Classical drug resistance in the molecular age

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MTC

ICORG
The All Ireland Cooperative Oncology Research Group

DCU

NICB

National Institute for Cellular Biotechnology (NICB)

- 3,200 m^2
- €20 million biomedical research centre
- Built from a previous centre (1987)
- Core Biological Themes
  - **Cancer**
  - Commercial Biotechnology
  - Diabetes
  - Occular Stem Cell Treatment
  - Molecular Virology
  - **Focus on Industrial & Clinical Translation**

www.nicb.ie

Core Research Facilities

- Transcriptomics
  - Affymetrix
  - Q-PCR
  - Bioinformatics
- Proteomics
  - MS/ LC-MS
  - DIGE
  - Bioinformatics
- Cell characterisation
  - Confocal microscopy
  - Laser capture microdissection
  - Immunocytochemistry
  - FACS
- LC-MS Drug quantitation
- 13 Class C Cell culture suites
  - Associated core services & cell lines (~350)

ICORG

The all Ireland Cooperative Oncology Research Group

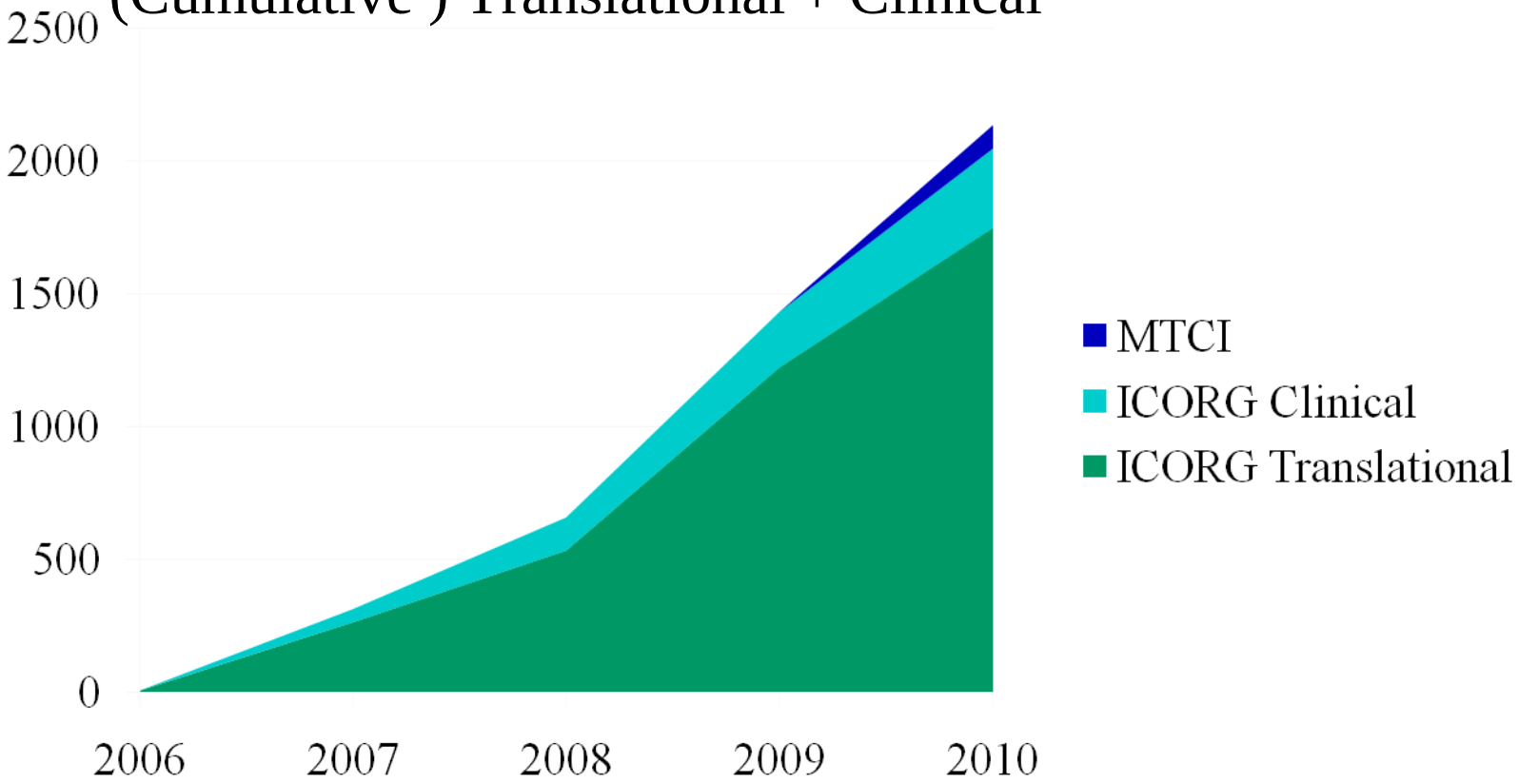
- 32 members in October ’96
- 2011 Membership 404 including >95% of the Island’s Medical, Radiation, Haematological and Surgical Oncologists. A similar percentage of the Specialist site staff and an increasing cohort of leading Translational Scientists
- >100 in translational group

www.icorg.ie

ICORG
The All Ireland Cooperative Oncology Research Group

Number of Patients from Whom Translational Samples Collected

(Cumulative) Translational + Clinical



Approximately 5% of all Irish patients diagnosed with cancer are enrolled in ICORG studies

ICORG
The All Ireland Cooperative Oncology Research Group

- Now to the science!!

Drug resistance

Drug resistance - *Tumour cells do not respond to drug treatment*

Causes – **Multiple and often in combination**

- *Apoptosis Resistance*
- *Metabolic alterations*
- *Tumour biochemistry changes*
- *Target mutations*
- Drug transport alterations
  - **Overexpression of drug efflux proteins**

Overall classification of major resistance mechanisms

Pharmacokinetic resistance	Pharmacodynamic resistance
Description of resistance mechanism	***Description of resistance mechanism***
Resistance due to factors largely outside of individual tumour cells	
Blood perfusion alterations – reducing delivery of drug	Alterations in oxygen tension
Tumour encapsulation	Alterations in redox potential
Reduction in tumour permeation	
Alterations in tumour and cellular pH	
Alterations in whole body metabolism, distribution and/or elimination	
Resistance factors evident within tumour cells	
Reduced cellular drug uptake (active or passive mechanisms)	Decreased expression of target enzyme/receptor
Increased expression/activity of drug efflux pumps	Increased expression of target molecules
Vesicular drug localisation/sequestration	Mutation of target
Increased expression of metabolic enzymes	Alterations in apoptotic cascade
Decreased expression of metabolic enzymes (for pro-drugs)	Alterations in tumour cell growth rate (inc. senescence)
	Alterations in cellular repair mechanism
	Stromal/autocrine/paracrine factors secreted which protect tumour cells from drug treatment

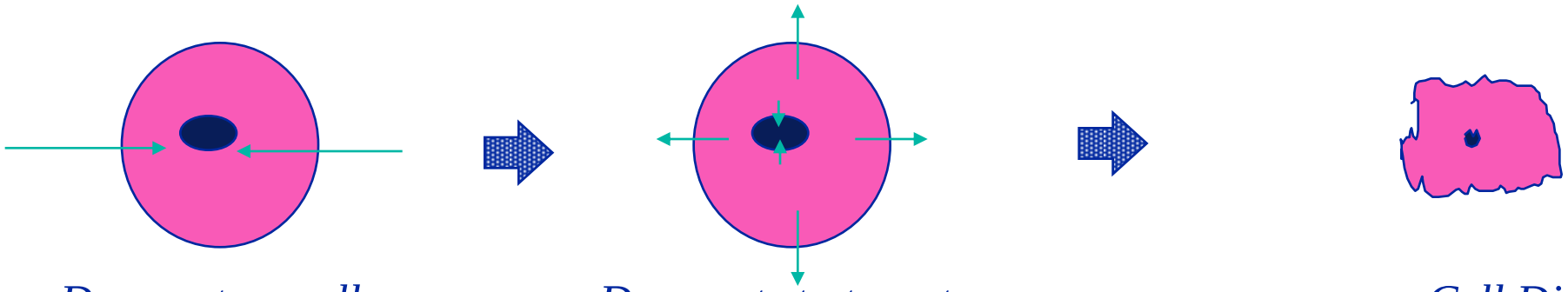
Major Multidrug Resistance Transporters in Cancer

