An ISO Certified/GLP Laboratory Operating in an Academic Setting: Pharmacokinetic Enhancements Combined with Targeted Drug Delivery

BIOMEDICAL RESEARCH FOUNDATION, ACADEMY OF ATHENS (BRFAA)
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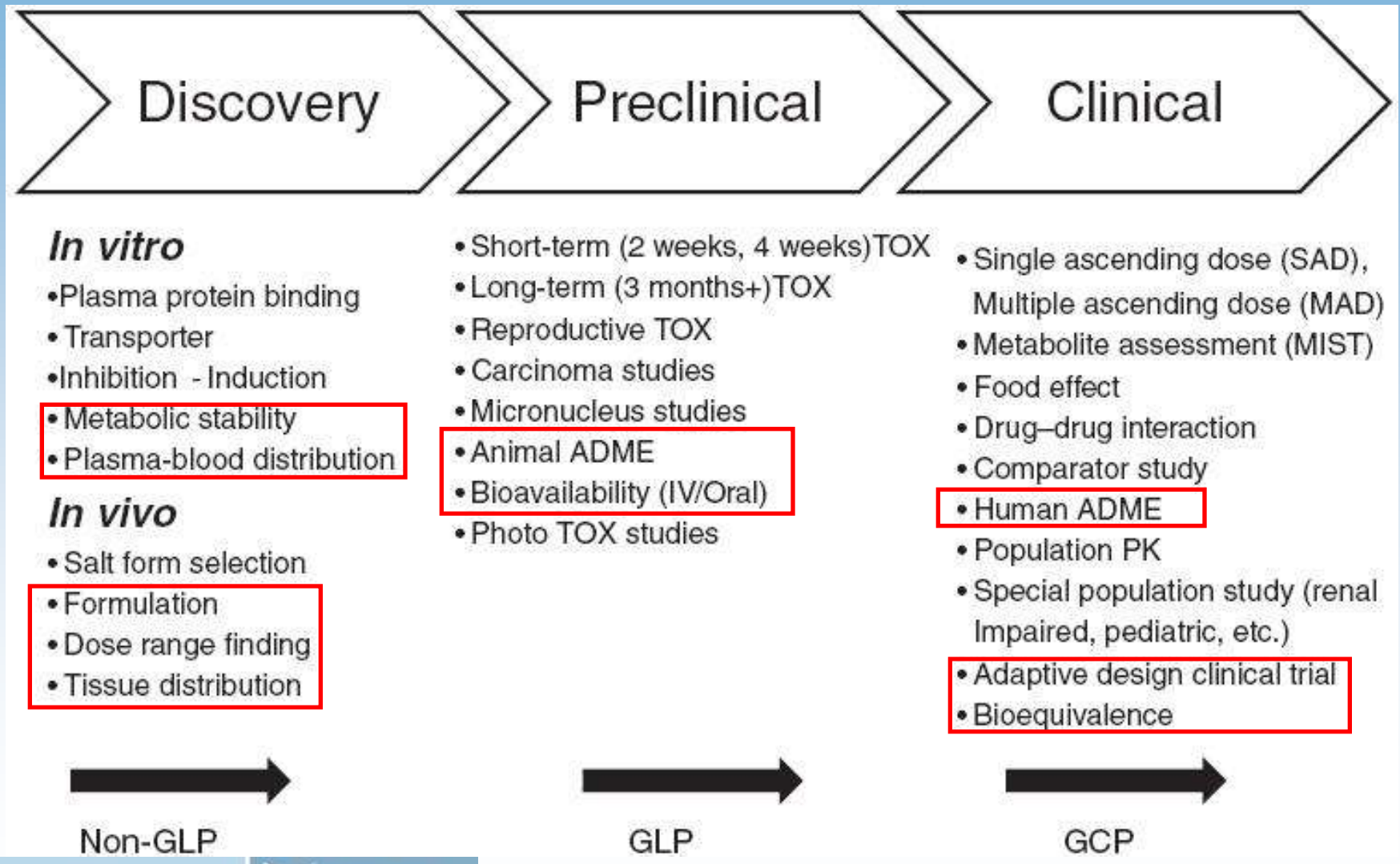
Bioanalytical laboratory supported studies



DIVISION OF PHARMACOLOGY - PHARMACOTECHNOLOGY

The laboratory operates according to the Good Laboratory Practice (GLP) standards and has been awarded an ISO/IEC 17025:2005 certificate.

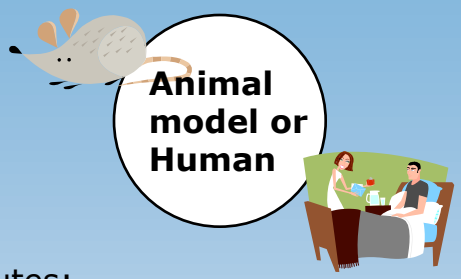
The lab is equipped with Hybrid system of Liquid Chromatography – Mass Spectroscopy of triple quadrupole – Ion trap (LC-MS/MS), and all the other equipment that are required to perform bioanalysis and validation of drugs.

