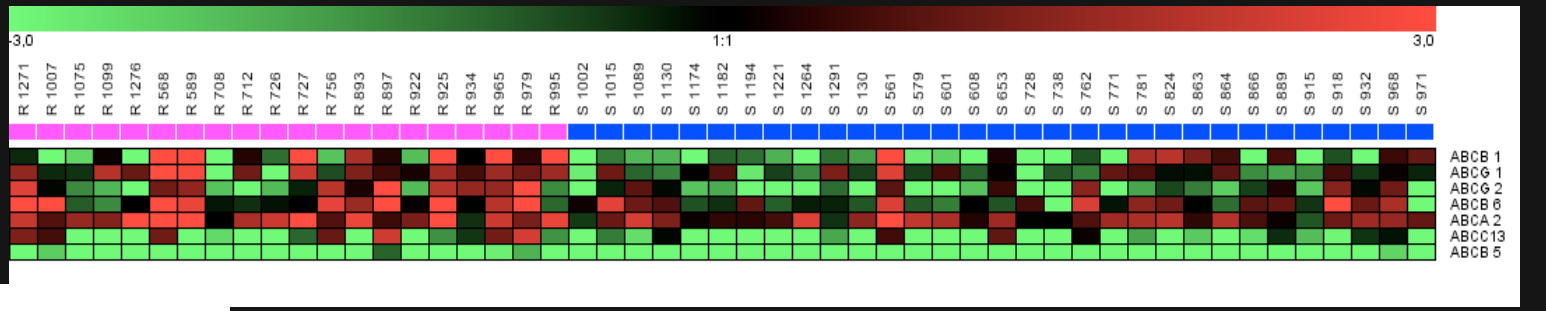


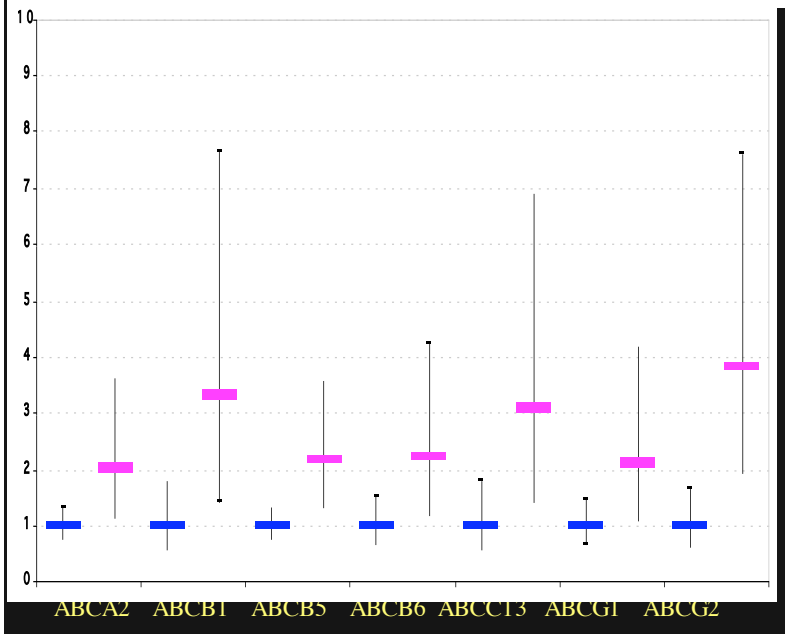
- Probes (Taqman) are lyophilized *in situ* on microfluidic chip
- 12 à 384 well/transcripts including 1 housekeeping ge
- 1 to 8 samples
- The « mix » is dropped on top and diffuse in wells by centrifugation
- chip dedicated to human ABC mRNA
- Normalisation and quantification for each mRNA

ABC proteins in Acute Myeloid Leukemia: cDNA screening



Þ*Detection of differentiel expression (>2) of ABC mRNA in « expreme » populations*





