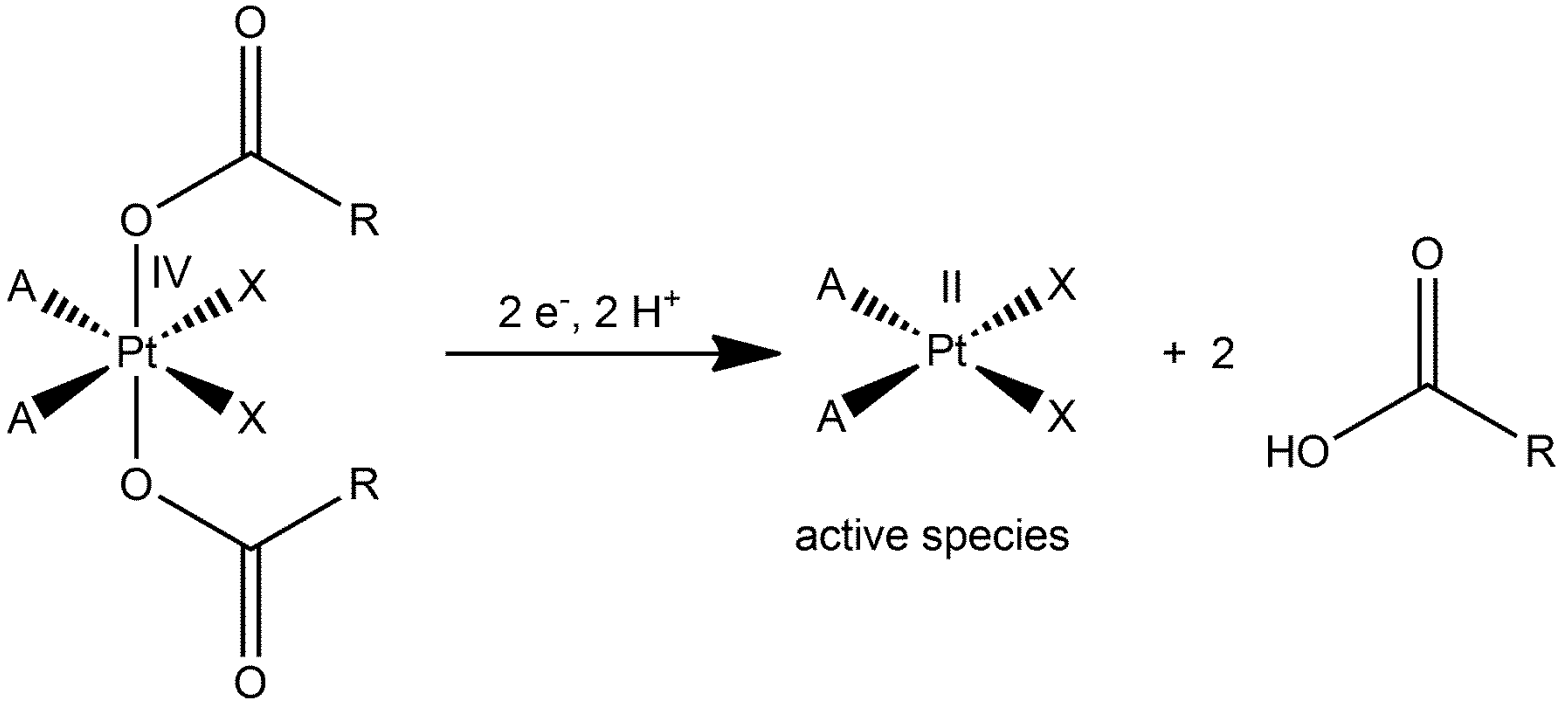
The long way to DNA: the impact of the axial ligands on the biological properties of Pt(IV) complexes.