- Qualitative/ Quantitative analysis of drugs in biological fluids/tissues
- Pharmacokinetics (PK) of drugs in pre-clinical/clinical stages
- Drug metabolism
- Bioavailability and bioequivalence studies
- Quantitative determination of biomarkers (*e.g.* peptides, proteins) in various matrices (*e.g.* plasma)

Pharmacokinetics and Drug Metabolism Background

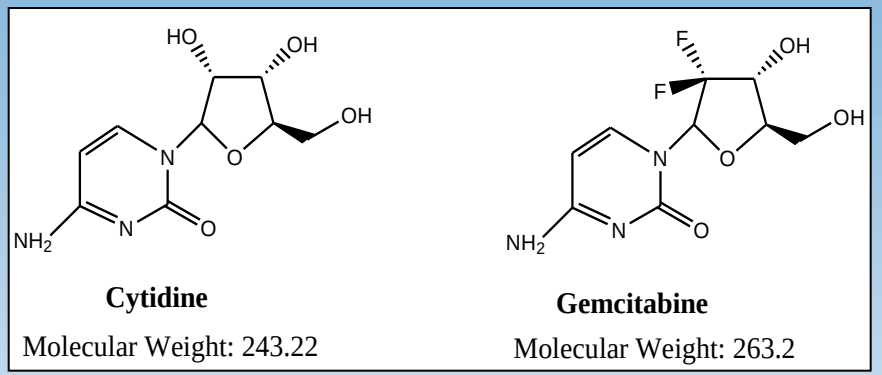
The LADMER system

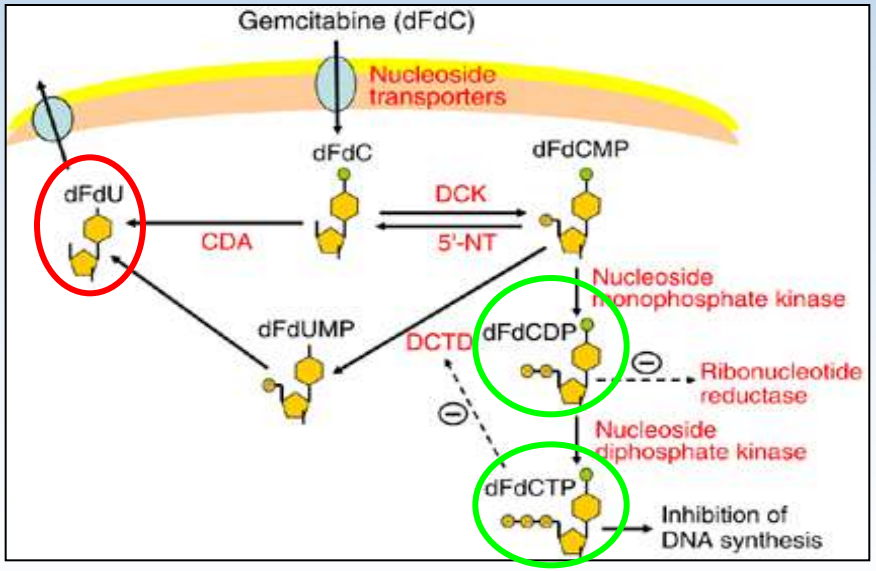
(Liberation, Absorption, Distribution, Metabolism, Elimination and Response)



Gemcitabine

- Potent anticancer agent used for the treatment of several solid tumors such as colon, lung, pacreatic cancer
- Transferred into the cell by nucleoside transporters, undergoes phosphorylation and blocks DNA synthesis
- **Main limitation: Rapid metabolic inactivation through deamination and formation of dFdU**
- Improving gemcitabine poor pharmacokinetics has become a field itself





Ueno et al, British Journal of Cancer (2007) 97, 145–151

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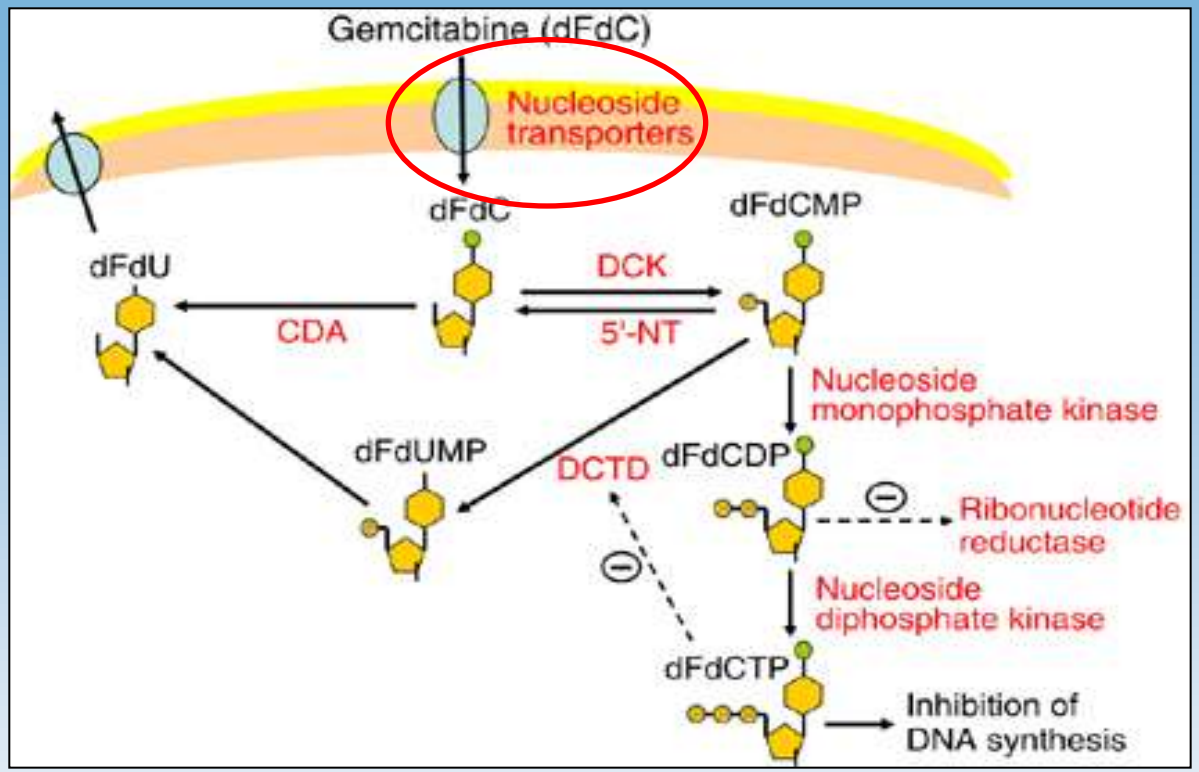
Gemcitabine Pharmacogenomics

