Applications and analytical aspects of microdialysis in oncology

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Microdialysis

What?	Analysis of antineoplastic agents and their metabolites
Where?	Tissue: interstitial fluid
How often?	Continuous monitoring
Where not?	Protein bound drug Intracellular space

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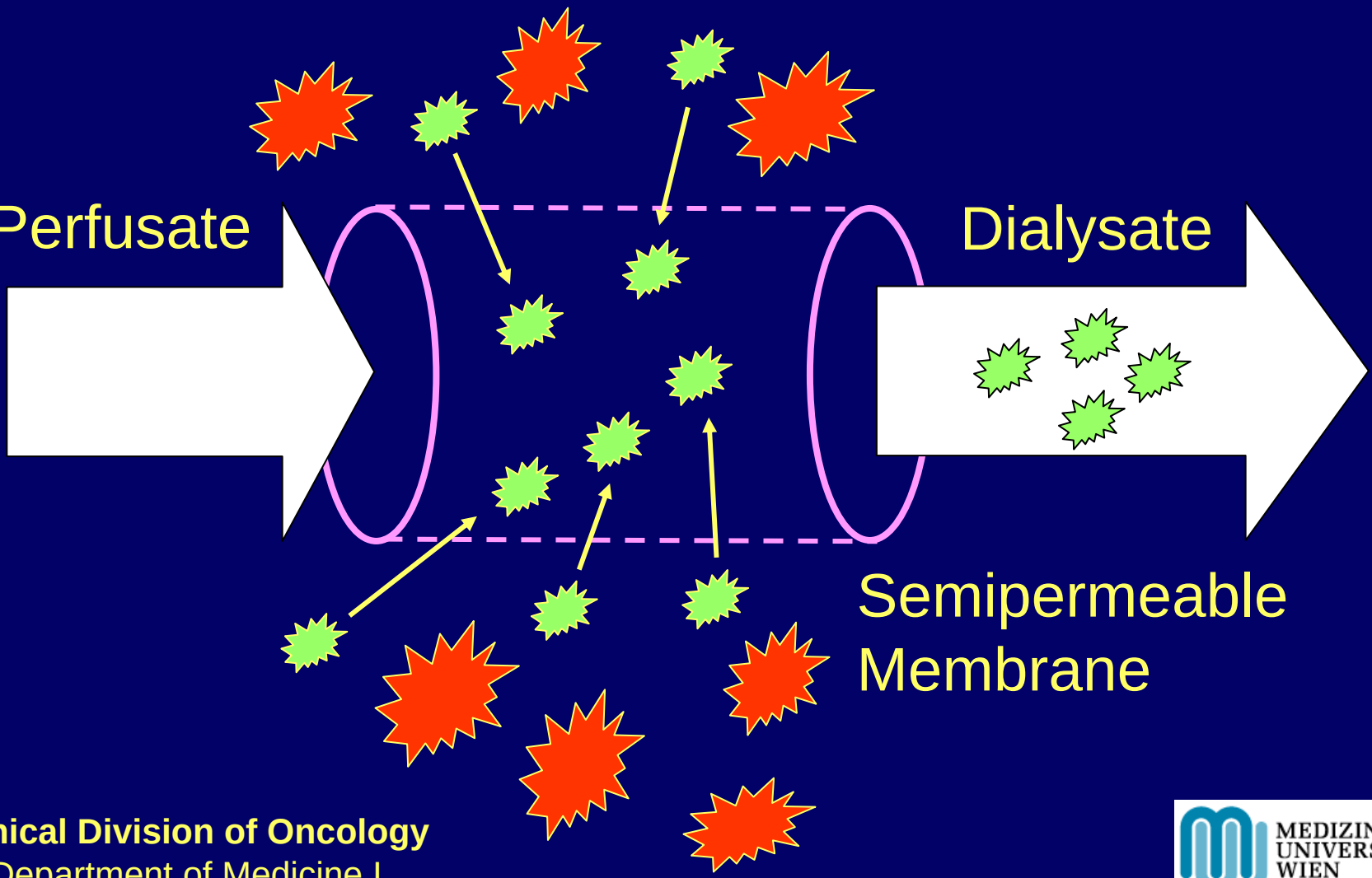
Principle



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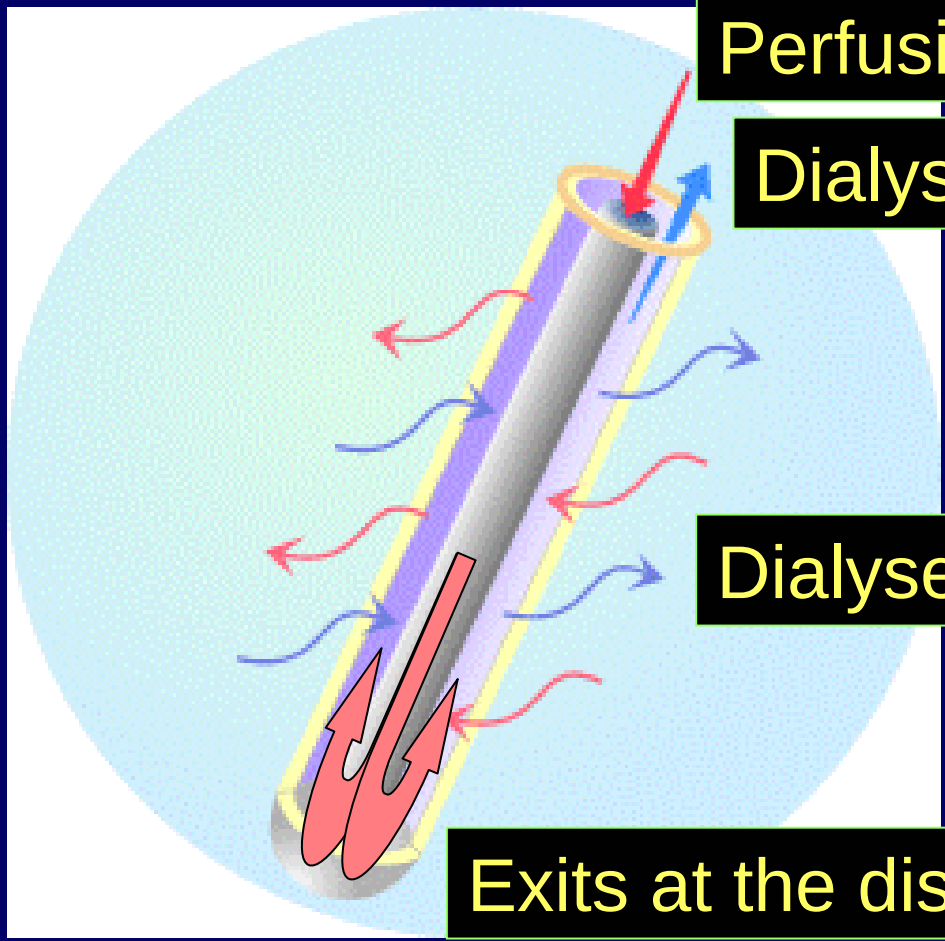
Probe



Perfusion fluid enters inner tube

Dialysate exits the probe

Dialyses takes place

Exits at the distal end

Probe characteristics

- Geometry: rigid - flexible
- Material: metal - plastic
- Semipermeable membrane: 6 - 100 kDa
- Volume considerations: 20 µl dialysate for capillary electrophoresis, even less for HPLC
- Syringe pump and a microfraction collector: typical sampling periods between 15 and 30 min