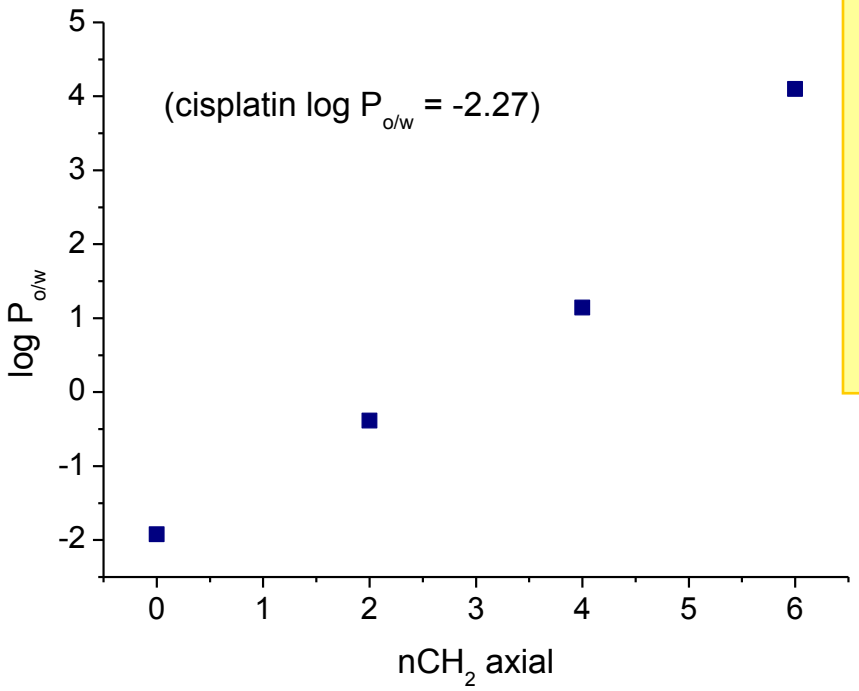
Mauro RAVERA, Elisabetta GABANO, Sabrina BIANCO, Ilaria ZANELLATO, Ilaria BONARRIGO, Domenico OSELLA.

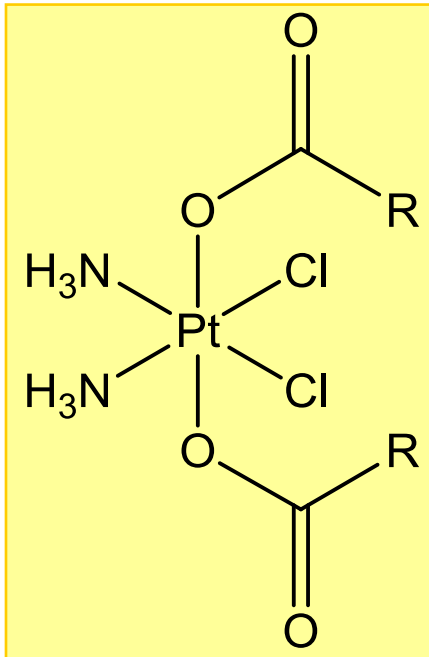
Dipartimento di Scienze e Innovazione Tecnologica,
Università del Piemonte Orientale,
Viale Teresa Michel 11,
15121 Alessandria (Italy)

Pt(IV) antitumor drug candidates are generally considered *prodrugs* since they can be reduced in the hypoxic (and then reducing) intracellular *milieu* of tumour cells to the corresponding cytotoxic Pt(II) metabolite with the (*usual*) loss of their two axial ligands (*activation by reduction*).



The relationship between lipophilicity, cell accumulation, DNA platination and antiproliferative activity (ovarian A2780) of a small series of homologous complexes has been studied and compared with that of cisplatin.