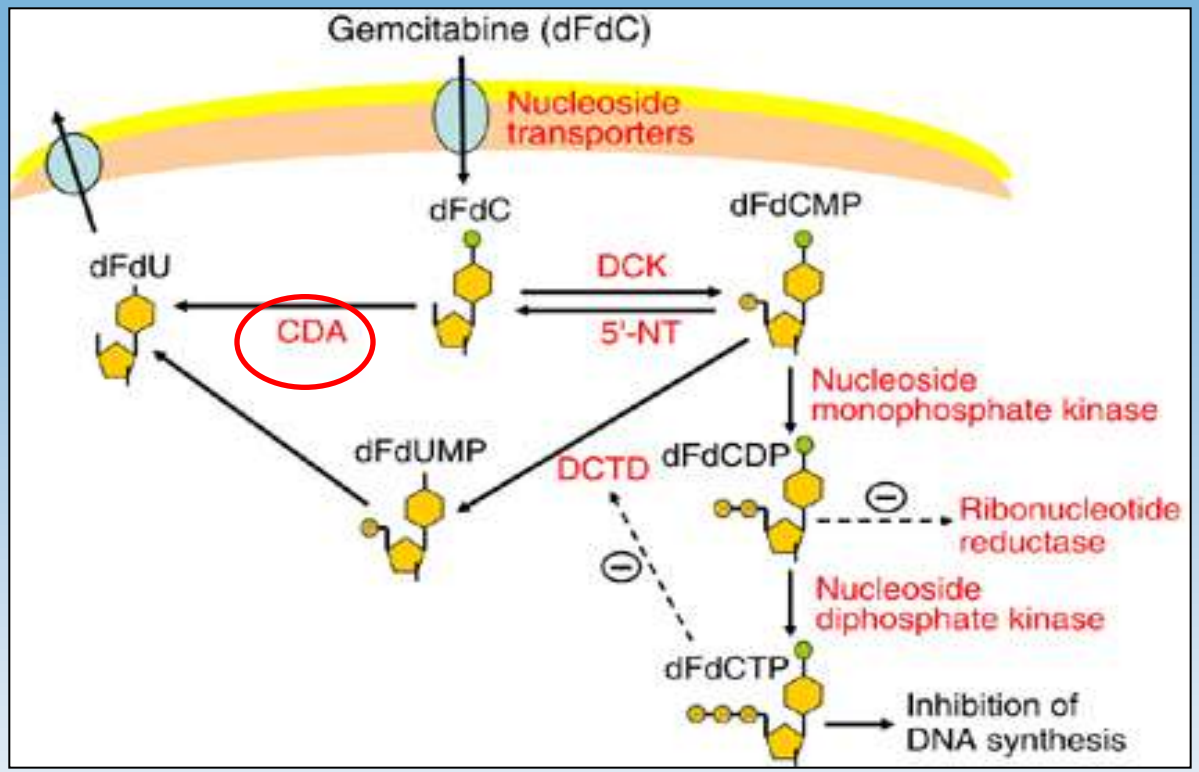


Ueno et al, British Journal of Cancer (2007) 97, 145–151

- Several studies suggest that nucleoside transporter expression in tumor tissues may be a good predictive marker of outcome in cancer patients receiving gemcitabine.

- The most common form of gemcitabine induced resistance is the one related to nucleoside transporter deficiency

Gemcitabine Pharmacogenomics



Ueno et al, British Journal of Cancer (2007) 97, 145–151

- CDA overexpression in tumor tissues might reduce the antitumor efficacy of this drug
- An *in vitro* study has demonstrated resistance to gemcitabine in cells overexpressing CDA
- Gemcitabine inactivation is the major impediment in its therapeutic use

Gemcitabine prodrug development

Designed gemcitabine prodrugs that would:

1) Reduce gemcitabine's metabolic inactivation

→ gemcitabine prodrugs can be designed specifically to affect its interaction with cytidine deaminase

2) lead gemcitabine specifically to the tumor site

→ through conjugation to a peptide with a strong affinity for a cell surface receptor over-expressed in the tumor cell.