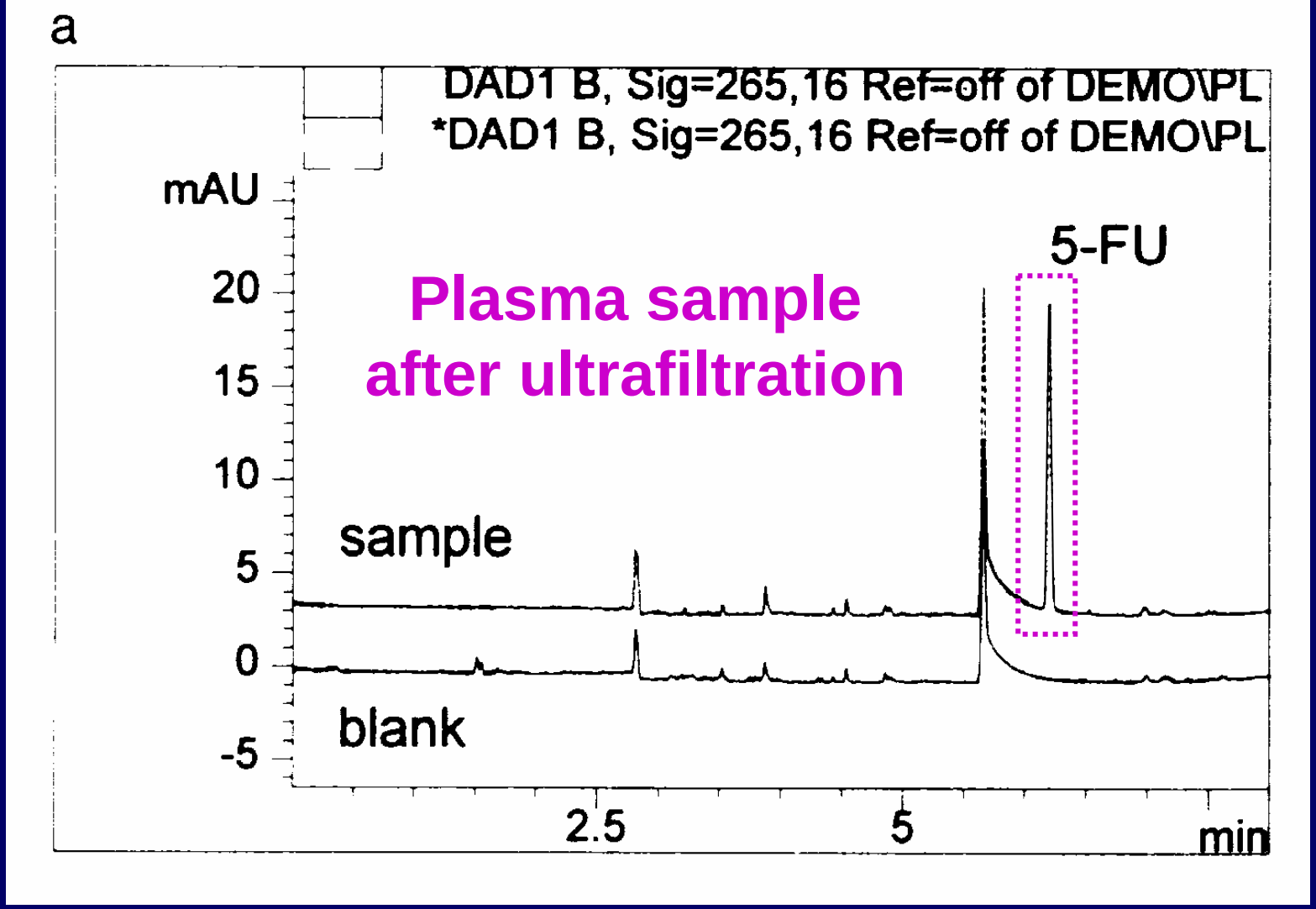
Recovery

- Depends on the diffusion coefficient of the analyte
- Depends on the flow rate (0.5 - 1.5 µl/min)
- Depends on the matrix
- Differs *in vitro* and *in vivo*
- In clinical studies assessed by the *in vivo* delivery method ➡ mass transfer is equal in both directions

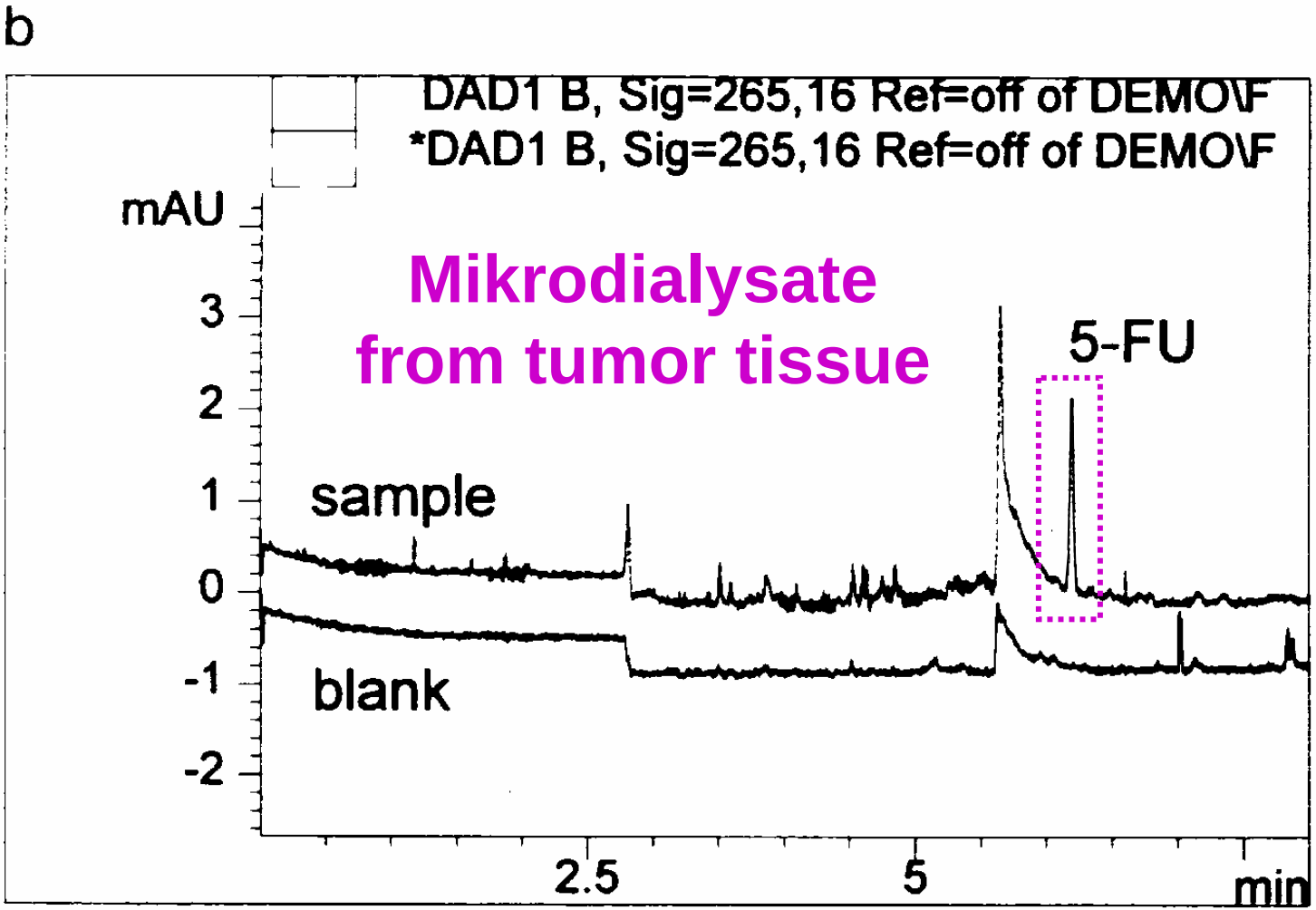
Analytical Matrix

	Blood	Urine	Mikrodialysate
Concentration of analyte	variable	high	variable
Protein content	6,5 – 8%	variable	minimal (cut-off 20 kDa)
Interferences	low	high	minimal
pH-value	7,4	4,8 – 7,5	7,4