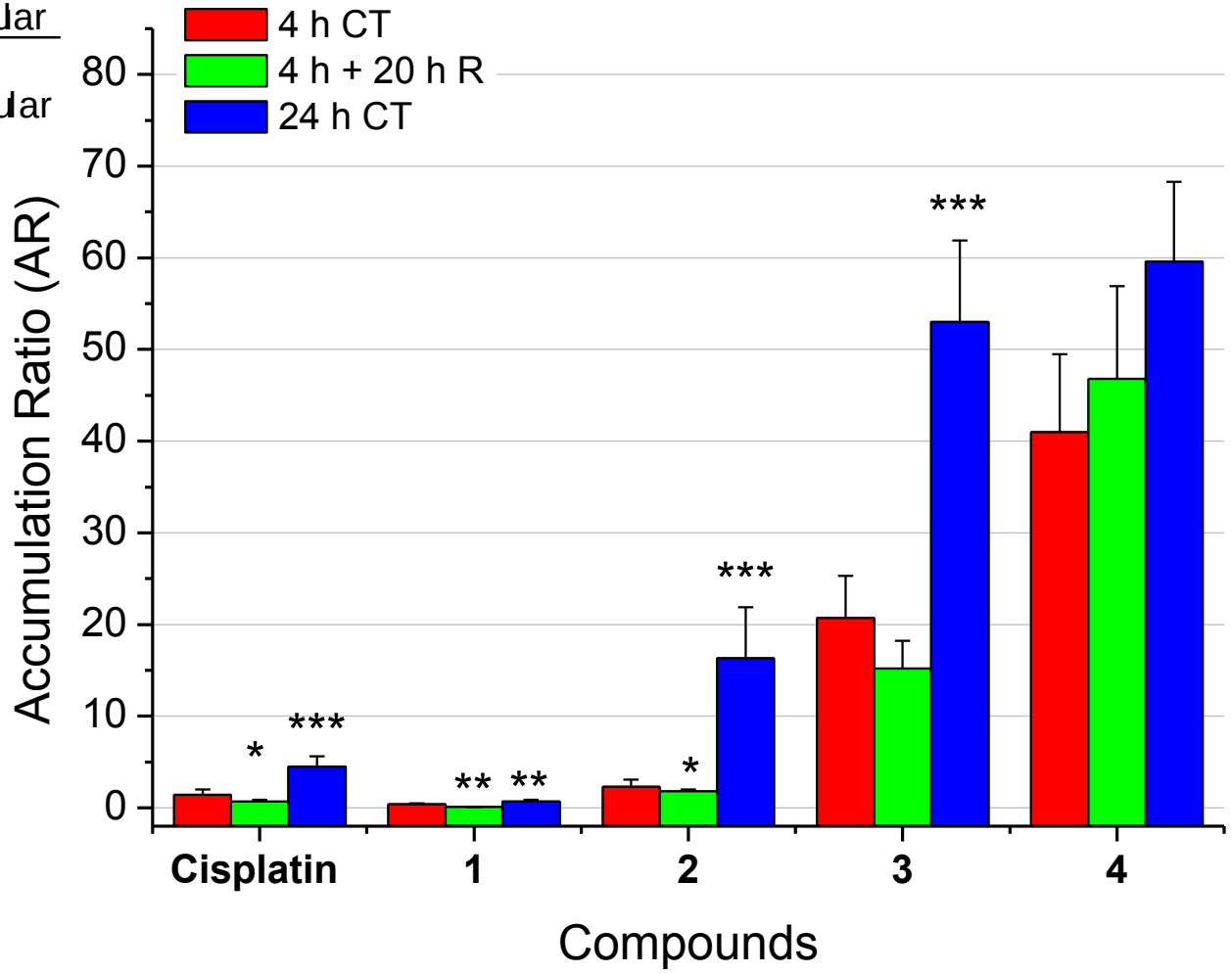




1 R = $-CH_3$
2 R = $-(CH_2)_2-CH_3$
3 R = $-(CH_2)_4-CH_3$
4 R = $-(CH_2)_6-CH_3$

Accumulation Ratio (AR)