- P-Glycoprotein (P-gp)
- BCRP
- MRP-1

Broad (and sometimes overlapping) cancer drug specificity

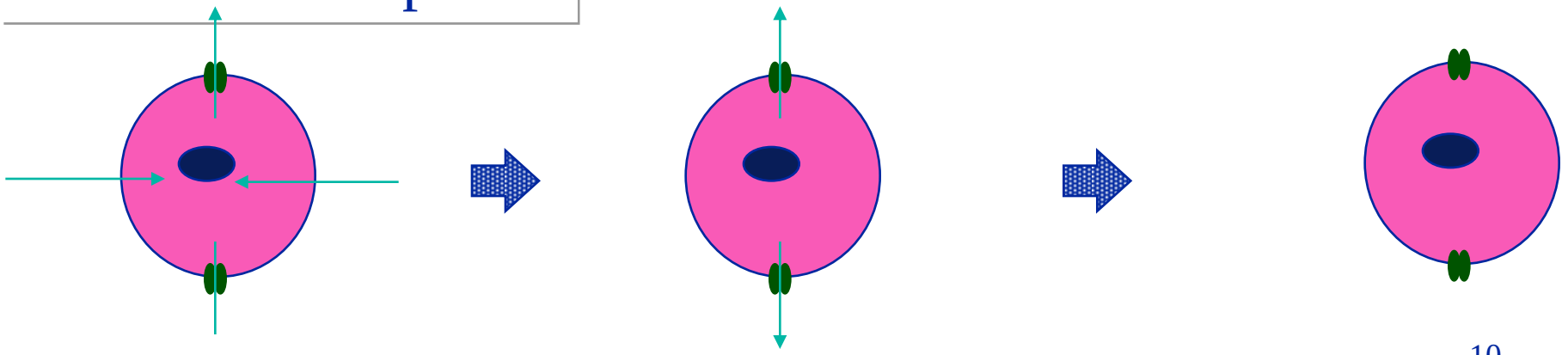
Mechanism of action in cancer resistance

Ideal Cancer Treatment



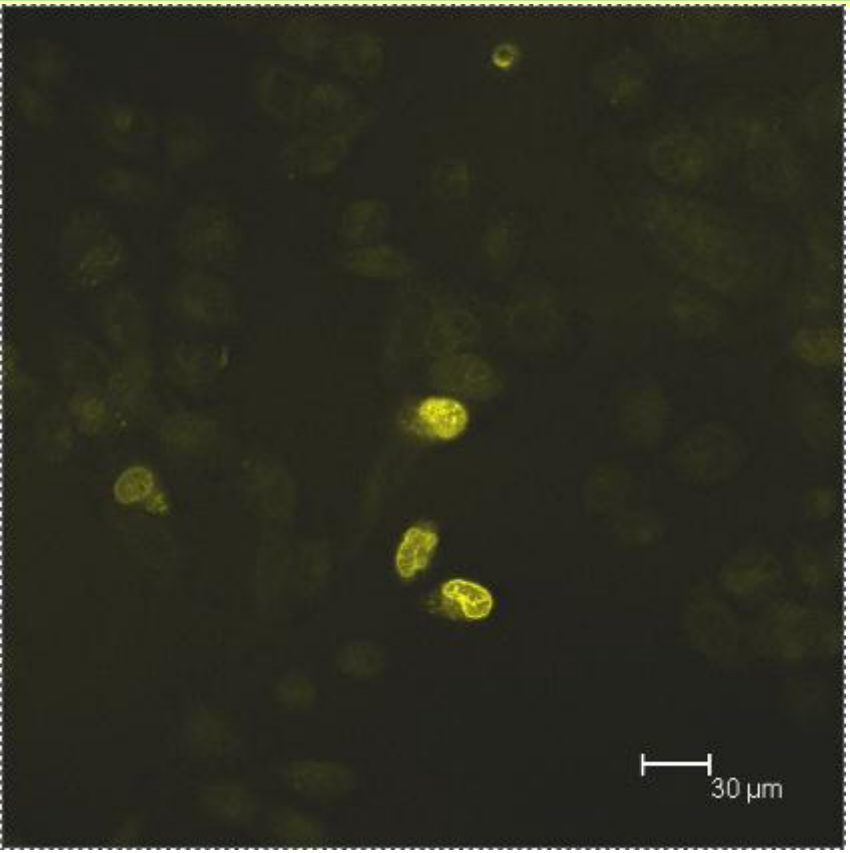
Drug enters cell *Drug gets to target* *Cell Dies*

Effect of Transporter

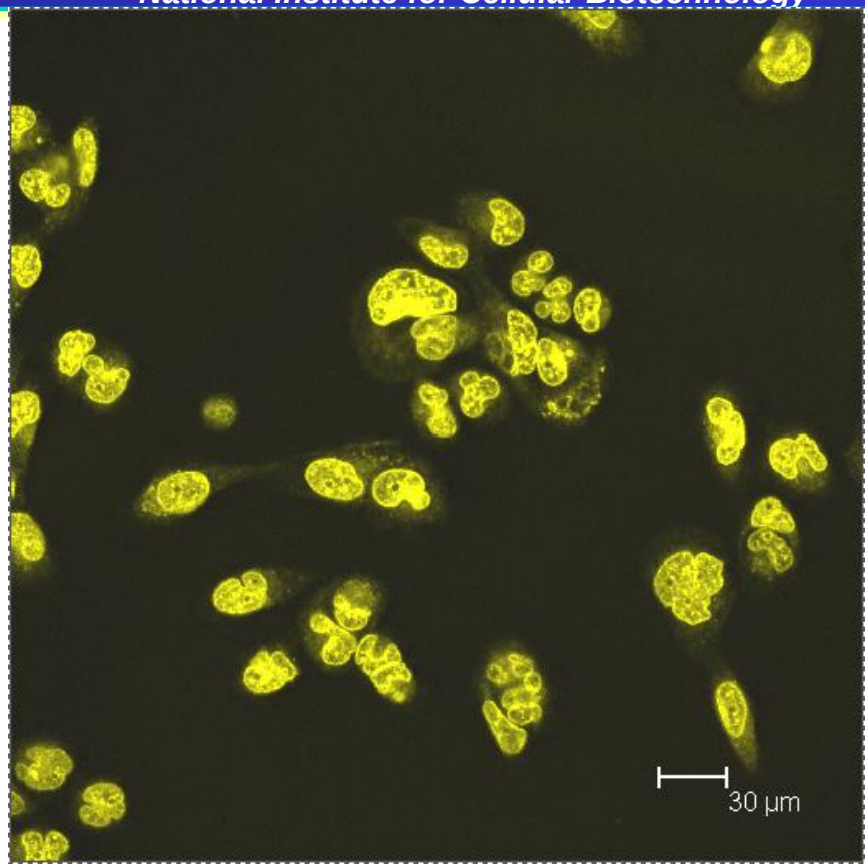


Drug enters cell *Drug pumped out* *Cell Survives*

P-gp resistance in live cells



Resistant DLKP-A cells
2uM epirubicin for 180 mins



Resistant DLKP-A cells
2uM epirubicin for 180 mins
PLUS resistance inhibitor

Physiological roles

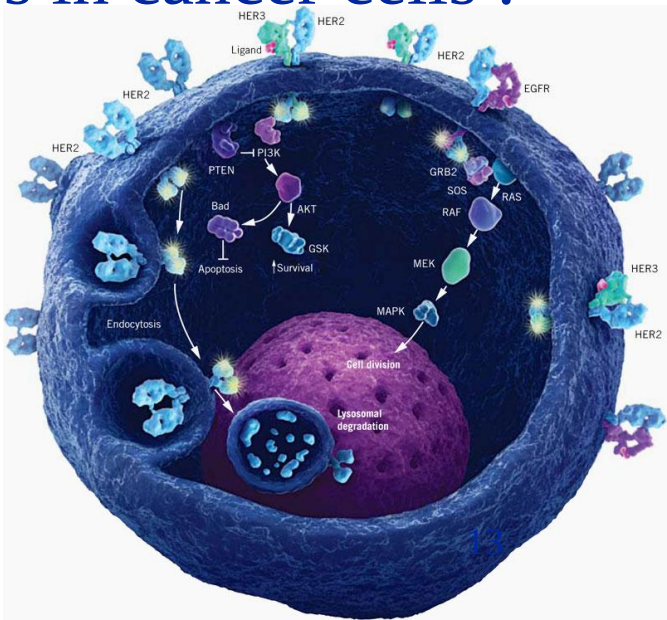
- Transporters play an important role in drug pharmacokinetics
  - Physiological barriers (usually have redundancy)
  - E.g. blood brain barrier, blood-testis barrier etc.
- P-gp particularly important for drug clearance
  - Urinary and biliary

- **Body-wide inhibition of P-gp may increase AUC and drug toxicity**

Drug Treatment in the Molecular Age

- A targeted therapy is a drug with a focused mechanism that specifically acts on a well-defined target or biologic pathway causing regression or destruction of the malignant cells.

- Targeting receptor tyrosine kinases in cancer cells :
  - Block signal transduction
  - Inhibit growth
  - Promote apoptosis

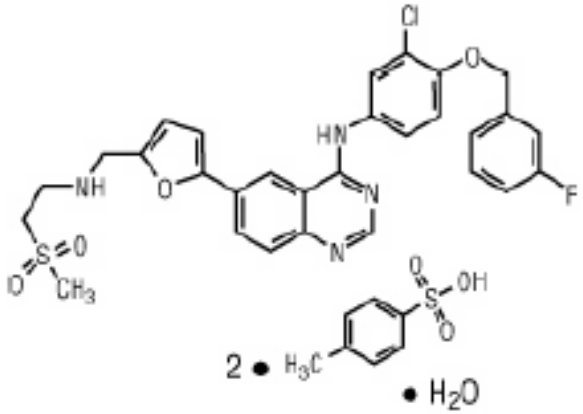


- How do these agents interact with Drug Transporters ?