Plasma and ...


a
DAD1 B, Sig=265,16 Ref=off of DEMO\PL
*DAD1 B, Sig=265,16 Ref=off of DEMO\PL
mAU
20
15
10
5
0
-5
5-FU
Plasma sample
after ultrafiltration
sample
blank
2.5
5
min

Microdialysates



Breast Cancer

Study design: 10 patients with advanced breast cancer

Therapy: CMF or FEC

- CMF
  - Cyclophosphamide 600 mg/m²
  - Methotrexate 40 mg/m²
  - 5-Fluorouracil 600 mg/m²
- FEC
  - Epirubicin 60 mg/m²

Probes: primary tumour and s.c. adipose tissue

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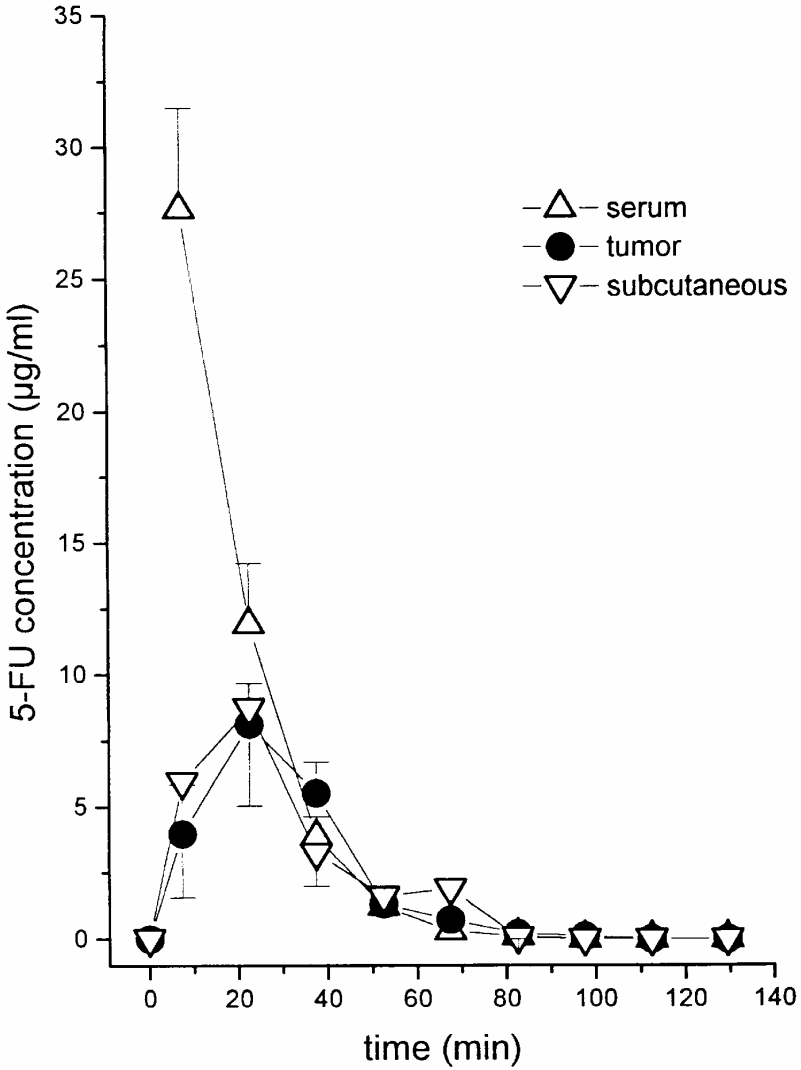
5-Fluorouracil

- Sample clean-up: ultrafiltration for blood, no clean-up for microdialysates necessary
- Sampling: microdialysate and blood in 15 min intervals (perfusate: 1.5 µl/min)
- Analysis: Capillary electrophoresis in an uncoated fused silica capillary (56 cm x 50 µm i.d.); Detection: absorbance at 265 nm
- Background electrolyte: 30 mM sodium tetraborate buffer (pH = 9.0)

Electrophoresis 19, 2981-2985 (1998)

5-Flurouracil *in vivo*



- Rapid equilibration between plasma and tissue
- Identical kinetics after 30 min
- No difference between healthy and malignant tissue (AUC, ...)

Cancer Res 57, 2598-2601 (1997)

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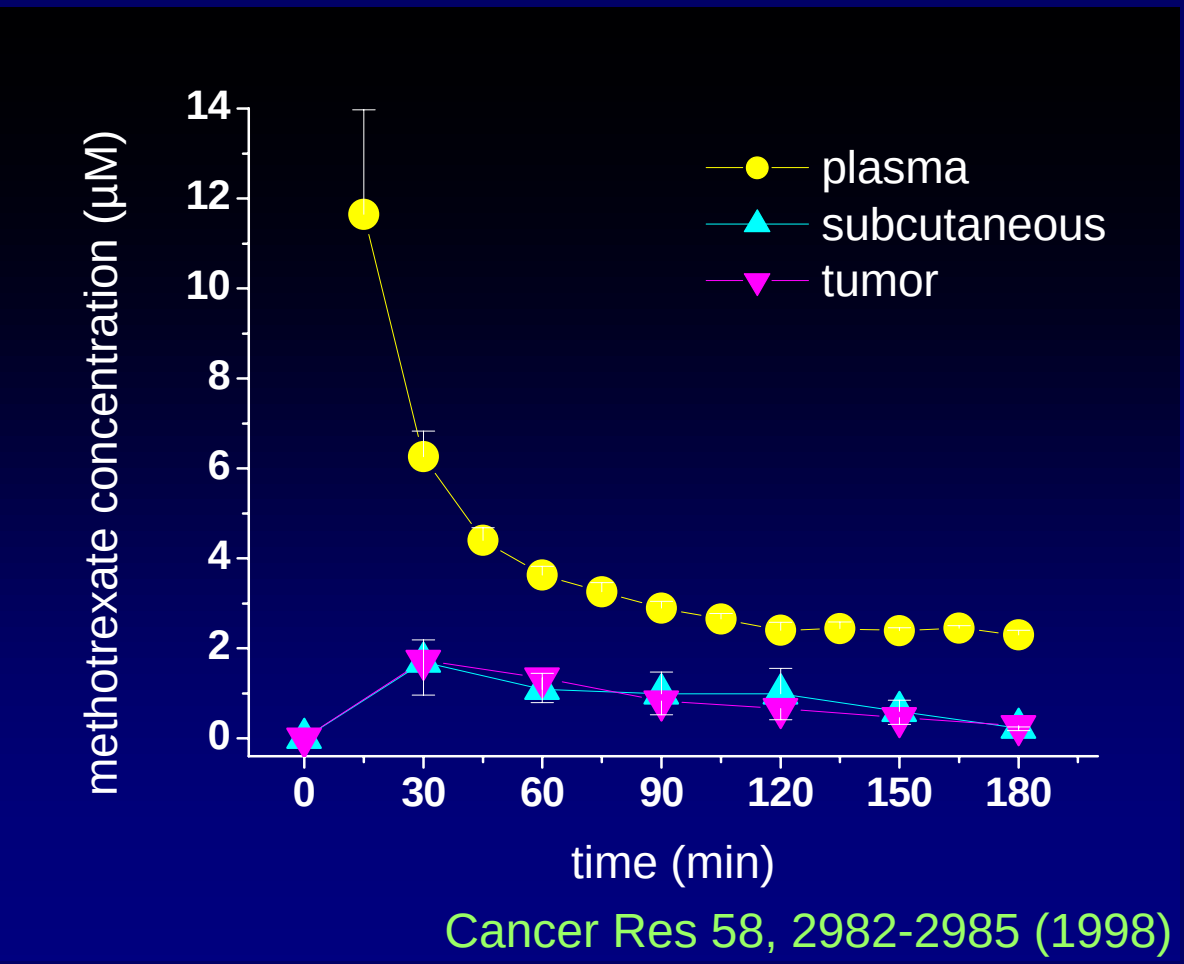
Methotrexate *in vivo*



Cancer Res 58, 2982-2985 (1998)

- Partial equilibration between plasma and tissue
- Ratio AUC_{tissue} / AUC_{plasma} ~ 0.4
- No difference between healthy and malignant tissue (AUC, ...)