$$AR = \frac{[Pt]_{intracellular}}{[Pt]_{extracellular}}$$



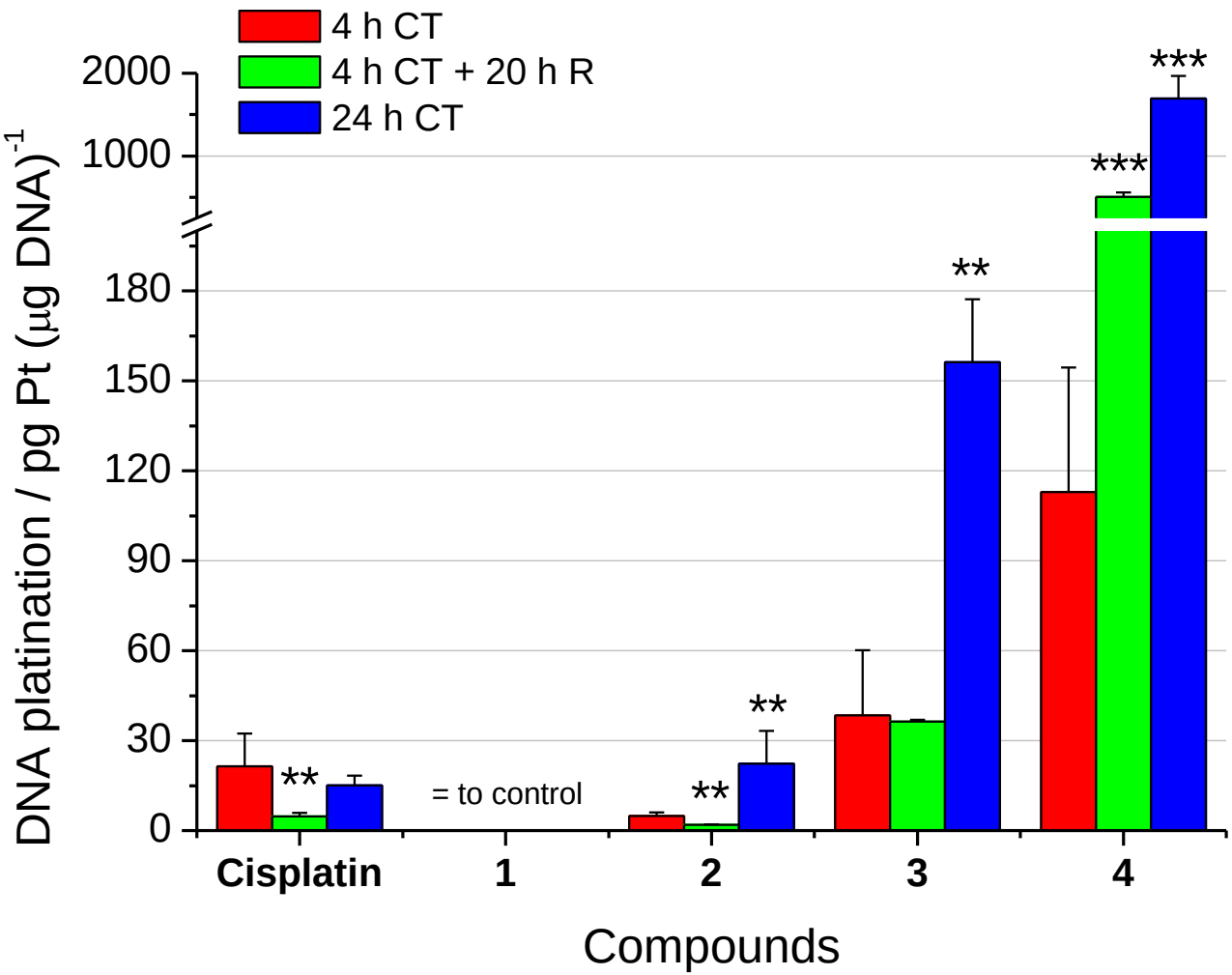
AR of A2780 treated with 10 μM of all the Pt-based complexes.

Data are means ± SD of at least 3 independent replicates and were compared to those obtained for each drug after 4 h CT by means of the two sample t-test (*p<0.05;** p<0.01; ***p<0.001).

Accumulation Ratio (AR)

- Relationship between log $P_{o/w}$ and AR for **1**-**4**. ➡ *passive uptake*
- Despite lower log $P_{o/w}$, AR of cisplatin is slightly higher than that of **1.** ➡ *involvement of active uptake for cisplatin?*
- AR of cisplatin and **1**-**3** increased from 4 h to 24 h CT. Complex **4** needed only 4 h to reach maximum accumulation. ➡ *saturation*
- AR of cisplatin, **1** and **2** dropped during 20 h recovery (R). On the contrary, the AR of **3**-**4** remained almost unchanged during R. ➡ *efflux seems to have almost no effects on the most lipophilic complexes*