- ***Its Complex!!***

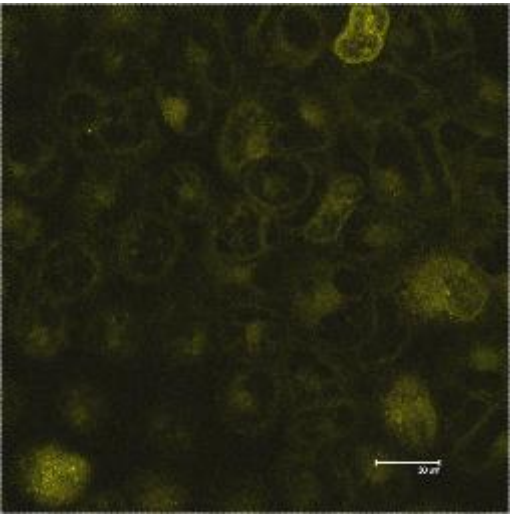
Lapatinib

- HER2 and EGFR-targeting TKI
- Used in HER2^{+} breast cancer

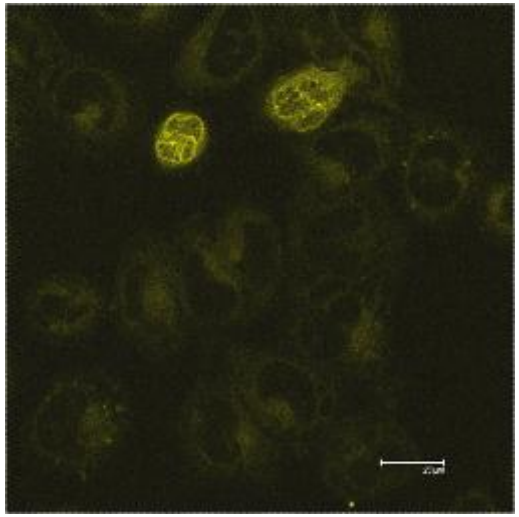


Lapatinib inhibits P-gp-mediated resistance

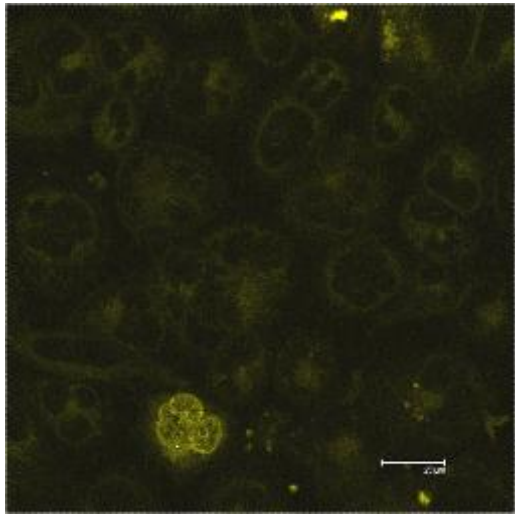
Epirubicin



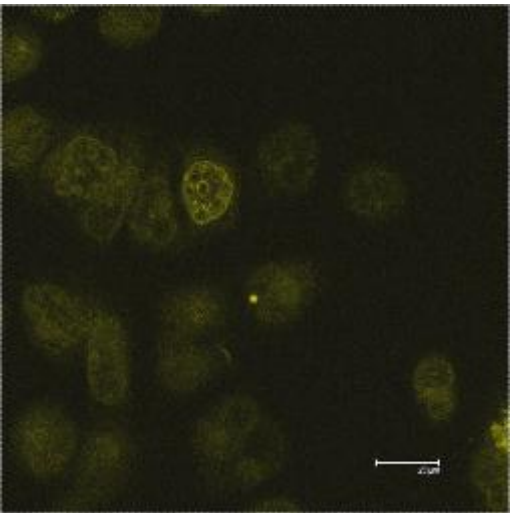
Epi & DMSO



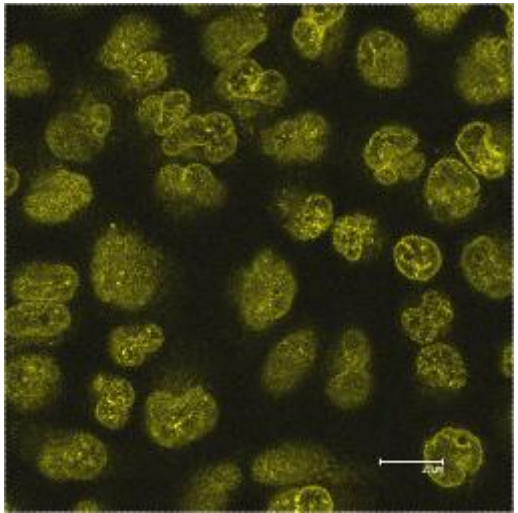
Epi & 0.25uM Lapatinib



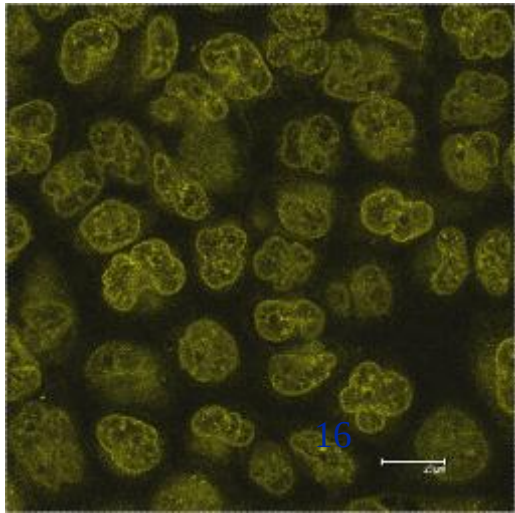