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Melanoma

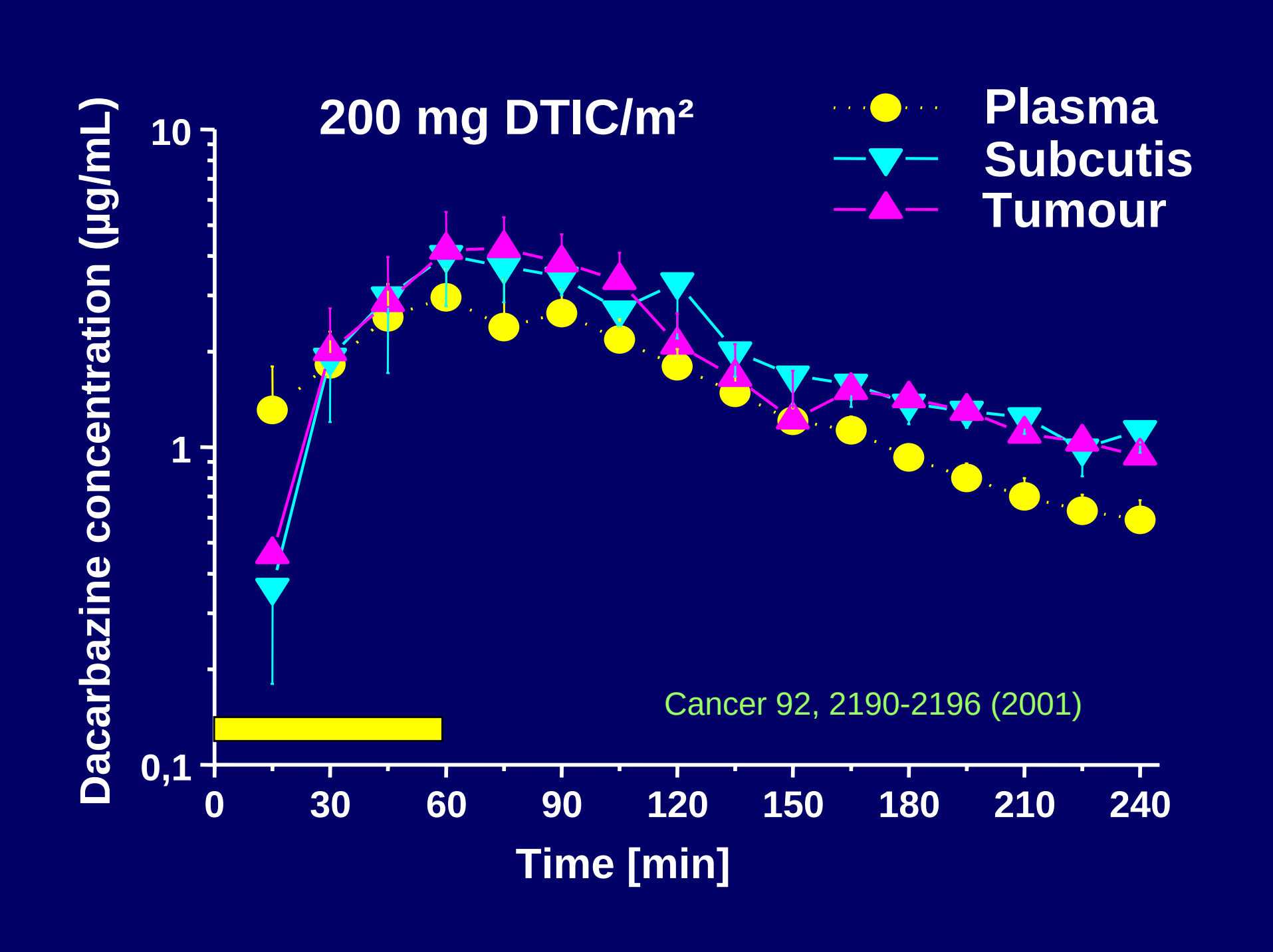
- Study design: 8 patients with advanced disease
- Dose escalation study: 200 - 800 mg Dacarbazine/m²
- Probes: cutaneous metastases from melanoma and s.c. adipose tissue
- Sample clean-up: ultrafiltration for blood, no clean-up for microdialysates necessary
- Sampling: microdialysate and blood in 15 min intervals

Dacarbazine and AIC

- Analysis: RP-HPLC (Lichrospher 100 RP18e)
- Eluens: Gradient elution in acetonitrile / 16 mM ammonium formate buffer (pH = 5.5)
- Detection: Absorbance at 330 nm (DTIC) and 273 nm (AIC)
- Protein binding

  DTIC: 26%

  AIC: 13%


200 mg DTIC/m²
Plasma
Subcutis
Tumour
Dacarbazine concentration (µg/mL)
10
1
0,1
0
30
60
90
120
150
180
210
240
Time [min]
Cancer 92, 2190-2196 (2001)

Melanoma

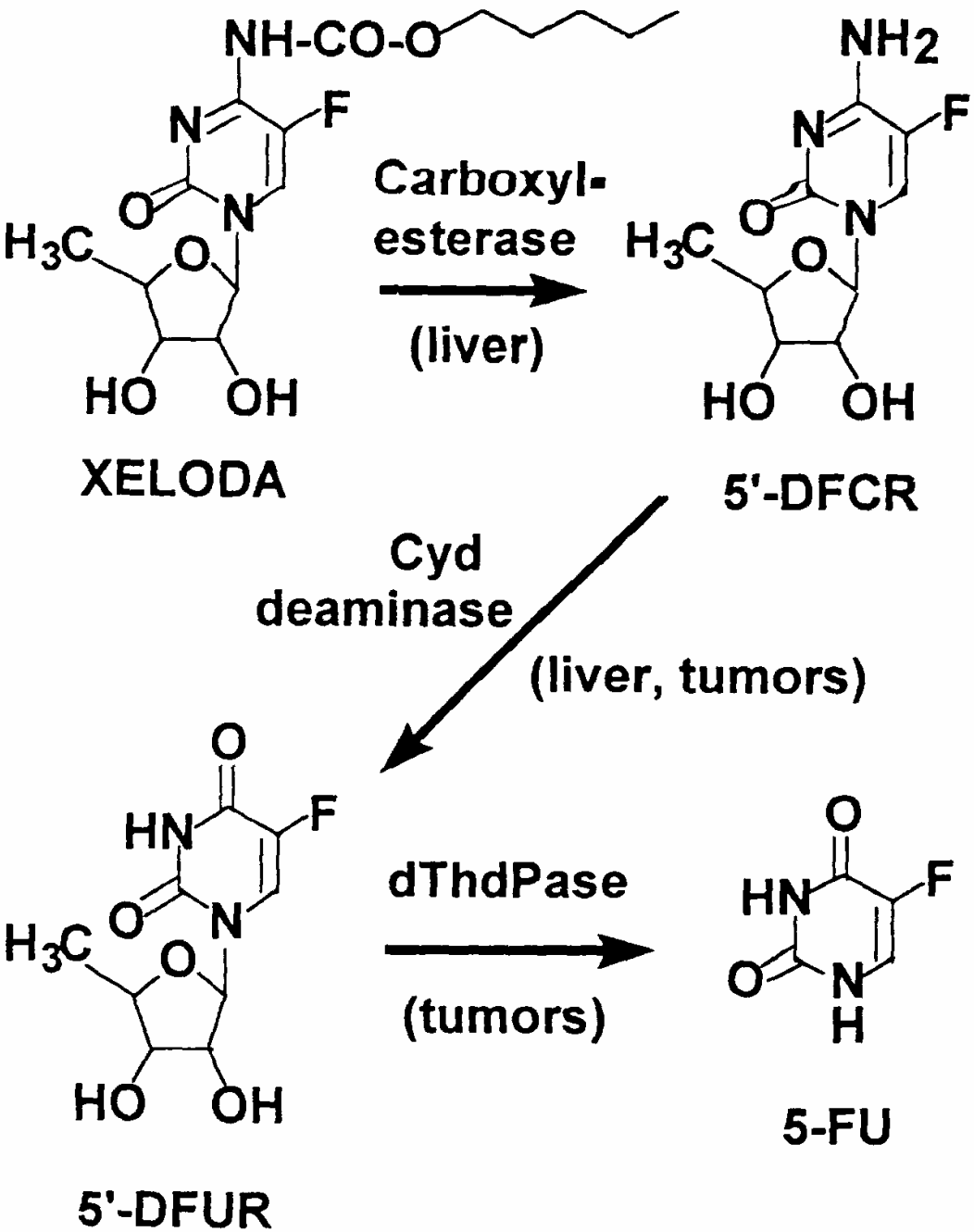
- No significant difference in the AUC between plasma and tumour
- Rapid and complete equilibration between plasma and tissue for both DTIC and AIC
- Significant correlation between AUC in plasma and malignoma