3) Provide gemcitabine an alternative entrance route

→ through conjugation with a peptide that could enter the cell using an alternative route (e.g. a GPCR)

Peptides have taught us a great deal on how to target a receptor – The extrapituitary implication of GnRH in Cancer

- In advanced prostate cancer, after ADT, androgen receptor **does not depend on stimulation by androgens** (GnRH agonist therapy through the pituitary) leading to **androgen independent** state

- GnRH Receptors are expressed in various cancer cells related to the reproductive system (**Prostate**, Ovarian, Breast)

- GnRH-R gene expression is upregulated in patients with androgen-independent CaP

→ Opportunity to treat cancer by targeting the GnRH Receptor beyond the pituitary directly on the tumor

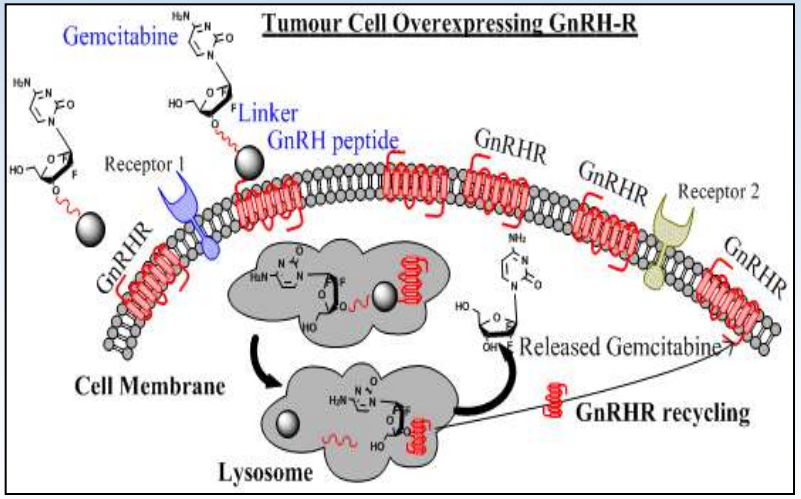
Tumor cell targeted therapy using GnRH conjugates

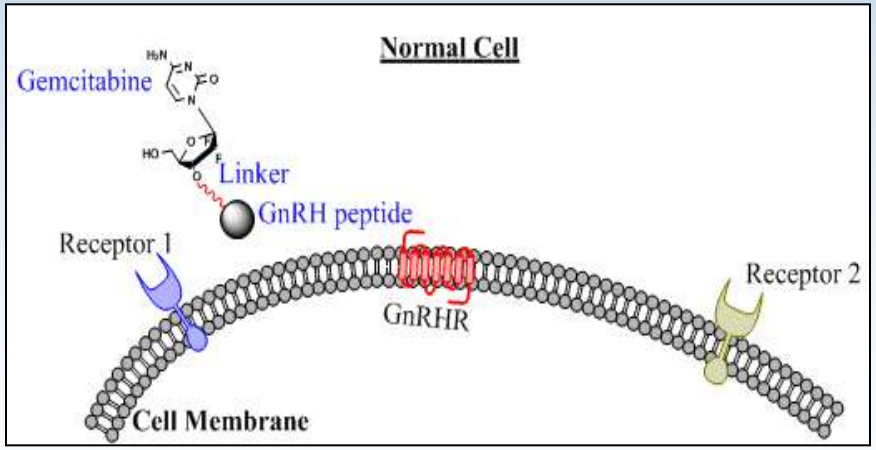
- GnRH conjugate = GnRH analogue + linker + small molecule anticancer drug

➢ GnRH Receptor is a GPCR

→ It can internalize into the cell together with its ligand upon activation

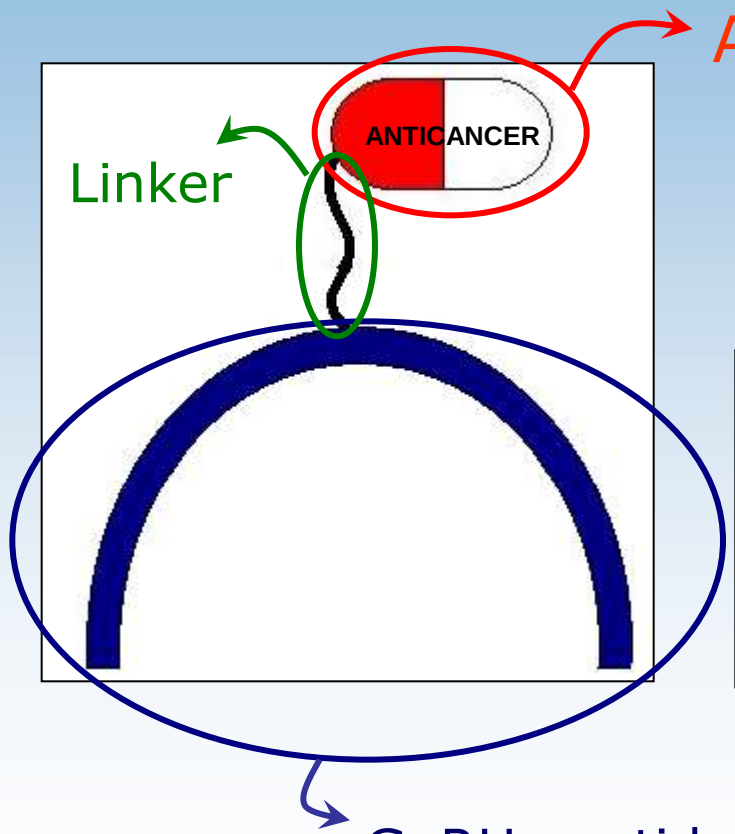
- Conjugation → Delivery of active drug selectively in cancer cells