Epi + 1uM lapatinib



Epi & 5uM Lapatinib

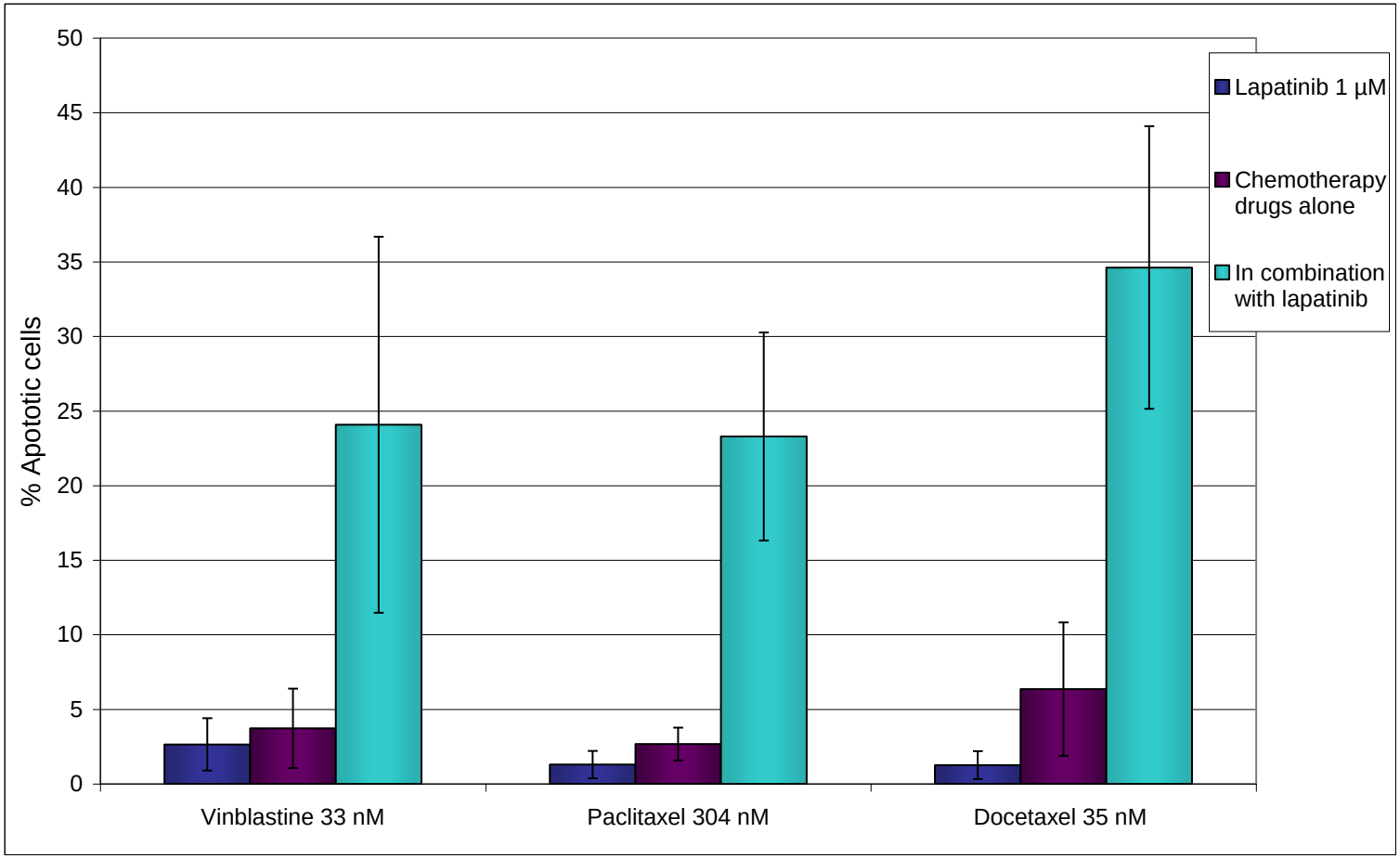


Epi & 10uM Lapatinib



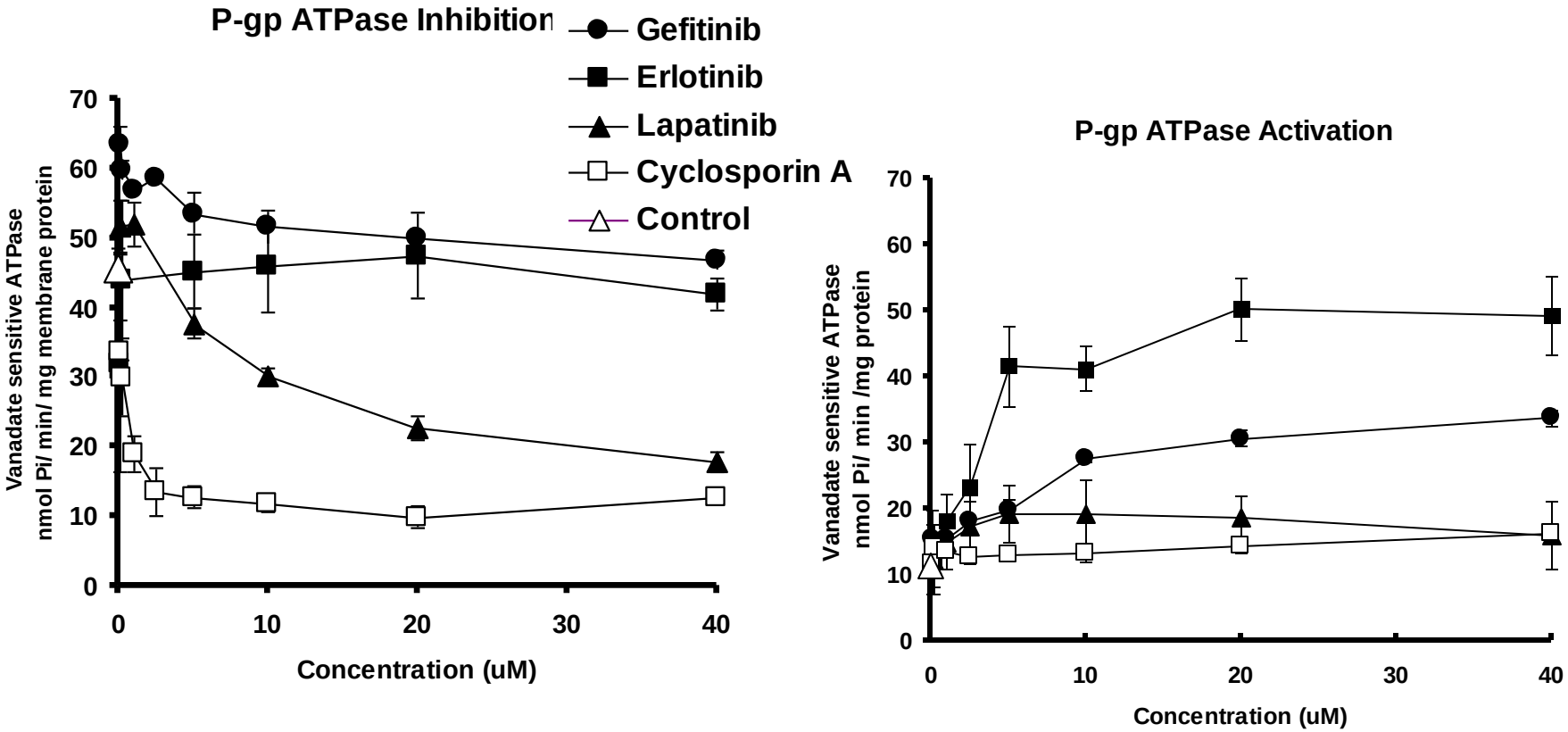
P-gp inhibition increases substrate-induced apoptosis

DLKP-A



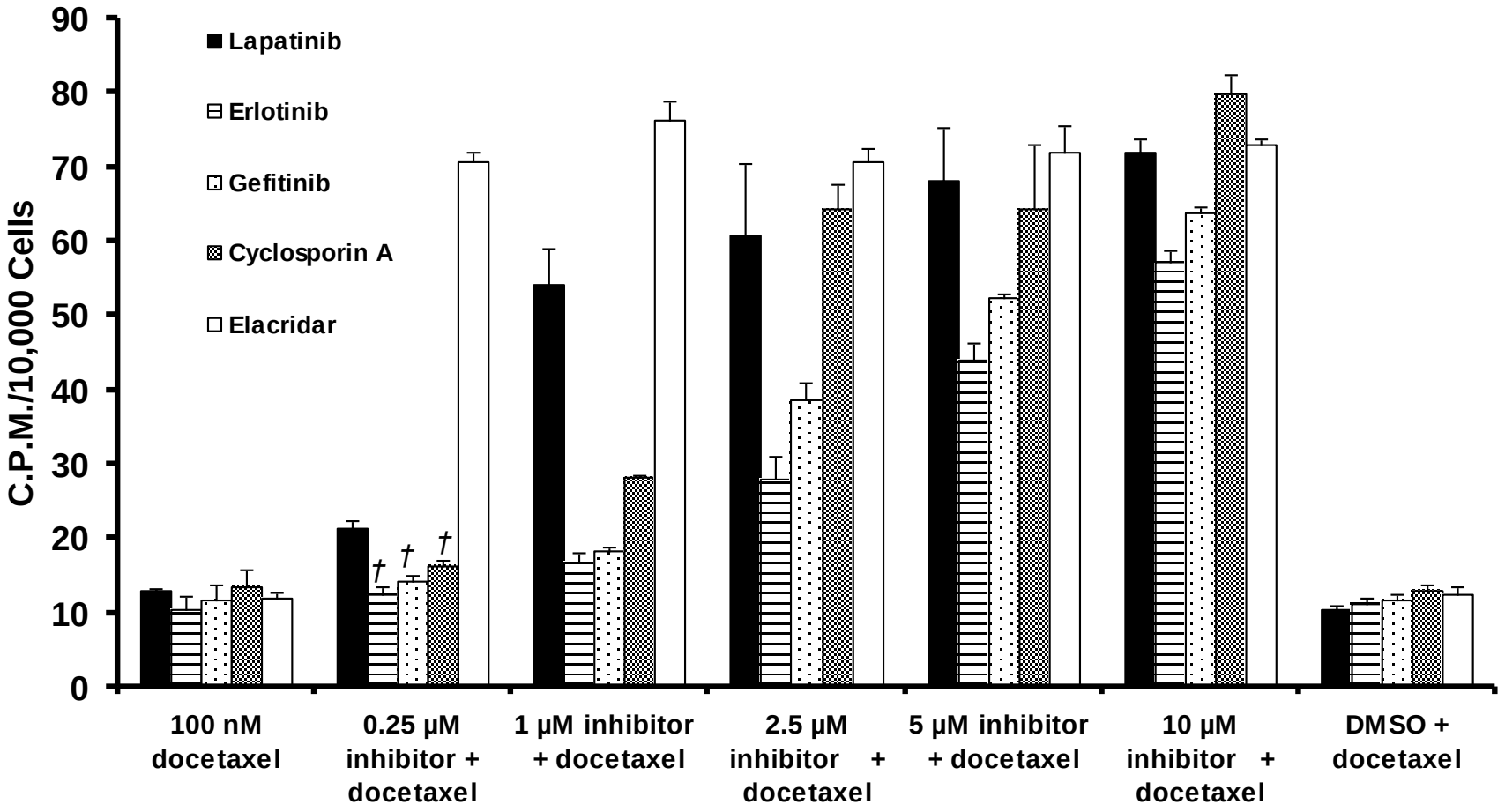
Apoptosis levels as determined by TUNEL staining in DLKP-A (P-gp positive) in response to 72 hour drug treatments of lapatinib in combination with vinblastine, paclitaxel and docetaxel. N=3