  DTIC: $r = 0.82$ ($p = 0.04$)

  AIC: $r = 0.90$ ($p = 0.04$)
- Resistance to therapy occurs at the cellular level

Capecitabine

- Study design: 10 patients with advanced breast cancer
- Therapy: 1250 mg Capecitabine/m² p.o. (2xd)
- Probes: skin metastases and s.c. adipose tissue
- Sampling: microdialysate and blood in 30 min intervals (perfusate: 1.5 µl/min)
- Analysis: Capillary electrophoresis using 200 mM sodium tetraborate buffer (pH = 9.0)
- Detection: absorbance at 266 nm, 282 nm, 297 nm



5'-DFCR
= 5'-deoxy-5-fluorocytidine

5'-DFUR
= 5'-deoxy-5-fluorouridine

5-FU = 5-fluorouracil

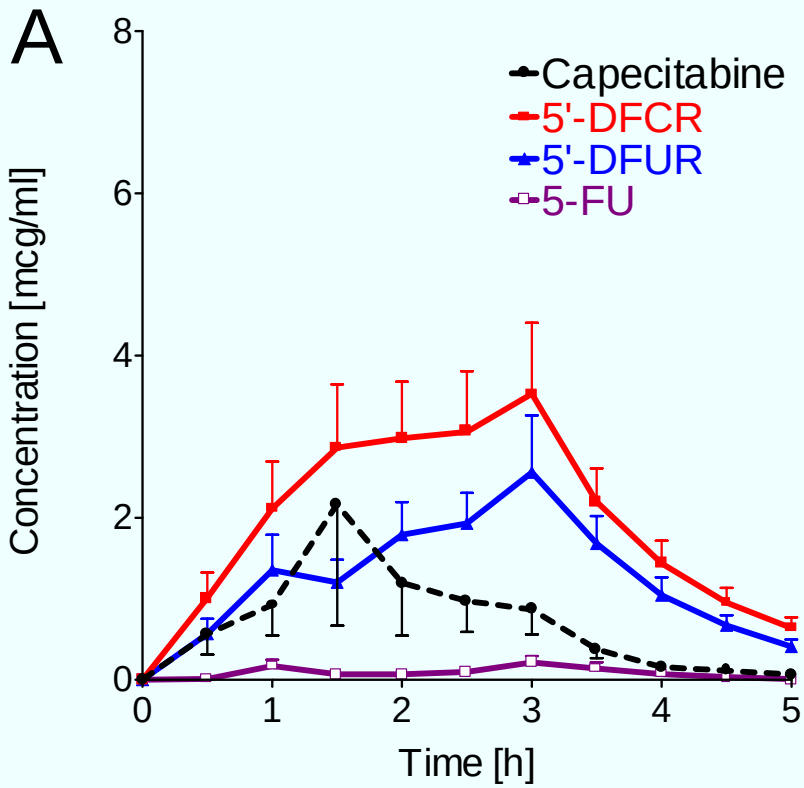
CE = carboxylesterase

CyD = cytidine deaminase

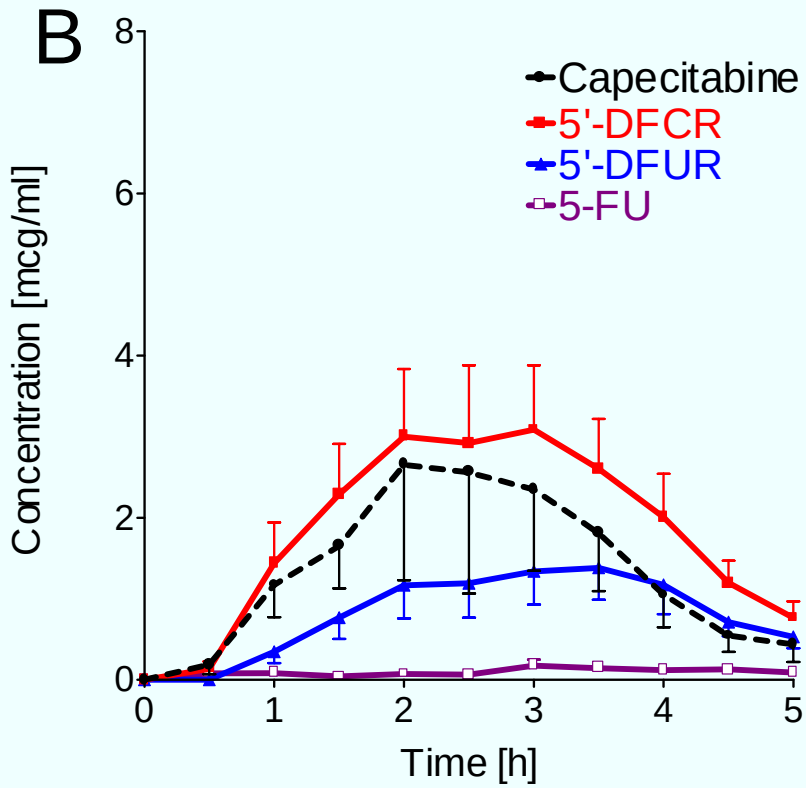
dThdPase =