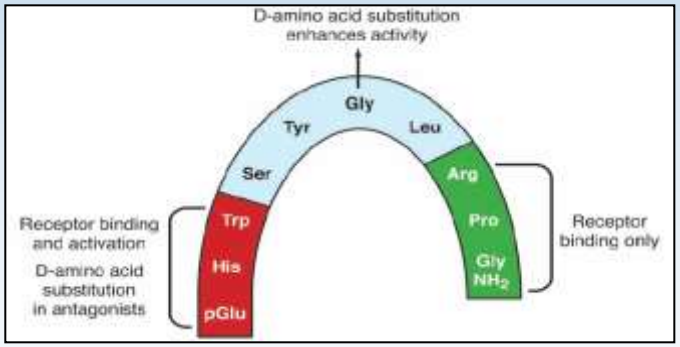




Targeted therapy using GnRH conjugates
Chemotherapeutic peptide conjugates

- GnRH conjugate = GnRH analogue + linker + small molecule anticancer drug

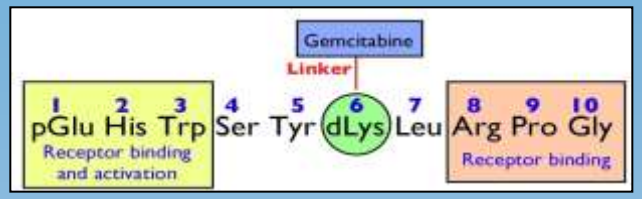




Millar, R.P. et al (2004) Endocrine Reviews, 25: 235

Evaluation of GnRH-gemcitabine conjugates

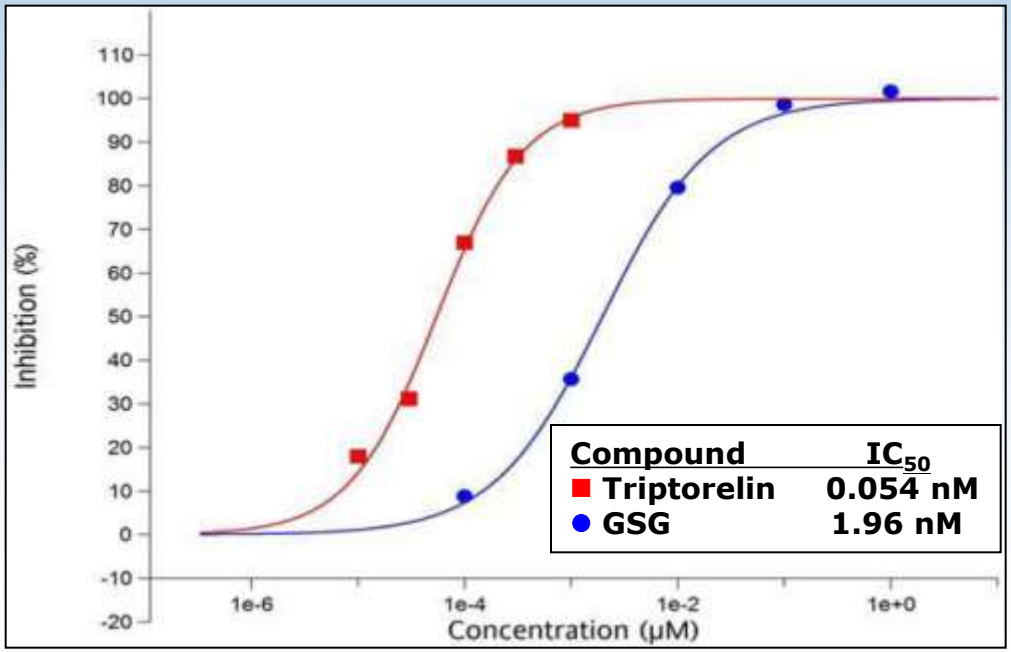
- Several GnRH-gemcitabine analogues have been synthesized and tested so far including different linkers and different conjugation sites



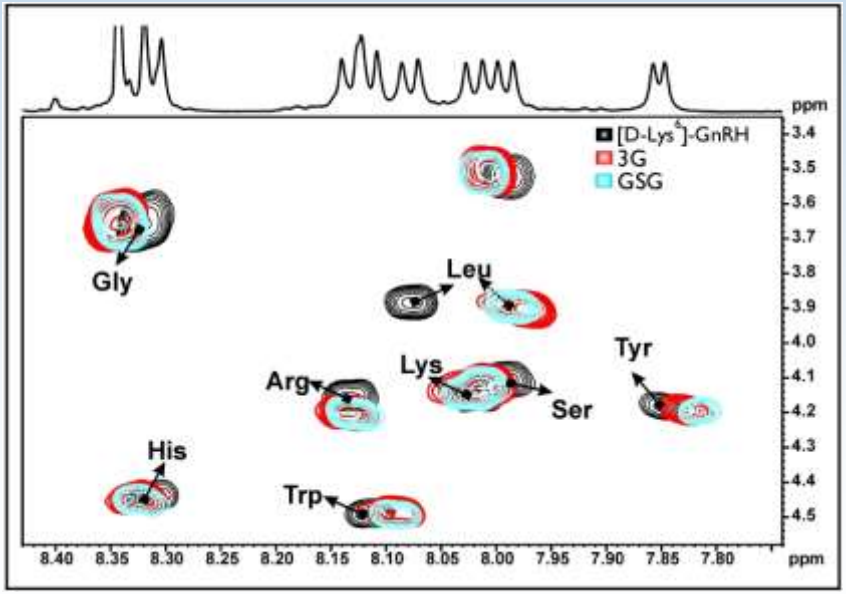
Evaluation in cell cultures

IC_{50} (nM)	Gemcitabine	3G	$3G_2$	GSG	GSG_2
DU145	231 ± 34	1171 ± 83	663 ± 273	308 ± 170	439 ± 217
PC3	585 ± 68	1161 ± 130	729 ± 193	670 ± 352	786 ± 125