Lapatinib- P-gp inhibitor but not a substrate



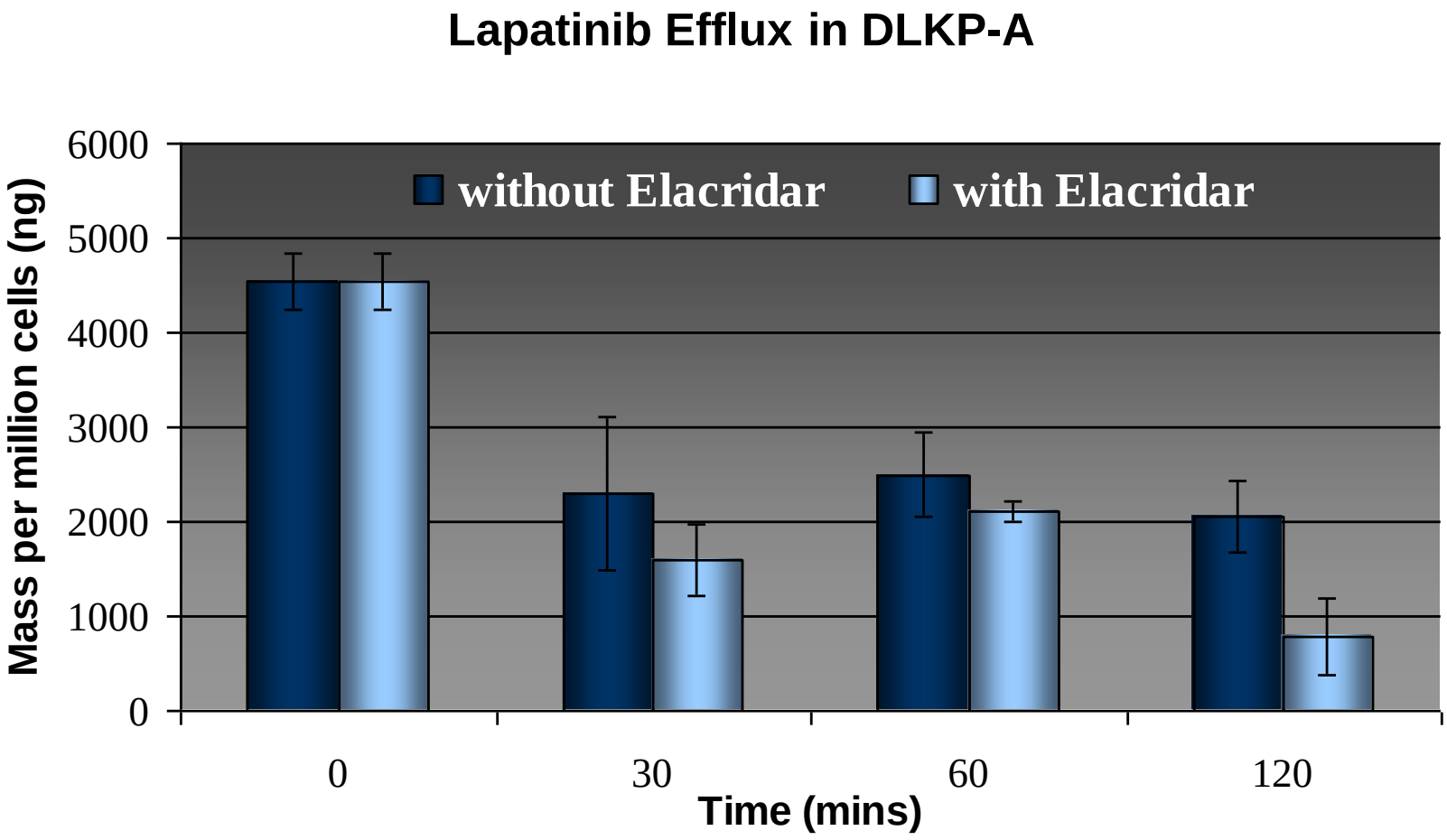
The effects of lapatinib, gefitinib, erlotinib and cyclosporin A on vanadate-sensitive P-gp ATPase inhibition (A) and activation (B). For (A) the control represents the ATPase activity measured in the presence of 45 µM verapamil (P-gp substrate) but in the absence of added test compounds. For (B), the control represents the ATPase activity measured in the absence of added test compounds. Each concentration was determined in duplicate. All compounds were dissolved in DMSO except cyclosporin A which was dissolved in ethanol. N=2

Lapatinib is potent



Accumulation of 100 nM ^{14}C radio-labelled docetaxel in DLKP-A over 90 minutes. All inhibitors were dissolved in DMSO, except cyclosporin A (ethanol). N=3

Lapatinib is not a good substrate



Cells exposed to 2uM lapatinib for 120 mins then cellular levels quantitated

Note the very large amounts of Lapatinib found in cells

Summary

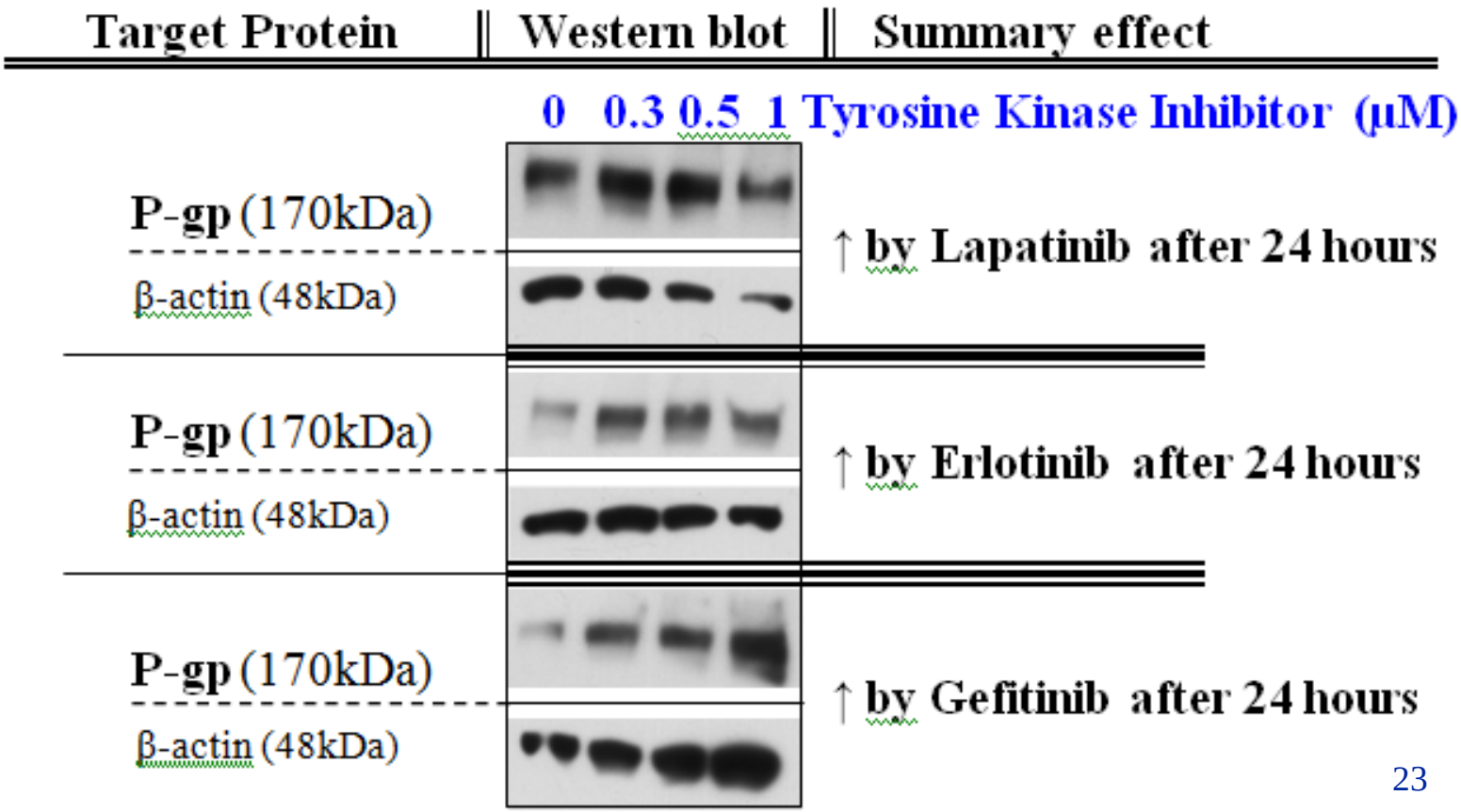
Lapatinib

- – potent inhibitor of P-gp
- – poor substrate of P-gp

Do Molecularly Targeted drugs have any indirect impacts on transporter activity?