DNA platination

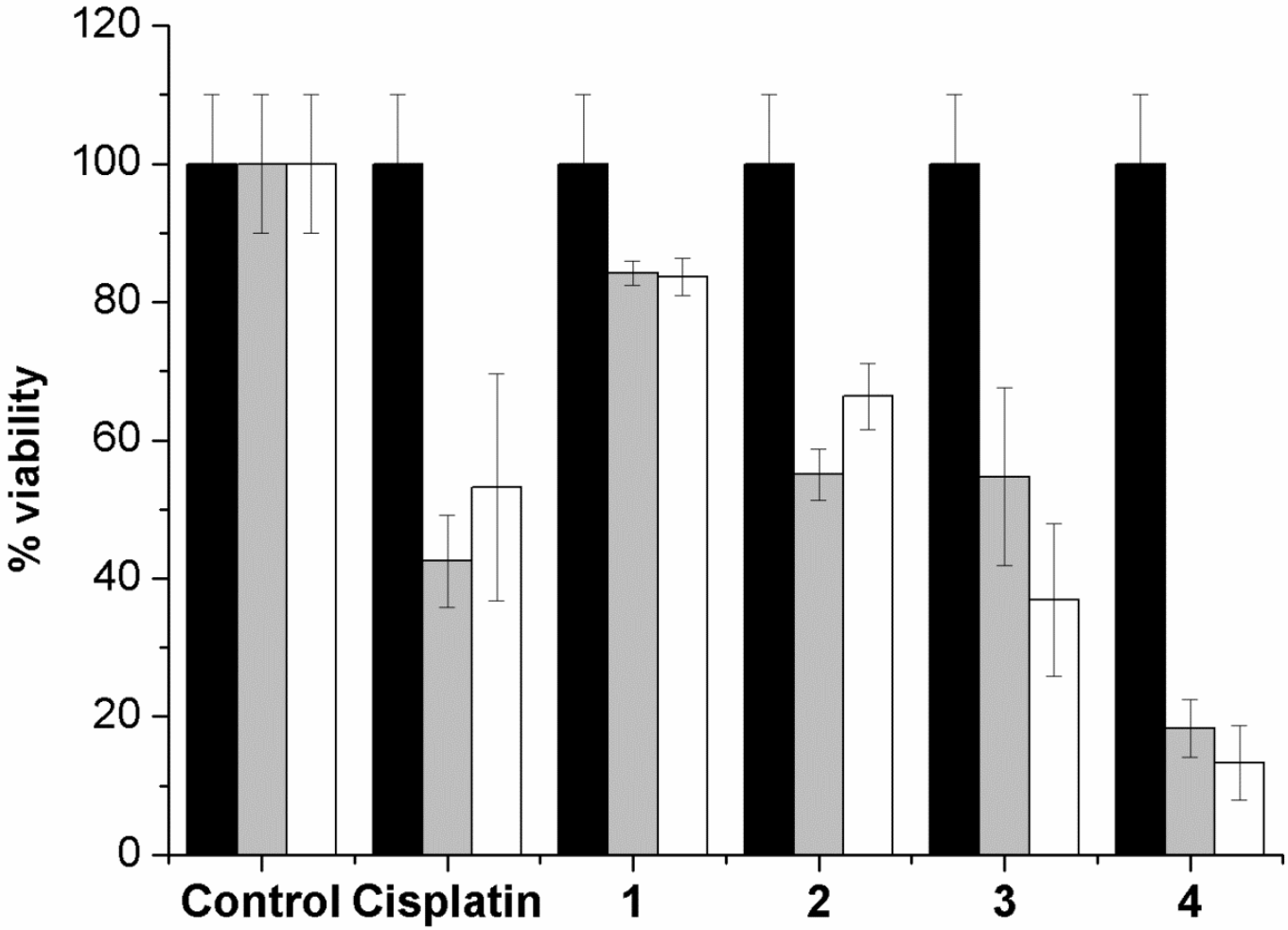


DNA platination [pg Pt (μg DNA)$^{-1}$] of A2780 treated with 10 μM of the Pt-based complexes for 4 h CT, 4 h+ 20 R and 24 h CT. Data are means ± SD of at least 3 independent replicates and were compared to those obtained for each drug after 4 h of treatment by means of the two sample t-test (*p<0.05;** p<0.01; ***p<0.001).

DNA platination

- DNA platination reflects AR data for **1**-**4** (Pt = to control for **1**); ➡ *DNA platination is ∝ to AR*
- For cisplatin, R decreased platination, whereas CT maintained it; ➡ *efflux and/or repair reduce platination?*
- DNA platination increased for **2**-**4** from 4 h to 24 h CT, but the R had different effects: **2** was significantly reduced, **3** was unchanged, while **4** was increased. ➡ *the reduced efflux observed for more lipophilic complexes accumulate Pt inside the cell?*

Citotoxicity



% viability of A2780 cells treated with 10 μM of the Pt complexes. Residual cell number was recorded after 4 h (black bars), 4 h + 20 h R (grey bars); 24 h CT (white bars).

- Platination → % viability.

Citotoxicity

	IC_{50} [μM], A2780 cell line		
	4h CT + 68h R	24h CT + 48h R	72h CT
Cisplatin	2.4±0.2	1.5±0.9	0.43±0.07 (*)
1	48.7±19.2	12.3±2.2	8.8±1.3 (*)
2	4.3±2.2	0.7±0.3 (*)	0.42±0.08 (*)
3	0.32±0.13	0.031±0.025 (*)	0.015±0.007 (***)
4	0.034±0.022	0.0035±0.0041 (*)	0.0019±0.001 (*)

- A more prolonged treatment increased the activity for all complexes, but in particular for the more lipophilic Pt(IV).