GnRH-R binding assay



Superimposed 2D-NMR



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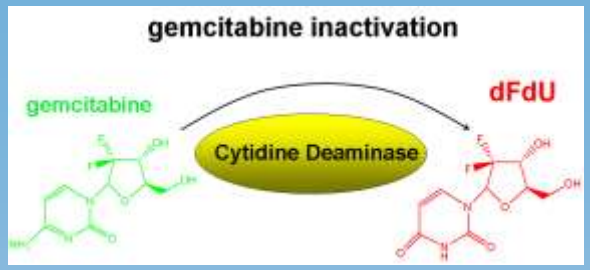
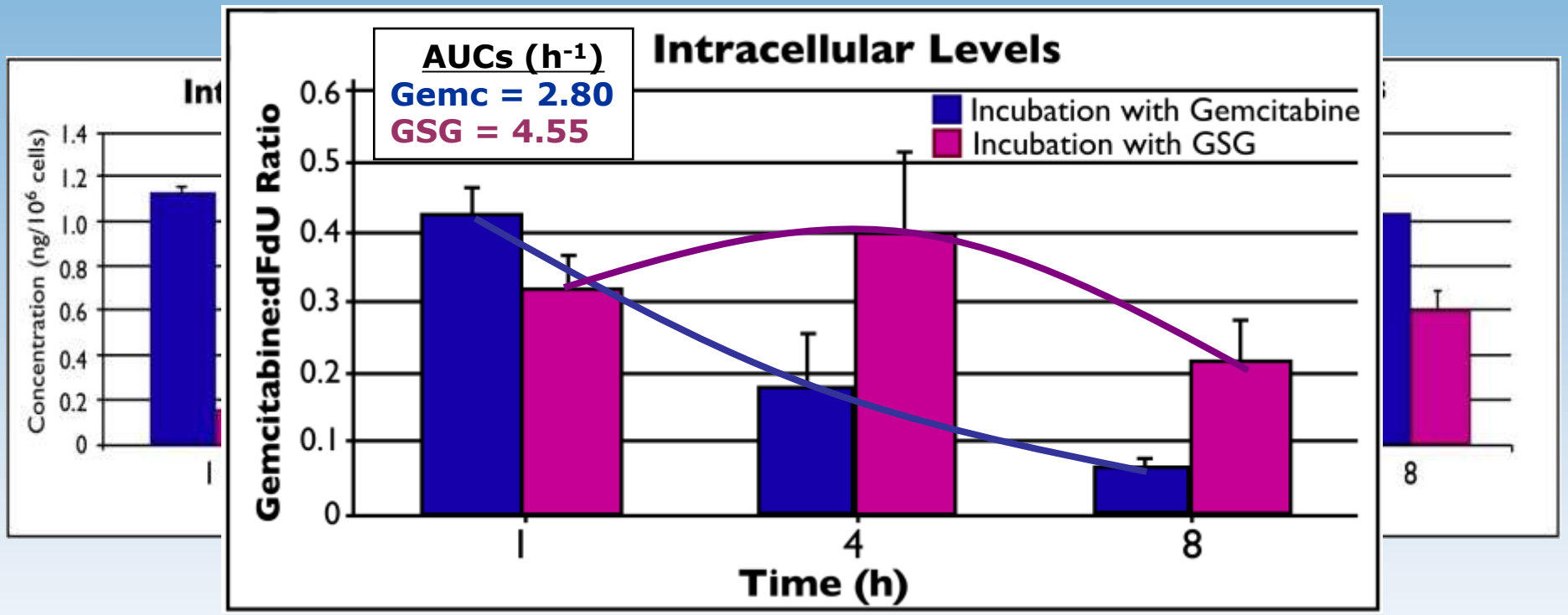
Measuring intracellular levels of gemcitabine, dFdU





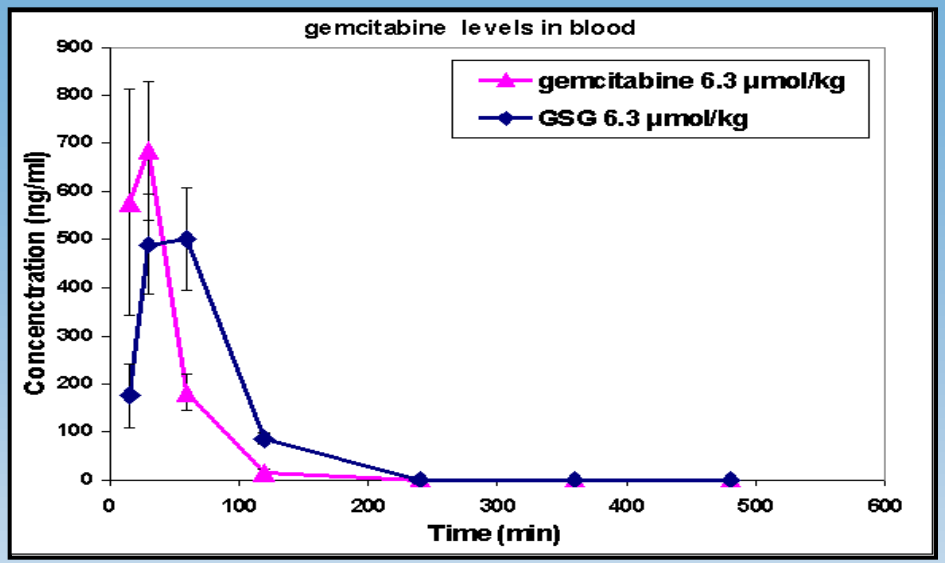
Incubated 10μM of Gemcitabine and Gemcitabine-GnRH conjugate in DU145 cells for 1,4,8 hr