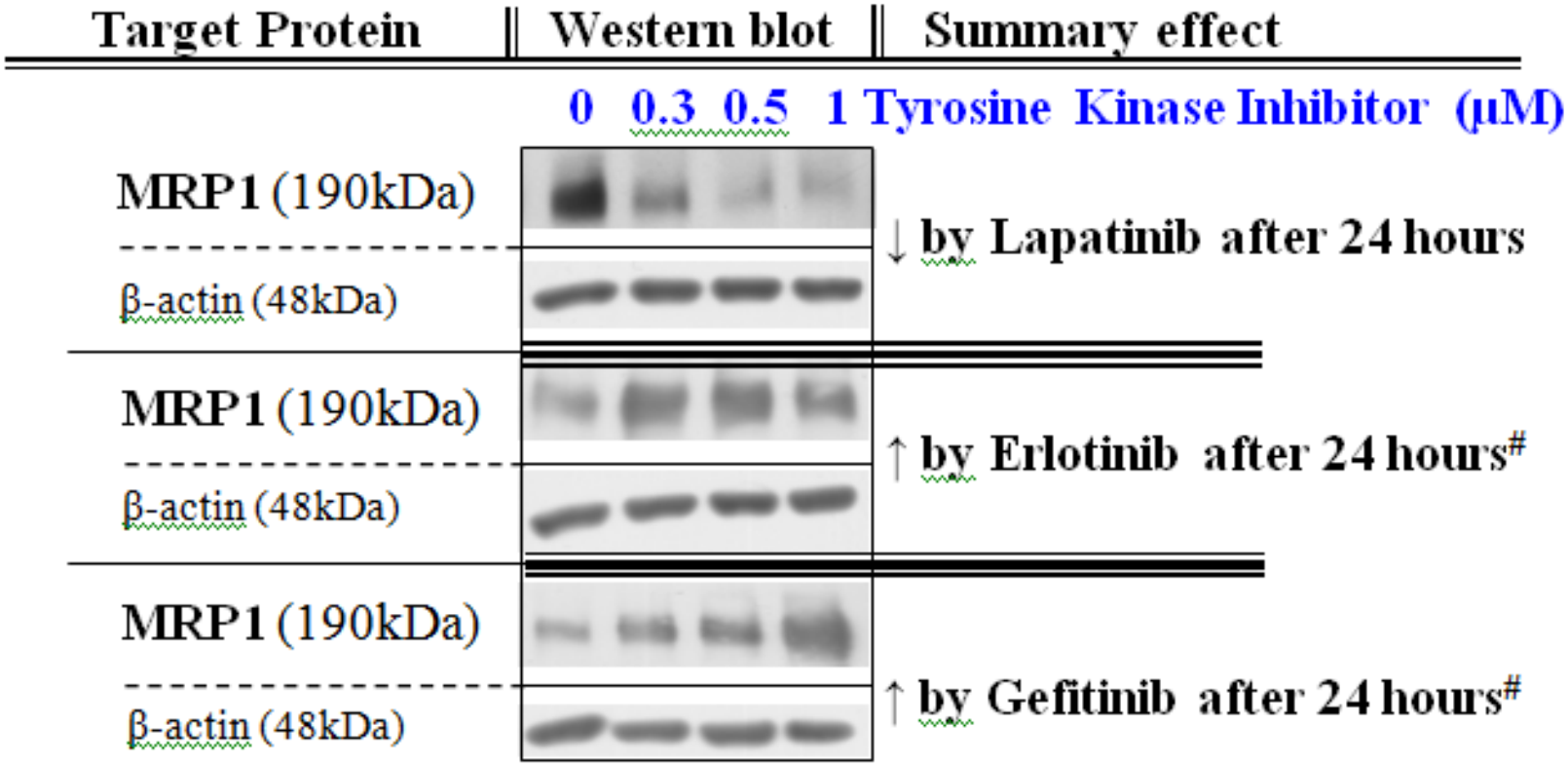
Impact of MTA treatment on P-gp expression

Effect of short-term TKI exposure on P-gp expression in the A549-Taxol cell line.



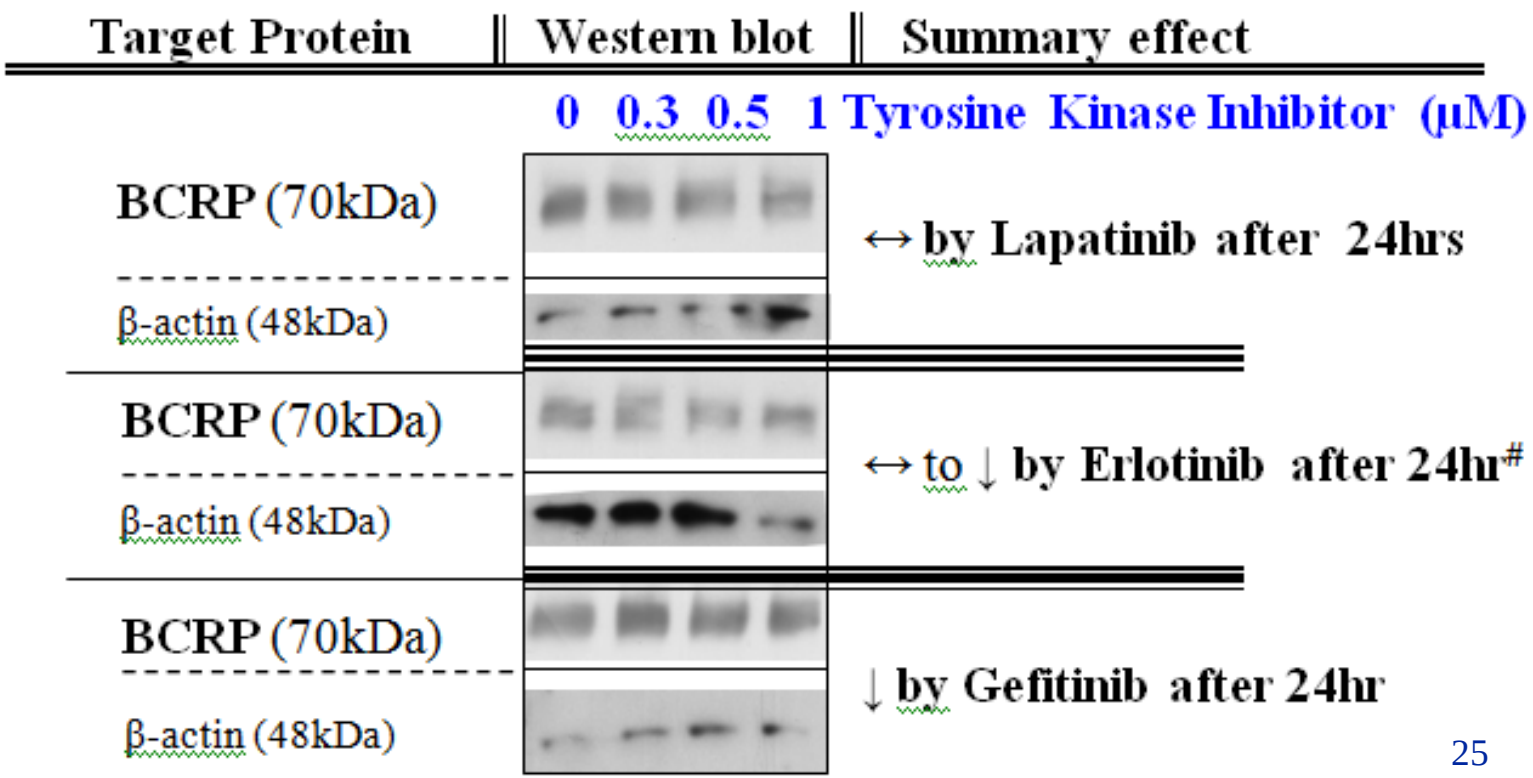
Impact on MRP-1

Effect of short-term TKI exposure on MRP1 expression in the A549 cell line.



Impact on BCRP

Effect of short-term TKI exposure on BCRP expression in the DLKP-SQ/mitox cell line.