thymidine phosphorylase

Tissue kinetics *in vivo*

Plasma



Malignoma



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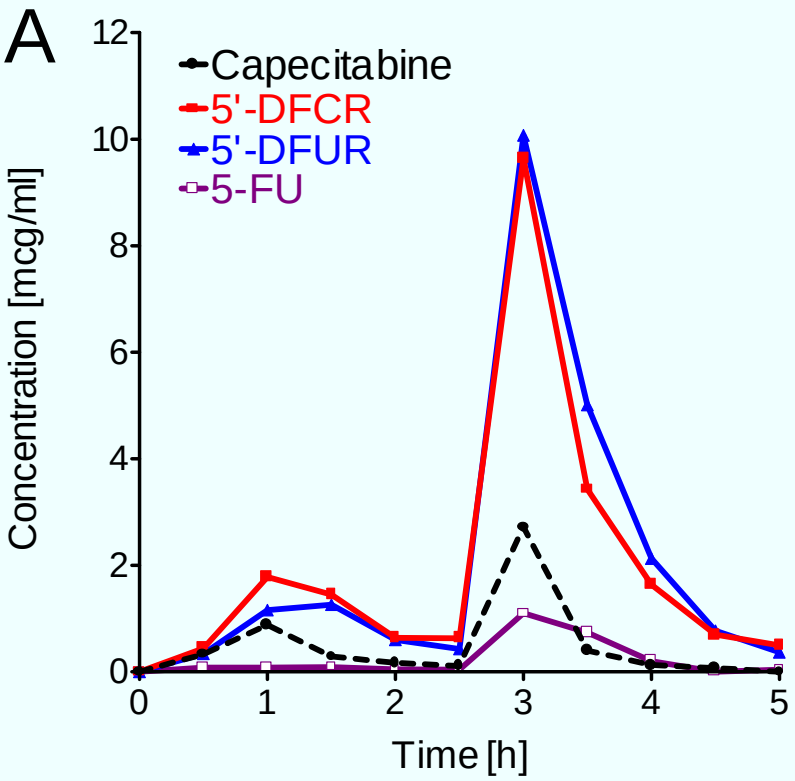
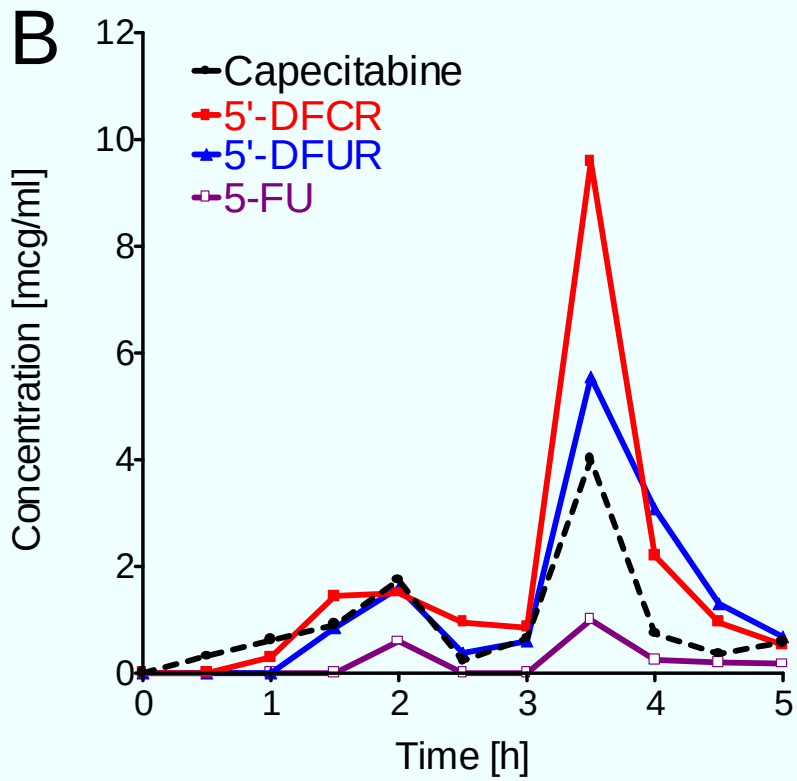
Repeated administration

Plasma



Malignoma



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Capecitabine

- Capecitabine and its metabolites easily penetrated healthy and malignant tissue
- Equilibration was completed within 45 min after p.o. administration
- Low concentrations of 5-FU in plasma and tissue interstitium
- Transcapillary transfer and metabolic pattern was not altered after repeated dosing

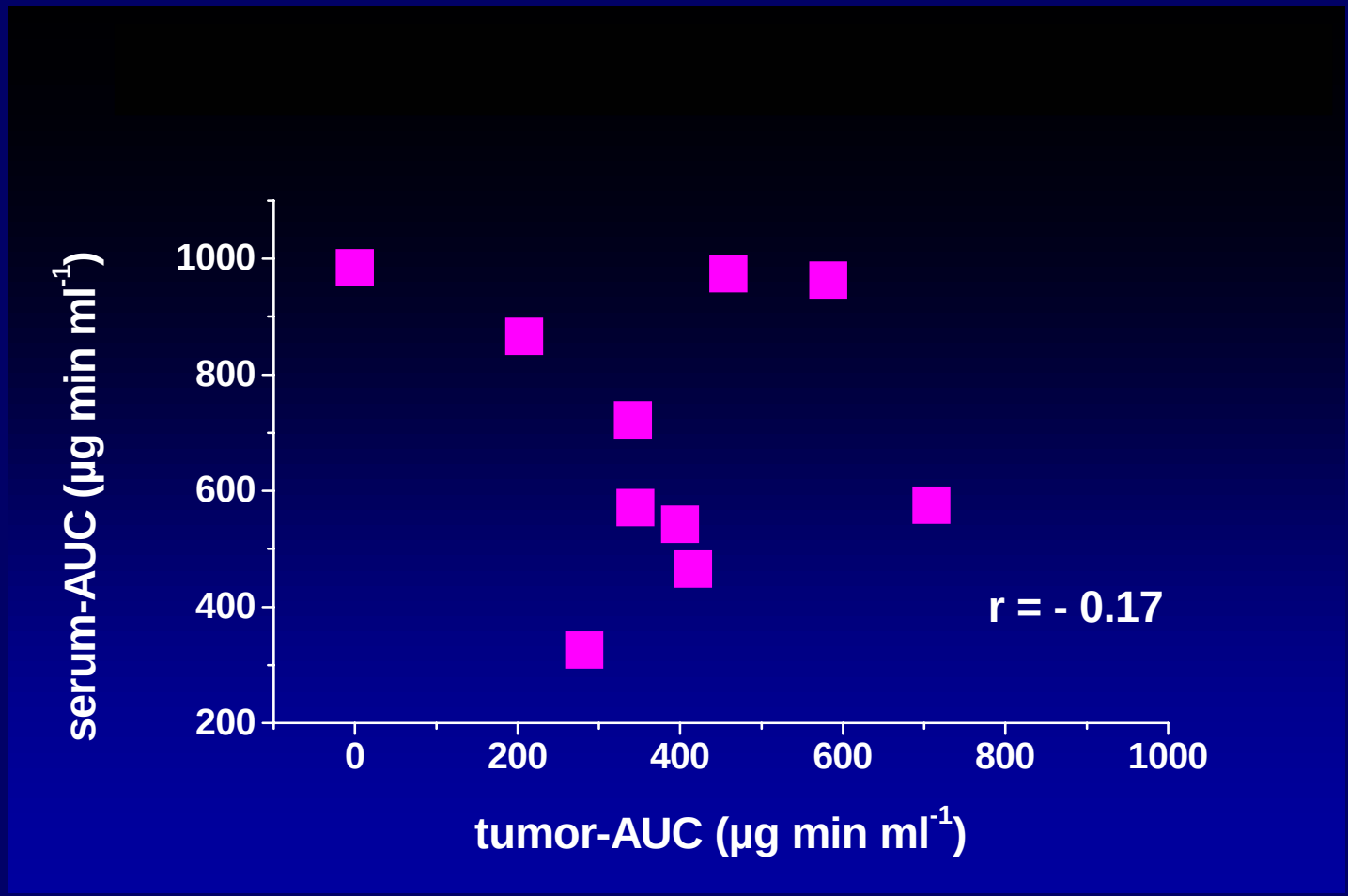
Br J Cancer 88, 782-787 (2003)

Pharmacodynamics

What characterises tumour interstitium?