→ gemcitabine degrades rapidly forming dFdU

→ GSG slowly forms gemcitabine

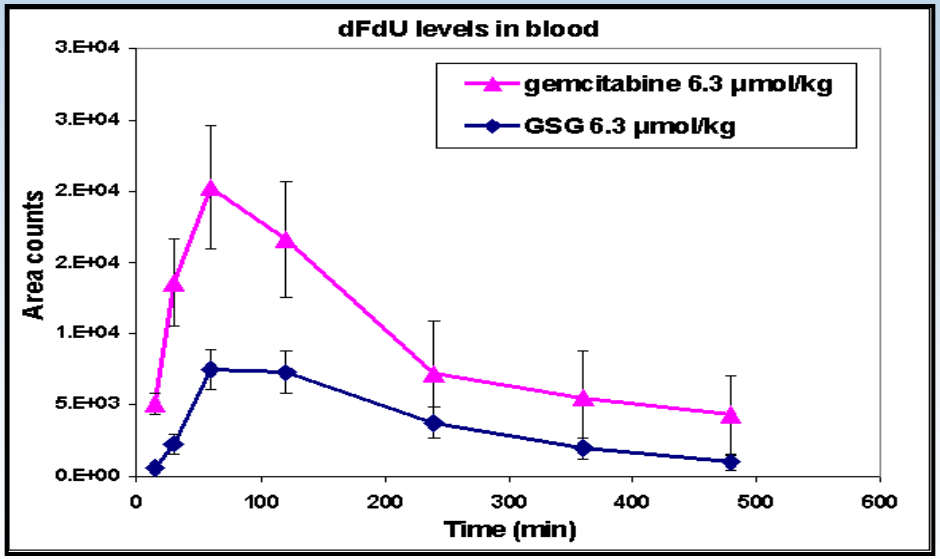
→ incubation with gemcitabine leads to higher dFdU levels in comparison to dFdU formed from GSG suggesting a "protective" effect of GSG on gemcitabine's rapid metabolic inactivation

PK studies of GSG vs gemcitabine in mice



→ **gemcitabine levels**

AUCs
(µg x min/mL)
Gem = 28374
GSG = 37412

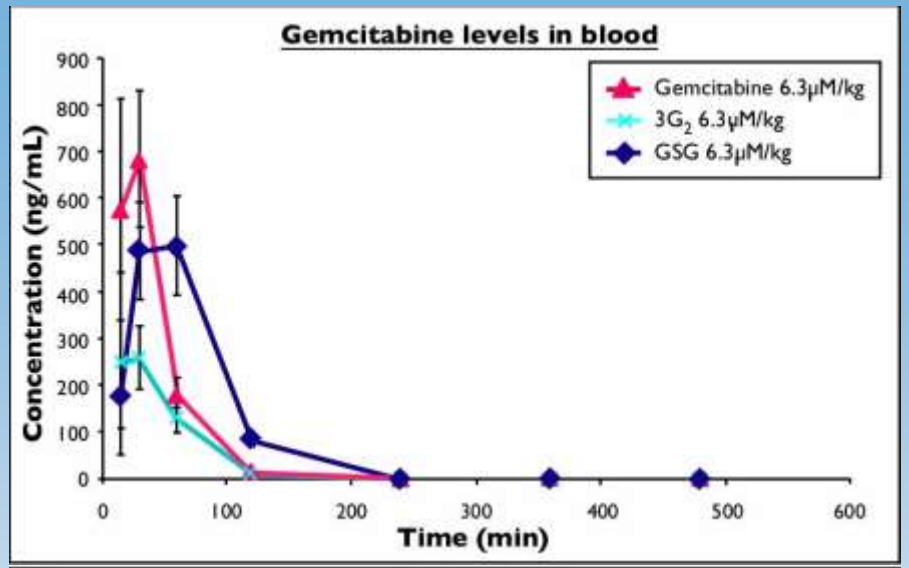


→ **dFdU (=inactive met.) levels**

AUCs
(Area Counts/min)
Gem = 4.53 x10^6
GSG = 1.79 x10^6

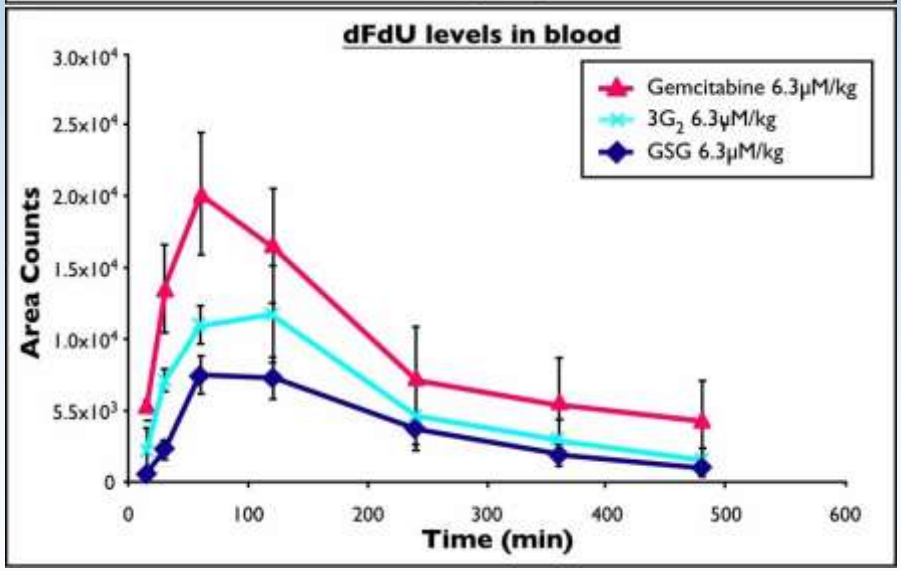
- <u>GSG</u> → High gemcitabine AND low dFdU levels