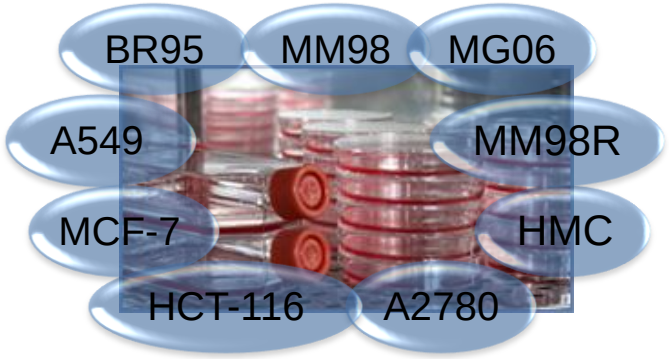
Considerations

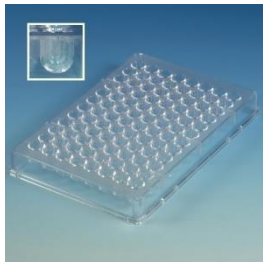
- Higher the lipophilicity, higher the AR, higher the DNA platination, and then the potency (*passive diffusion*).
- Low log $P_{o/w}$ are not enough to "charge" the cells of adequate amounts of Pt to have an important biological effect. Cisplatin may bypass this limitation through an active or assisted uptake (*just an hypothesis…*).
- Compounds with high log $P_{o/w}$ fill the cells by a double effect: high uptake and reduced efflux.
- In the latter case, "Pt" may be stored in some intracellular trapping site, permitting a gradual release and leading to a prolonged cytotoxic effect.

…but how prolonged?

2D vs. 3D cell cultures

2D methods are limited by cellular confluence to few days of treatment. On the contrary, multicellular tumor spheroids (MCTS) were used to perform drug screening for prolonged periods. The 3D architecture better reproduces a “true” tumor (cell–cell and cell–matrix interactions, proliferating/necrotic areas, etc.). Thus, MCTS may simulate the drug penetration into the tumor tissue.



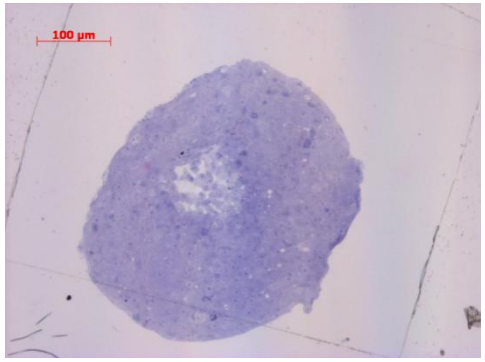


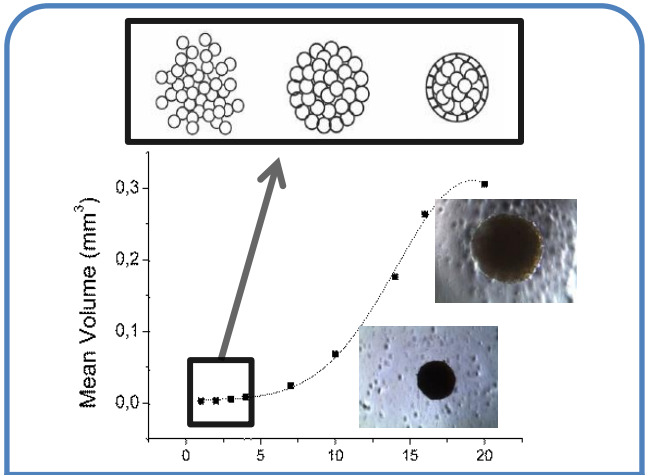
1. Cell seeding

2. Stirring

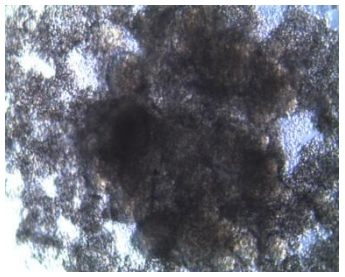
Multicellular tumor spheroids

Only certain cell lines are able to give **proliferating spheroids**





BR95; HCT-116

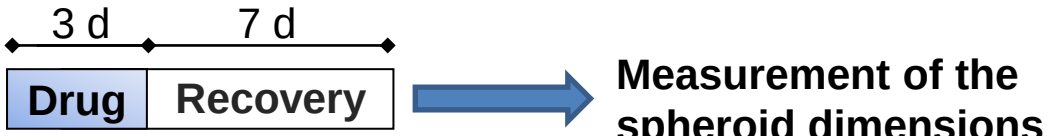


A2780
MCF-7
A549, etc.