Close to ABCA3. Surexpressed in HL60/AR

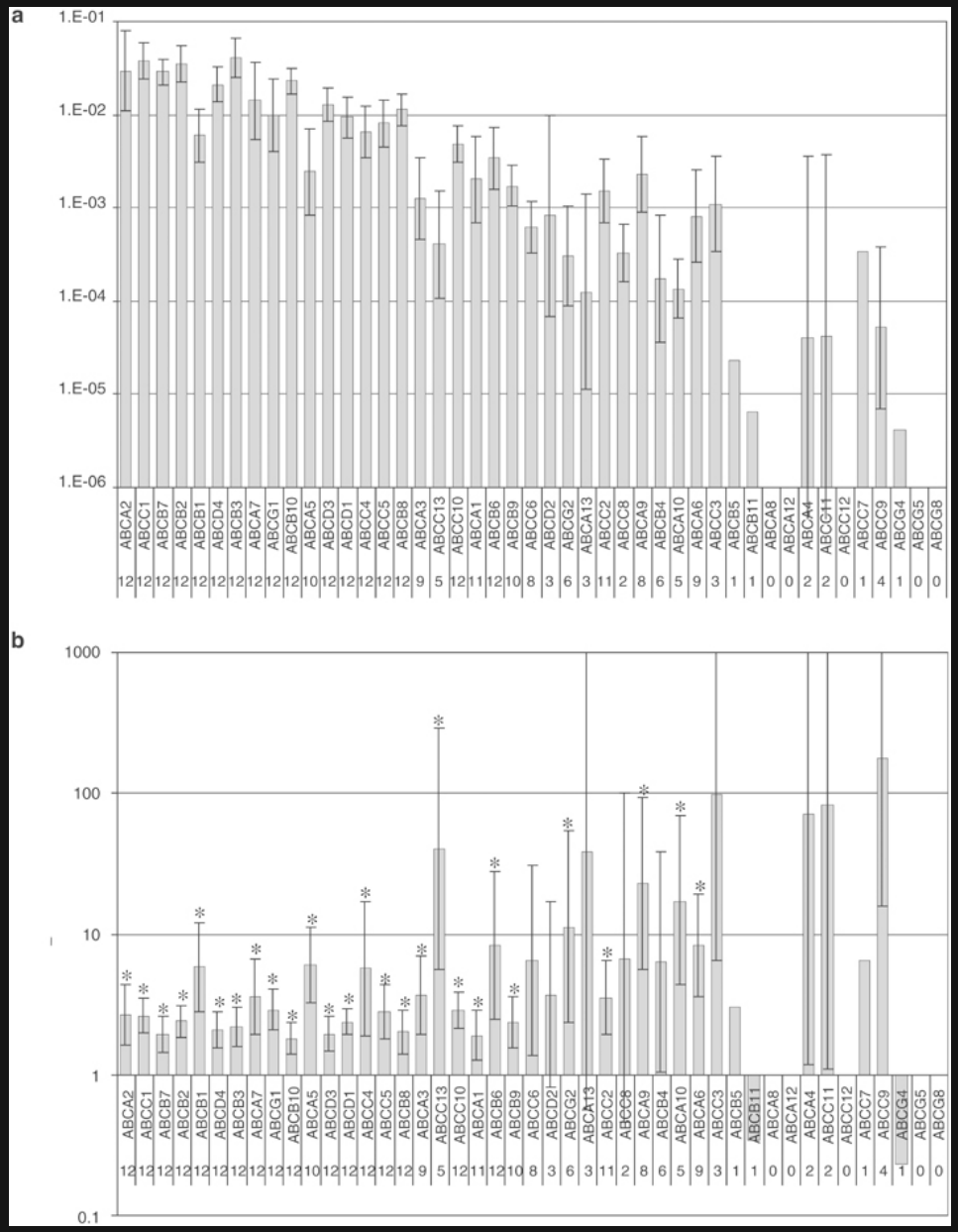
	RQ moyen	Ct normalisé moye du groupe R	P valeur
ABCA2	2,00	26,87	0,0108
ABCB1	3,32	28,88	0,0372
ABCB5	2,17	33,72	0,0071
ABCB6	2,23	27,54	0,0622
ABCC13	3,10	30,48	0,0354
ABCG1	2,11	28,30	0,0016
ABCG2	3,82	28,81	0,0033

Involved in doxorubicine transport in méla

Surexpressed in MCF-7/CH100

Non fonctionnal in mammals

ABC genes Expression in CD34+/CD38- (b m stem cells) and CD34+/CD38- (committed cell



Differencial expression between CD34+/CD38- and CD34+/CD38+ In normal bone marrow

Leukemia (2006) **20,** 750–754

Conclusions

- P-gp and BCRP are the most ABC pumps frequently expressed in « resistant » AML and are able to expel anthracyclines from leukemic cells
- Randomized trials using potent P-gp modulators demonstrated benefit only in cases with functional P-gp
- Numerous ABC pumps reduce the drug concentration in normal (and leukemic ?) cells: to eliminate leukemic stem cell expressing several ABC pumps is elusive when using inhibitors.
- Hu mRNA chips in « extreme » AML populations could be useful for detection of ABC pumps of interest in drug resistance