PK studies of GnRH- gemcitabine conjugates in mice



→ **gemcitabine levels**

AUCs
(μg x min/mL)
Gem = 28374
3G2 = 13864
GSG = 37412

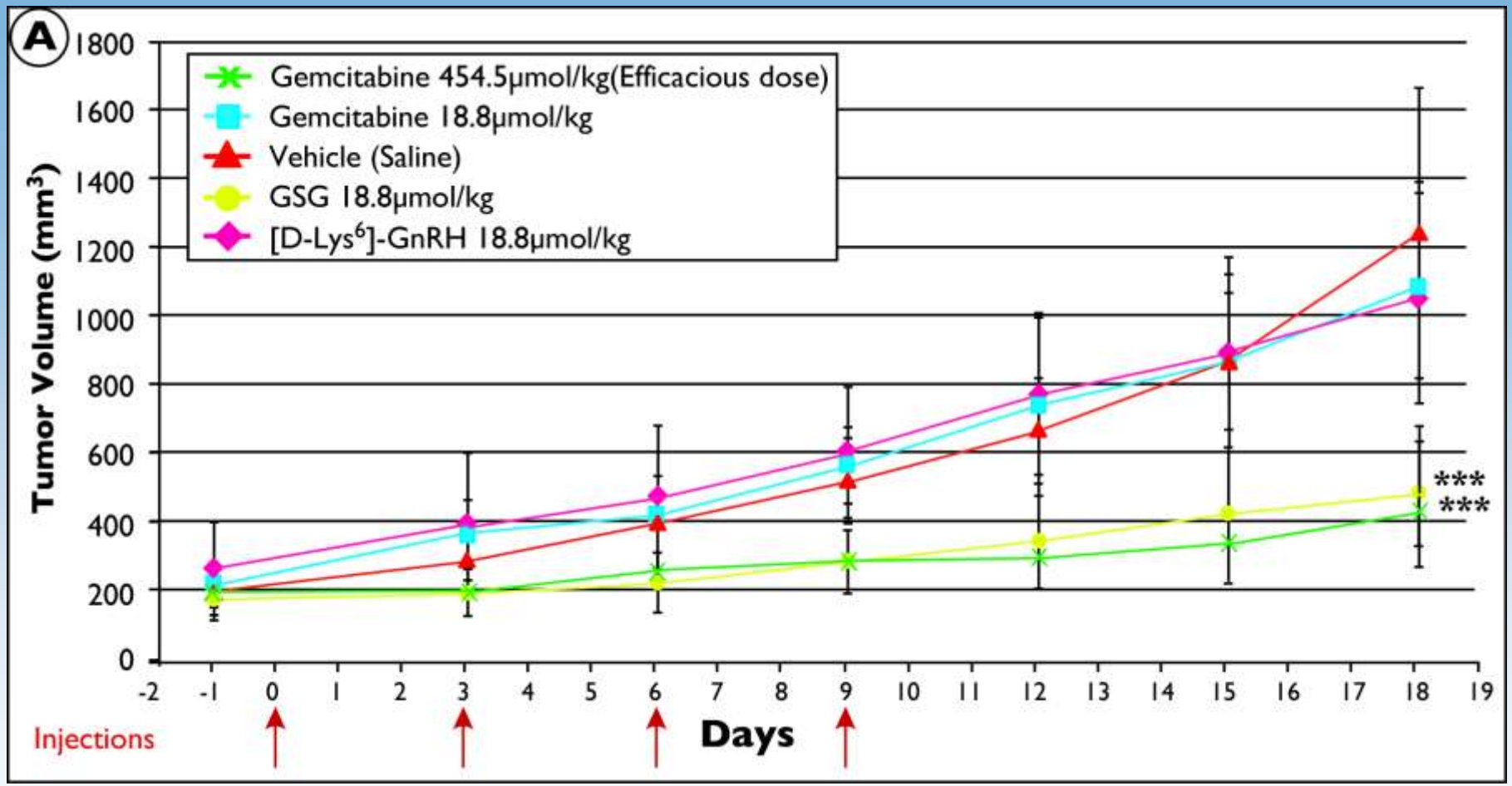


→ **dFdU (=inactive met.) levels**

AUCs
(Area Counts/min)
Gem = 4.53 x10^6
3G2 = 2.76 x10^6
GSG = 1.79 x10^6

- <u>GSG</u> → High gemcitabine AND low dFdU levels

Efficacy of GSG in DU145 xenografted mice



- DU145 cells were subcutaneously injected in NOD-SCID mice
- Treatment started when tumors were ~250 mm^3

Summary – Future Experiments and Plans

- Novel GnRH-gemcitabine conjugates were synthesized and evaluated against prostate cancer models

- The lead compound, GSG, shows a potent anticancer effect that appears to be associated with a dual mode of action:

1) improved efficacy due to reduced metabolic inactivation of gemcitabine
2) targeted delivery to cancer cells over-expressing the GnRH-R
3) Can enter the cell through a different route (GnRH-R)

Karampelas at al, 2014

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Acknowledgements

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