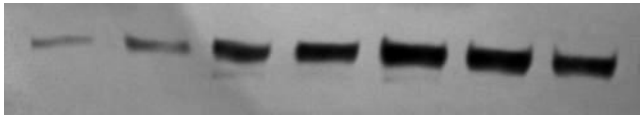
Lapatinib induction of P-gp is conc-dependent

P-gp expression in A549-T

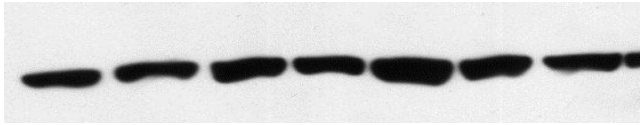
Lapatinib µM

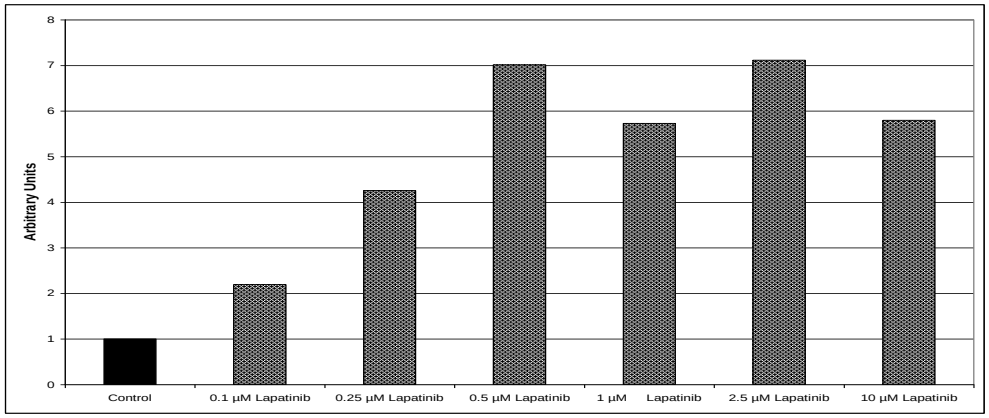
0 0.1 0.25 0.5 1 2.5 10

P-gp



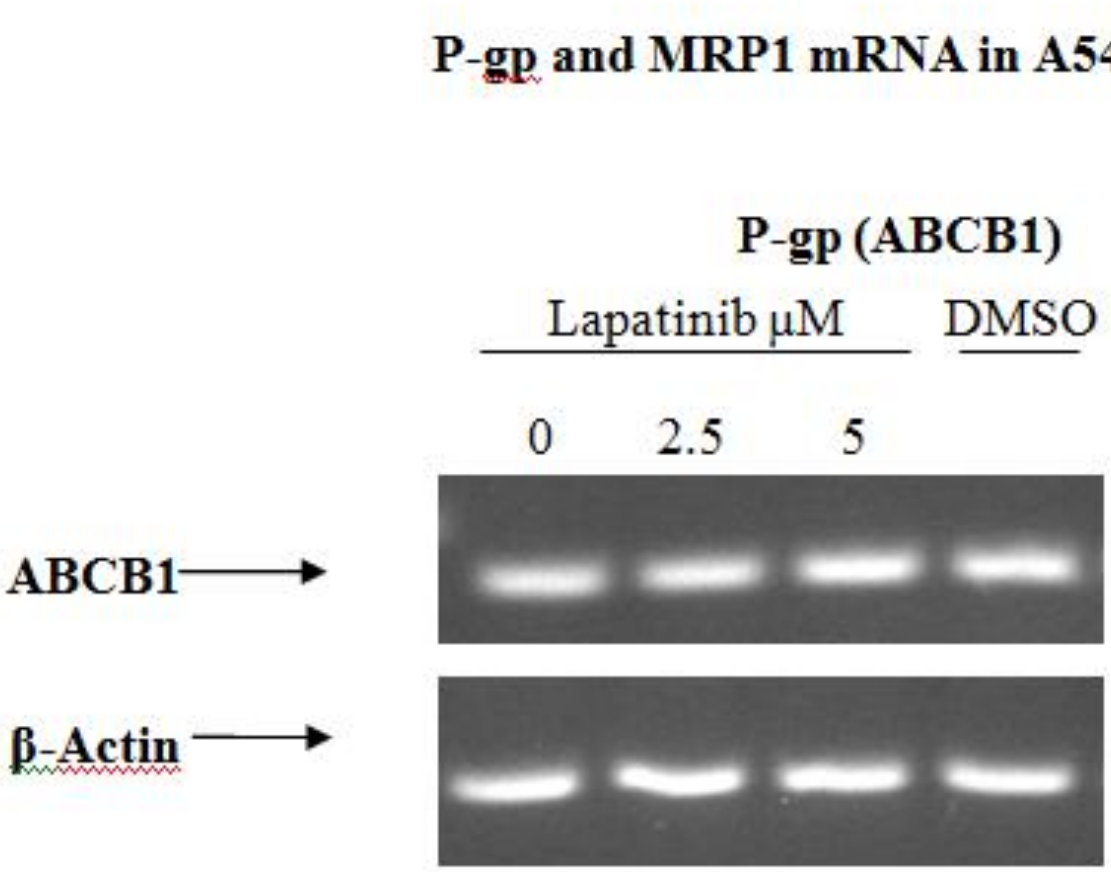
β-Actin





Western blot of P-gp expression following 48 hour 0.1 µM, 0.25 µM, 0.5 µM, 1 µM, 2.5 µM, and 10 µM lapatinib treatments in A549-T. Control was A549-T cells incubated with growth medium for 48 hours.