BR95 = malignant pleural mesothelioma
HCT-116 = colon carcinoma

Spheroid obtained from BR95 (malignant pleural mesothelioma) cell line.

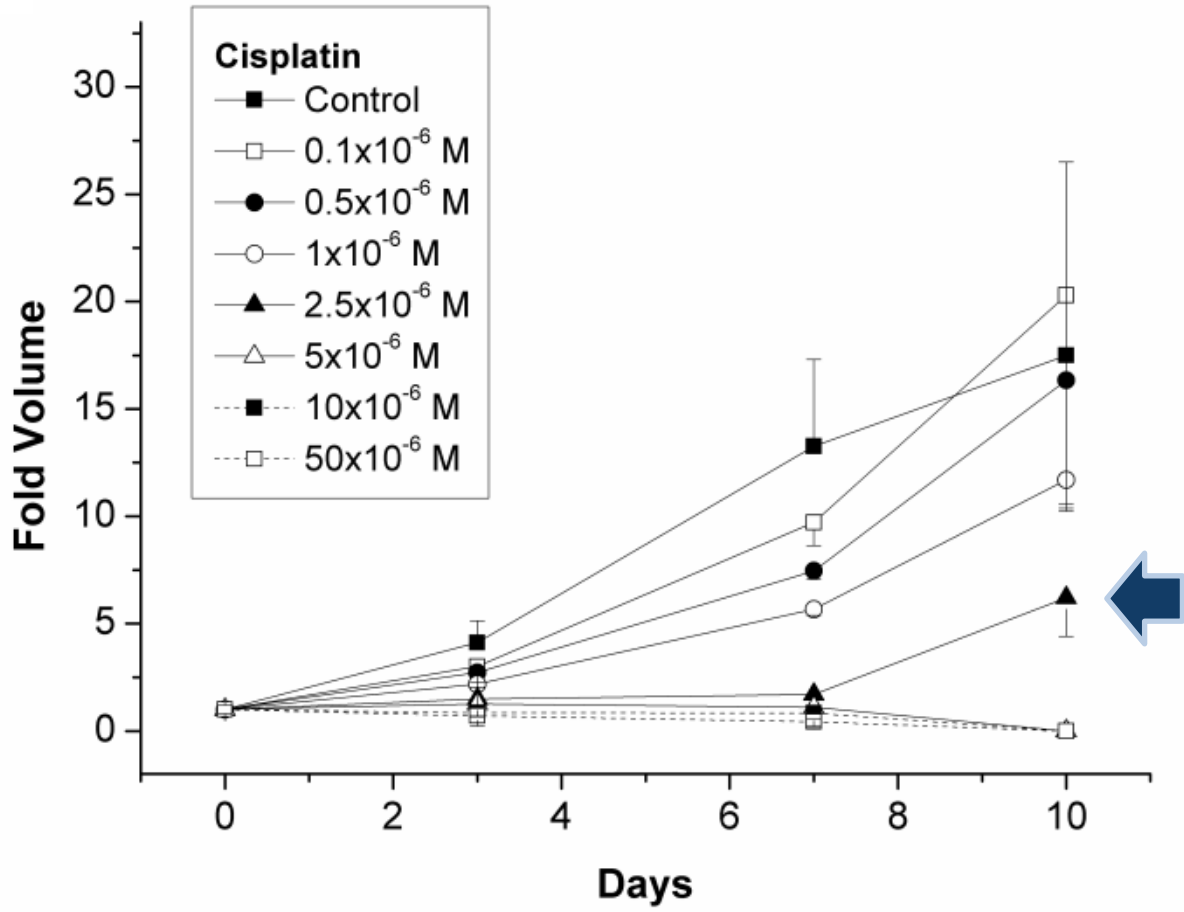
Treatment protocol:



Measurement of the spheroid dimensions

Treatment of HCT-116 MCTS with cisplatin

(IC_{50} monolayer HCT-116 = 2.3±0.3 μM, 72 h CT)