- High interstitial pressure
- Abnormal geometry of tumour vessels
- Decreased pressure in tumour venules
- High collagen content

5-FU in breast cancer



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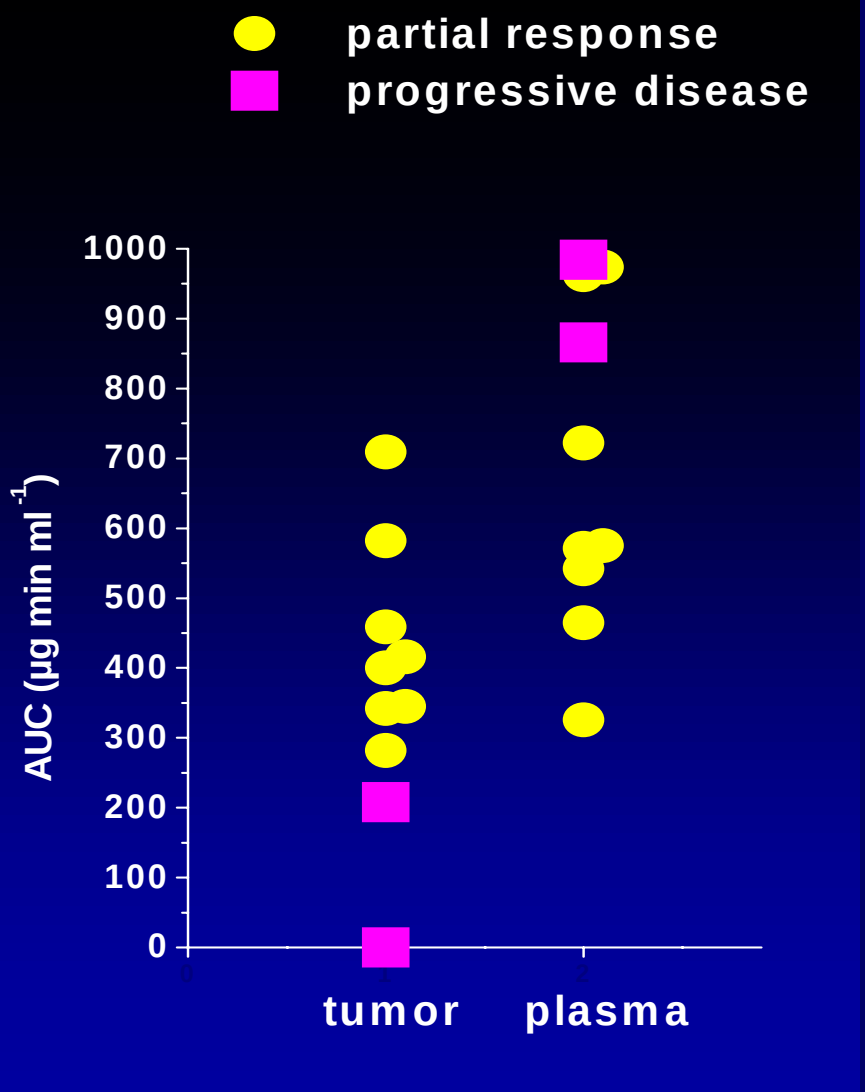
Response to 5-FU



- Plasma levels are not always predictive of intratumoural concentrations
- The penetration of 5-FU may be a rate-limiting step for the success of antineoplastic therapy

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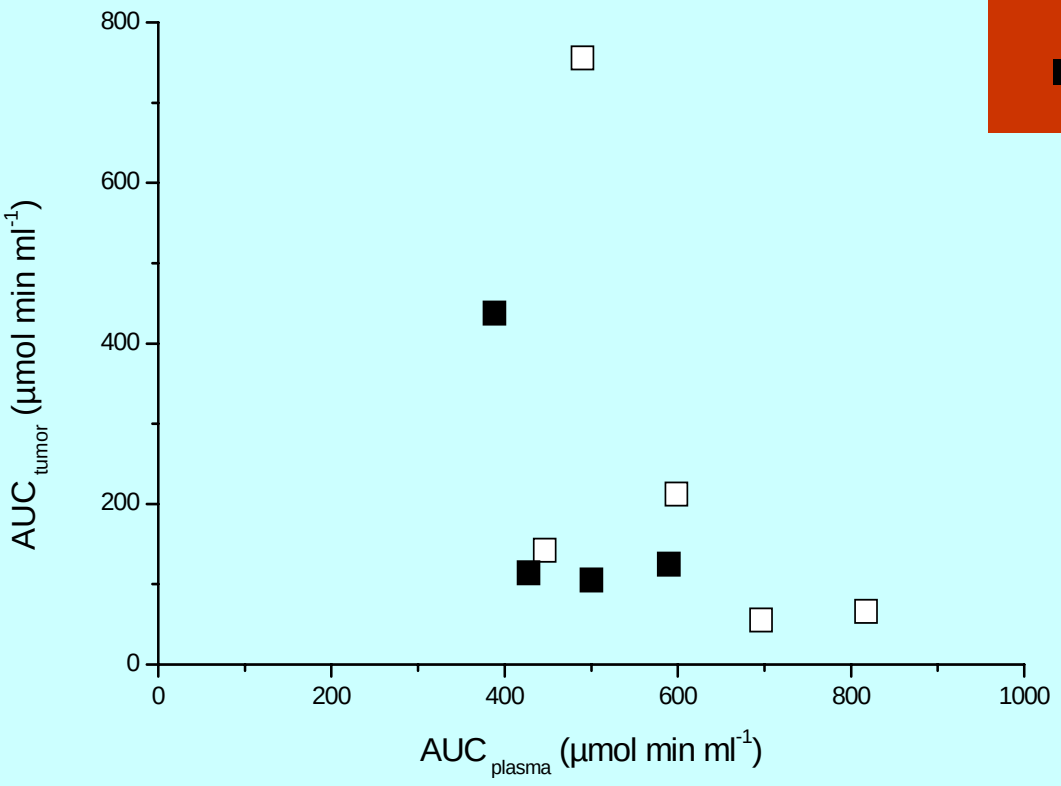
Response to MTX



- Partial remission
- Stable disease

- No correlation between plasma and tumour
- Tissue transfer is not a rate-limiting step

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PK-PD modeling

Interstitial concentration *in vivo*

≅

Concentration in cell culture *in vitro*

➡ Simulation of interstitial tissue pharmacokinetics using MCF-7 as a model for breast cancer and evaluation of cytotoxicity by MTT-assay