P-gp mRNA levels unaltered

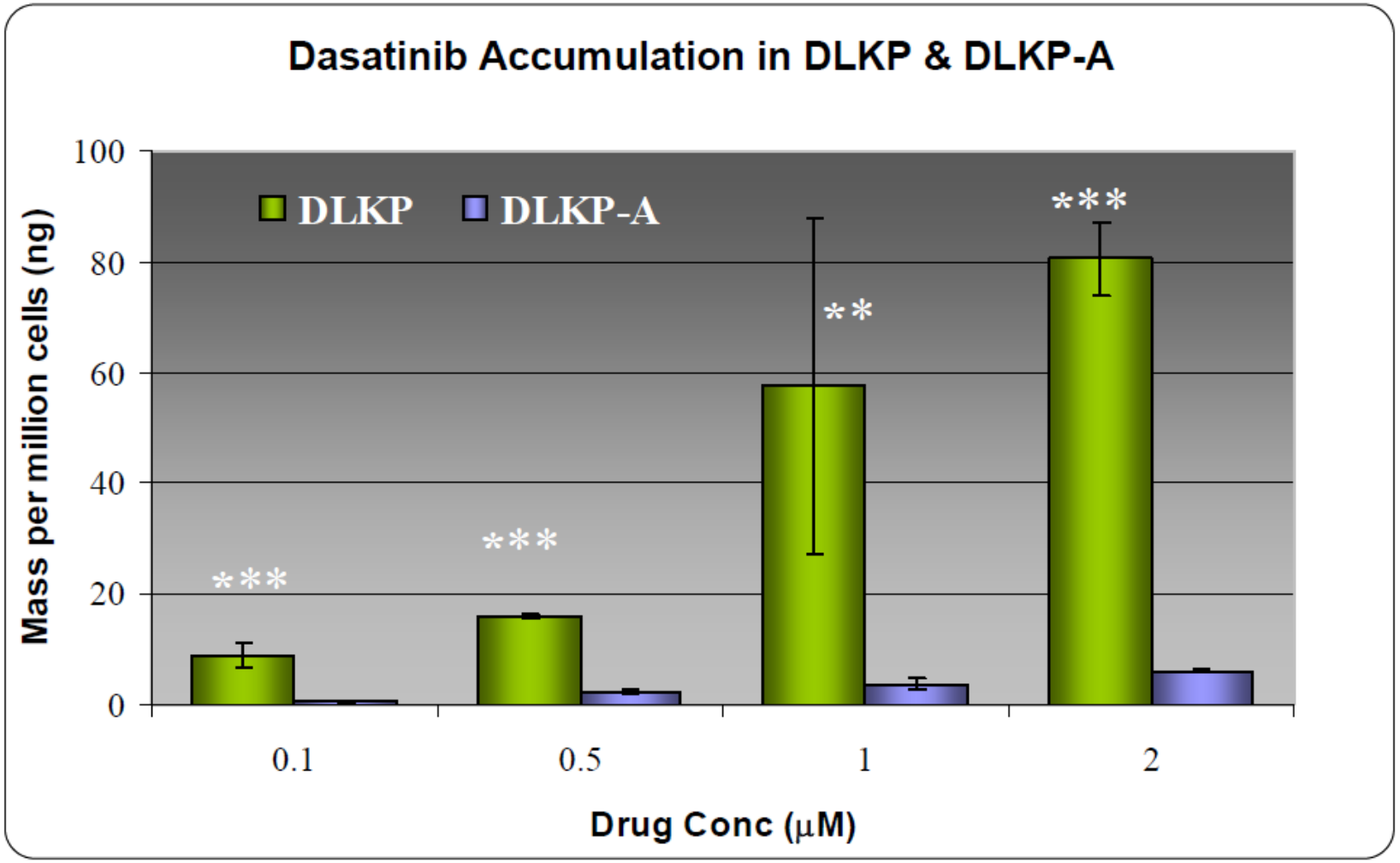


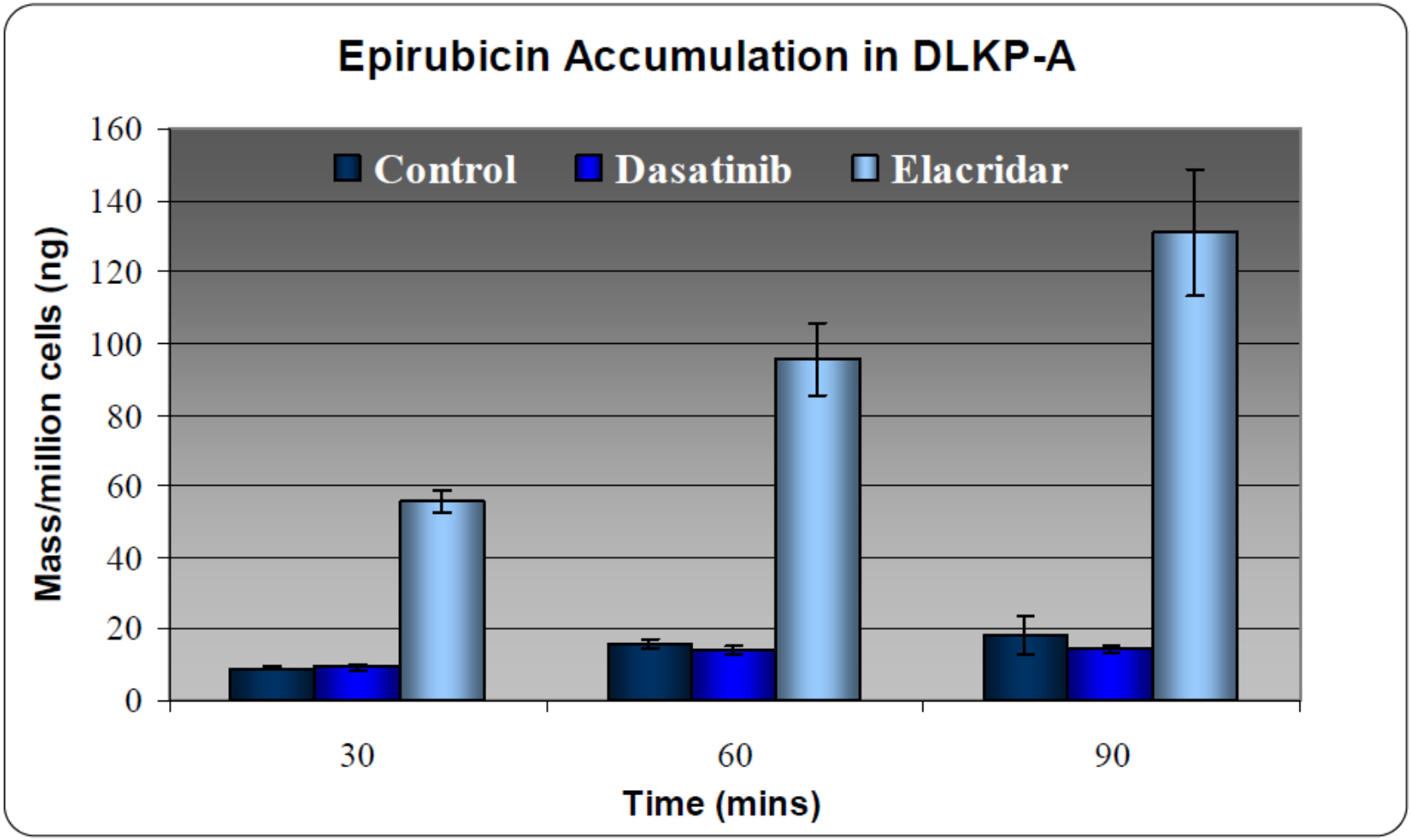
RT-PCR mRNA analysis of P-gp mRNA expression in A549-T following 24 hour 2.5 µM and 5 µM lapatinib

Summary

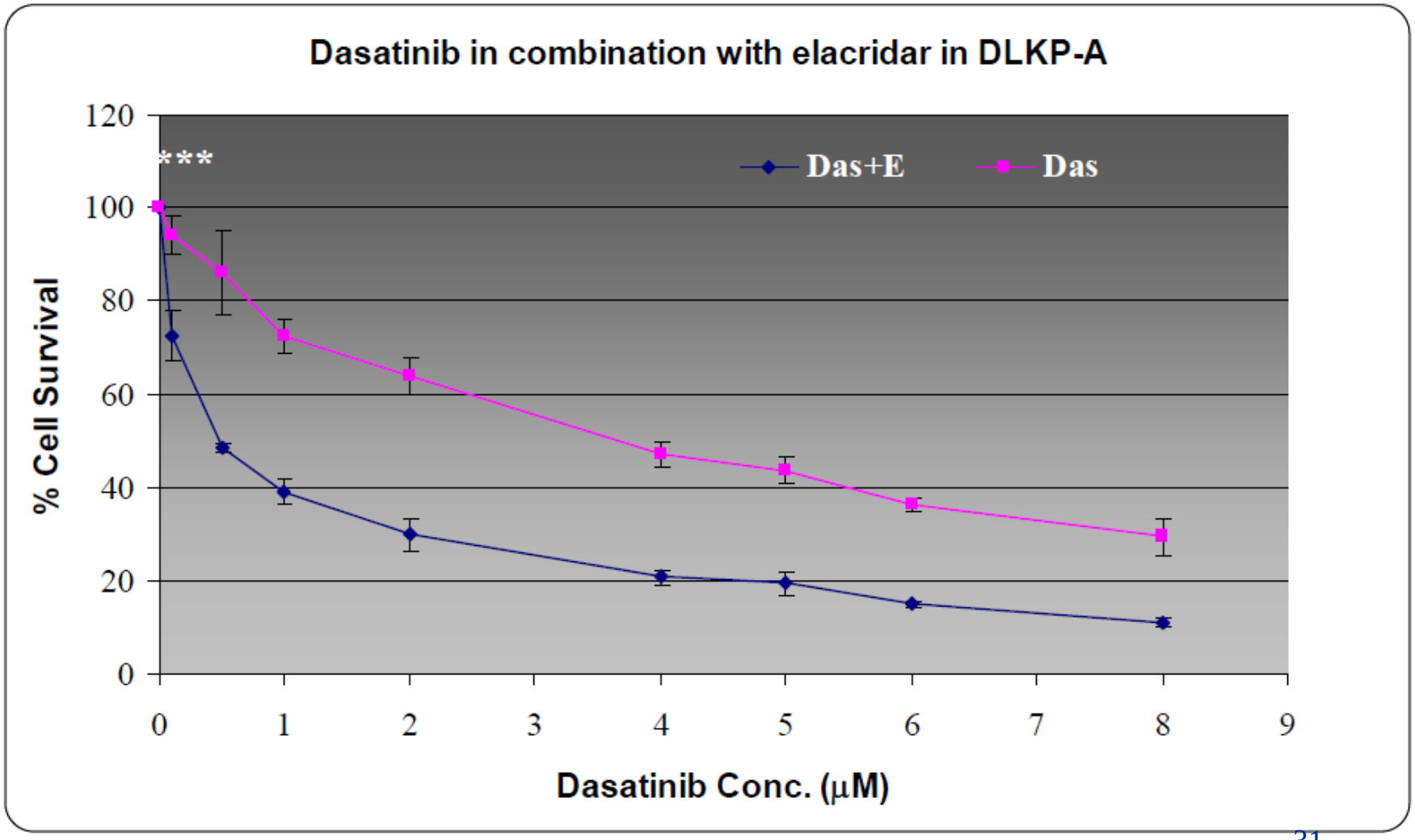
- Lapatinib induces a drug-dependent increase in P-gp expression
- This effect is negated since lapatinib is a potent inhibitor of P-gp
- Other MTAs (TKIs) induce various changes in transporter expression
- *These changes aren't permanent*

Dasatinib is substrate but poor inhibitor of P-gp




Epirubicin Accumulation in DLKP-A
Control
Dasatinib
Elacridar
Mass/million cells (ng)
160
140
120
100
80
60
40
20
0
30
60
90
Time (mins)

P-gp expression reduces efficacy of Dasatinib



Clinical investigations – MRP-1

- Phase I + II (melanoma) evaluations of sulindac (MRP-1 inhibitor) in combination with epirubicin
- No major increase in efficacy

Clinical Translation- Lapatinib

CT 1225/7/1 Phase I of Lapatinib with Epirubicin in metastatic breast cancer –

- 5 Centres
- ICORG collaboration, Investigator-led GSK study

Can Epirubicin be used with Lapatinib?

- Any indication of altered
  - Toxicity?
  - Efficacy?

Lap& epirubicin- Phase I results

- 75mg/m^2 Epi plus 1250mg lapatinib, tolerable (escalate epi)
- 80 mg/m^2 epirubicin 2 DLTs
  - Hepatotoxicity
  - Carditoxicity

- Otherwise clinical values are largely similar
- 9 patients – 7 response evaluable
  - 4- disease stabilisation >/= 3 cycles
  - 3 partial responses

So Lapatinib can be used with epirubicin but slightly increases toxicity.

- Phase II to start shortly

Conclusions

Emerging small molecularly targeted agents (MTAs/TKIs) can have major interactions with "classical" drug resistance mechanisms.

- Agents can be inhibitors or substrates
  - *Substrates- Potential for resistance to MTA*
  - *Inhibitors- Potential to alter PK of concomitantly administered drugs- careful clinical examination*
- Can alter expression of transporters
  - *Interpretation complex*
- Can we use MTA-mediated transporter modulation????

Acknowledgements

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Obrigado!