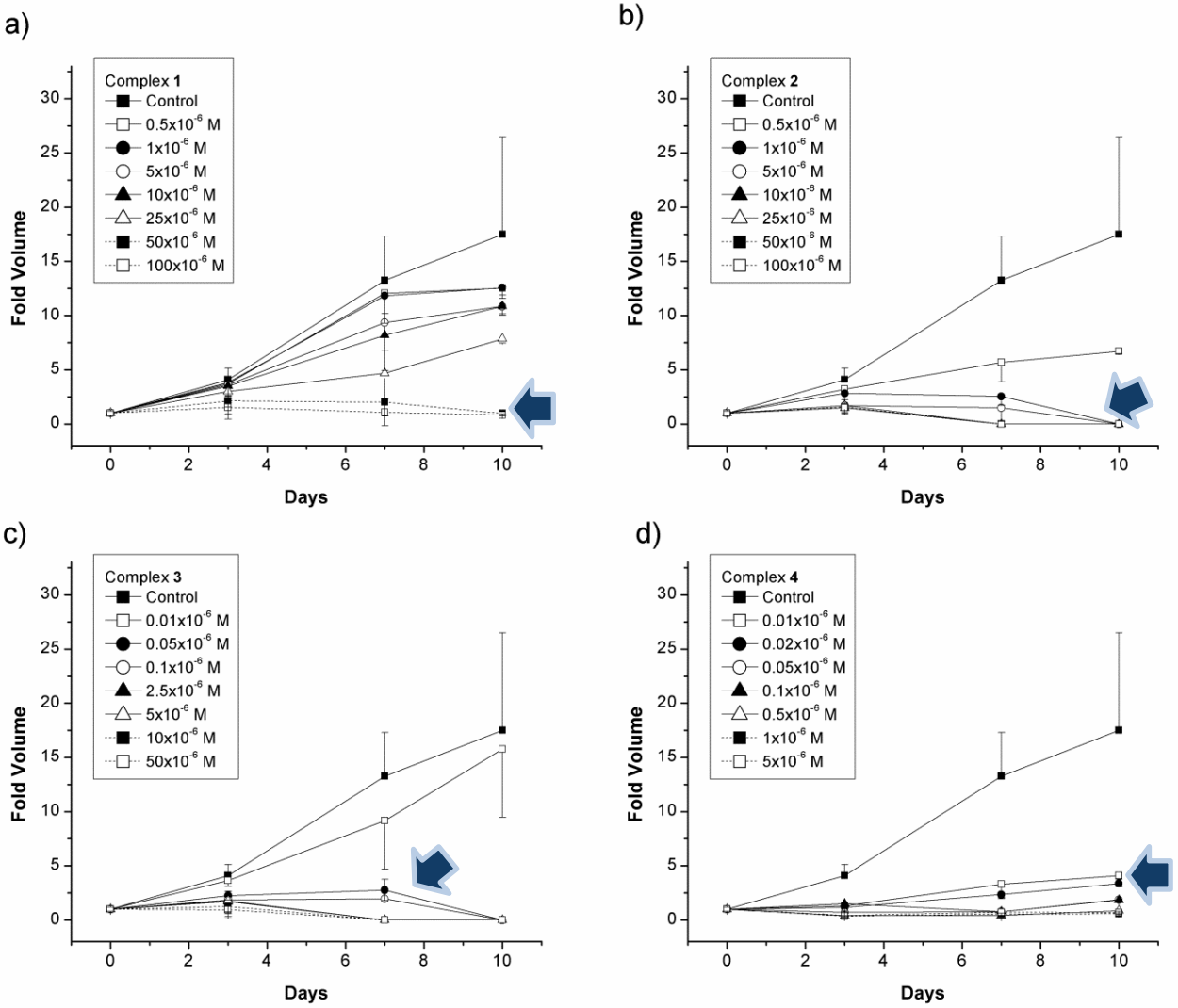


IC_{50} monolayer

Treatment of colon carcinoma HCT-116 MCTS with **1-4**

(IC_{50} monolayer HCT-116 (μM, 72 h CT) =

a)	**1**	55.3±1.7
b)	**2**	1.8±0.3
c)	**3**	0.042±0.010
d)	**4**	0.0090±0.0005



Cisplatin and HCT-116 MCTS

- Cisplatin and **1-4** complexes gave a concentration-dependent response in HCT-116 MCTS with a potency in the order **1** < cisplatin = **2** < **3** < **4** as observed for 2D experiments;
- Complexes **1-4** exert a prolonged antiproliferative action even when the drug is removed from the culture medium;

…but…

- Preliminary *in vivo* data show that high lipophilicity is detrimental of the oral absorption (*work in progress*).

Thanks to:

COST Action CM1105

***C**onsorzio **I**nteruniversitario di **R**icerca in **C**himica dei **M**etalli nei **S**istemi **B**iologici (Bari)*

FONDAZIONE
CASSA DI RISPARMIO DI ALESSANDRIA

FONDAZIONE CRT
Cassa di Risparmio di Torino