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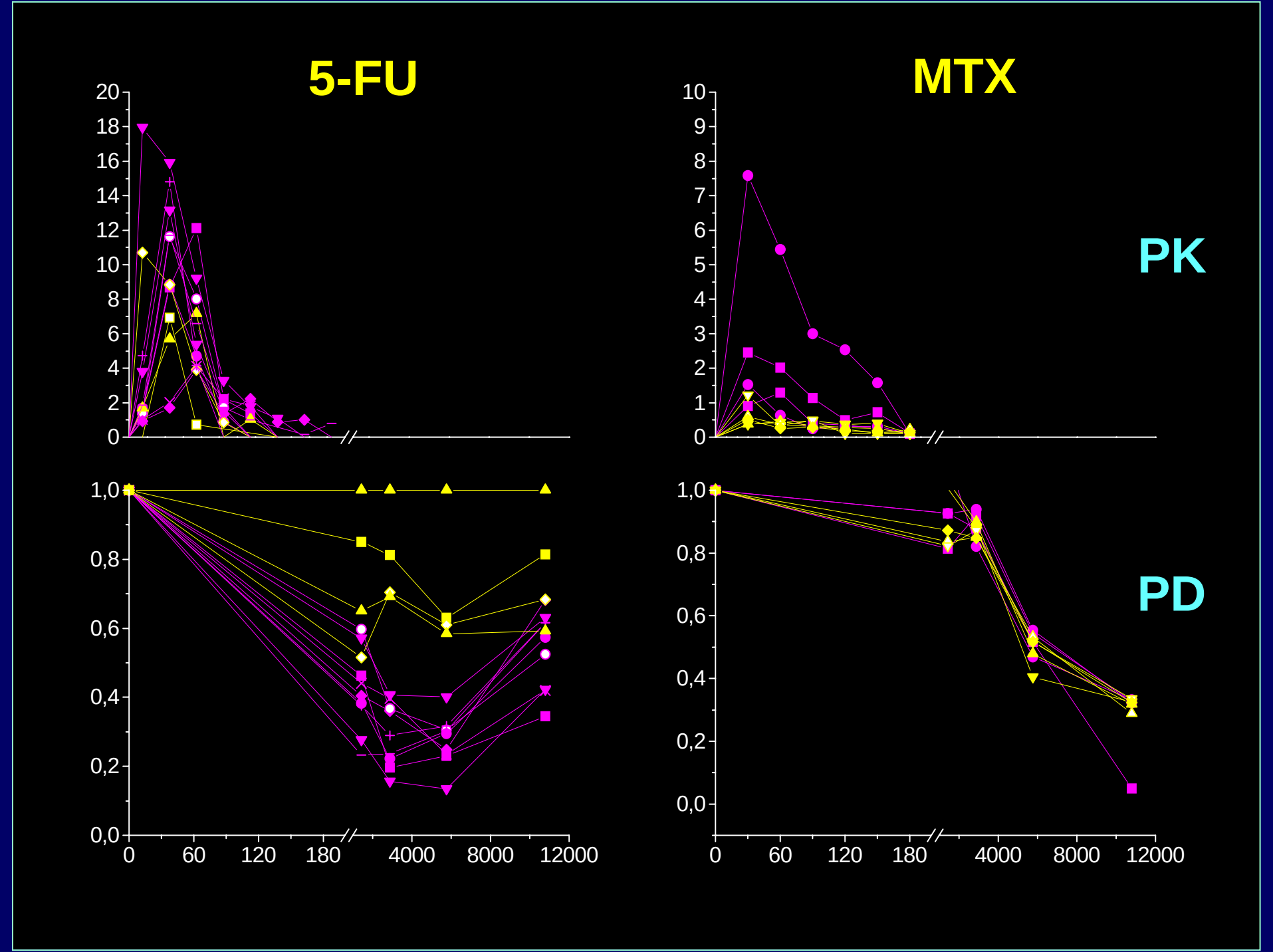
5-FU
MTX
PK
PD
20
18
16
14
12
10
8
6
4
2
0
10
9
8
7
6
5
4
3
2
1
0
1,0
0,8
0,6
0,4
0,2
0,0
0
60
120
180
4000
8000
12000

Results

- Significant correlation between the antitumour effect of 5-FU and the intratumoural AUC ($r = 0.82$, $p = 0.005$)
- No correlation for MTX ($r = 0.05$, $p = 0.88$)

➡ Poor tumour penetration of 5-FU may limit response, but not that of MTX!

The effect was highly dependent on the initial cell count

Breast Cancer Res Treat 60, 211-217 (2000)

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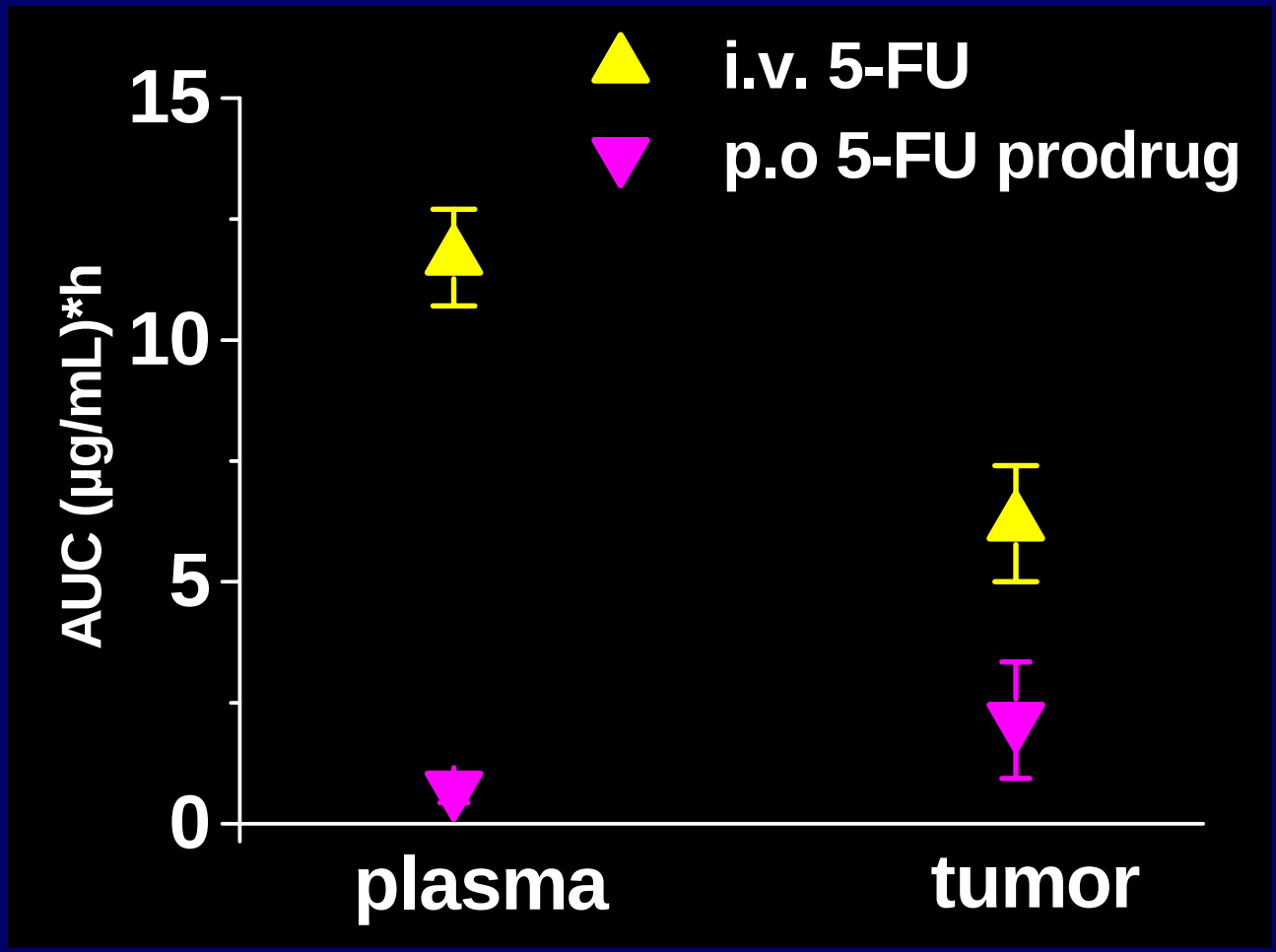
Exposure to 5-FU



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Hand-foot syndrome

No statistically significant difference between plasma and tissue pharmacokinetics after capecitabine p.o., but ...

... there are subgroups of patients with

- Increased distribution of capecitabine from plasma to subcutaneous tissue (Ratio AUC > 2; n=4)
- Increased distribution/re-distribution of 5-FU in subcutaneous tissue (c_{max} > 2; n = 6)

Conclusions

- Microdialysis is a very useful tool to assess tissue distribution and/or metabolism using HPLC or CE
- Microdialysis may help to individualize the relevant factors of drug resistance *in vivo*
- The data obtained by microdoalysis are an excellent starting point to develop PK-PD models dealing with response to therapy and related side-effects