Circadian disruption or induction in cancer therapy

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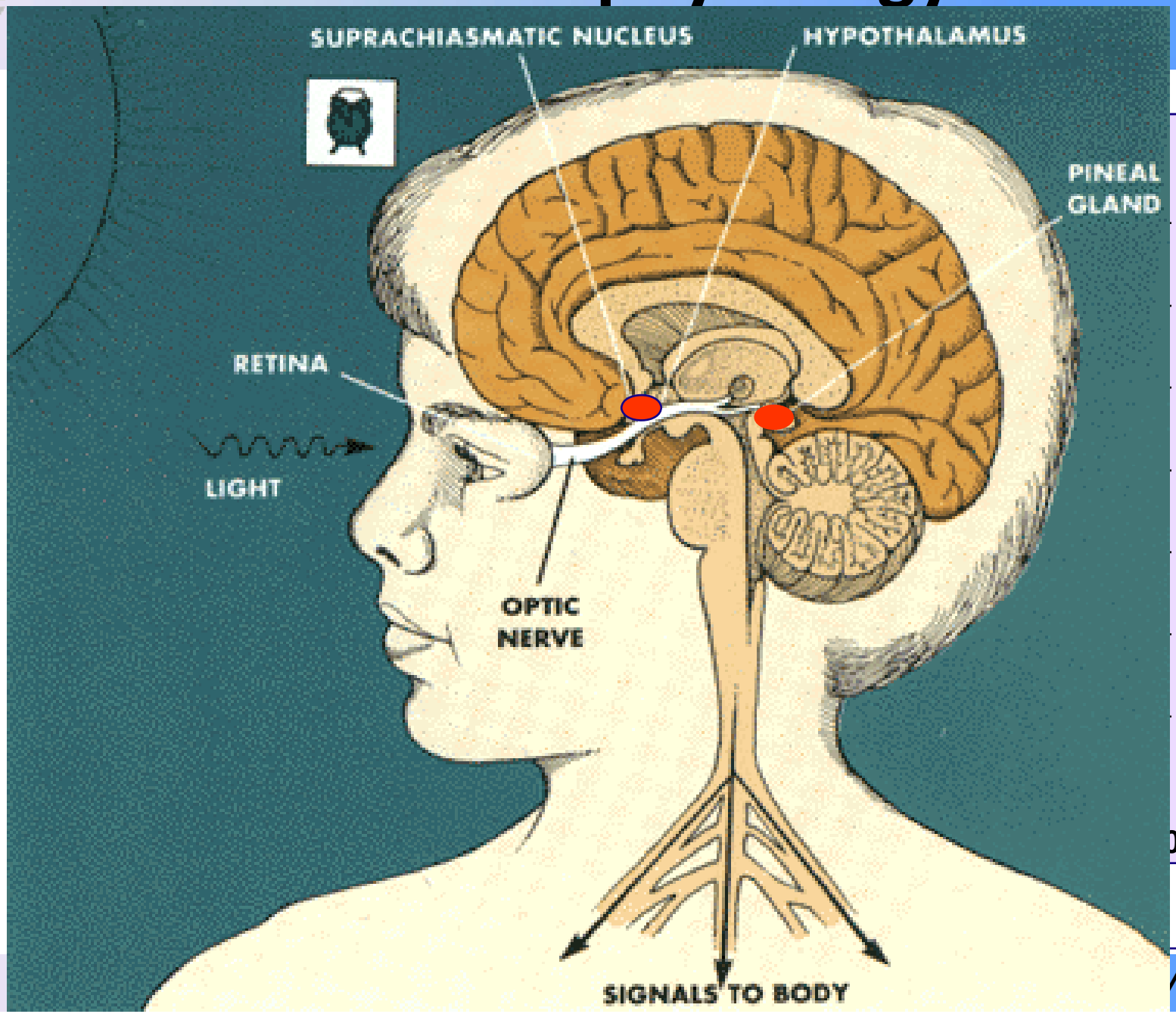
Outline of this talk

- **The circadian timing system**
  (circadian biomarkers and molecular clocks)
- **Circadian disruption**
  (cancer processes and treatments)
- **Circadian induction**
  (host and cancer clocks)
- **Conclusions**

Circadian physiology



160
140
120
100
80
60
40
20
0

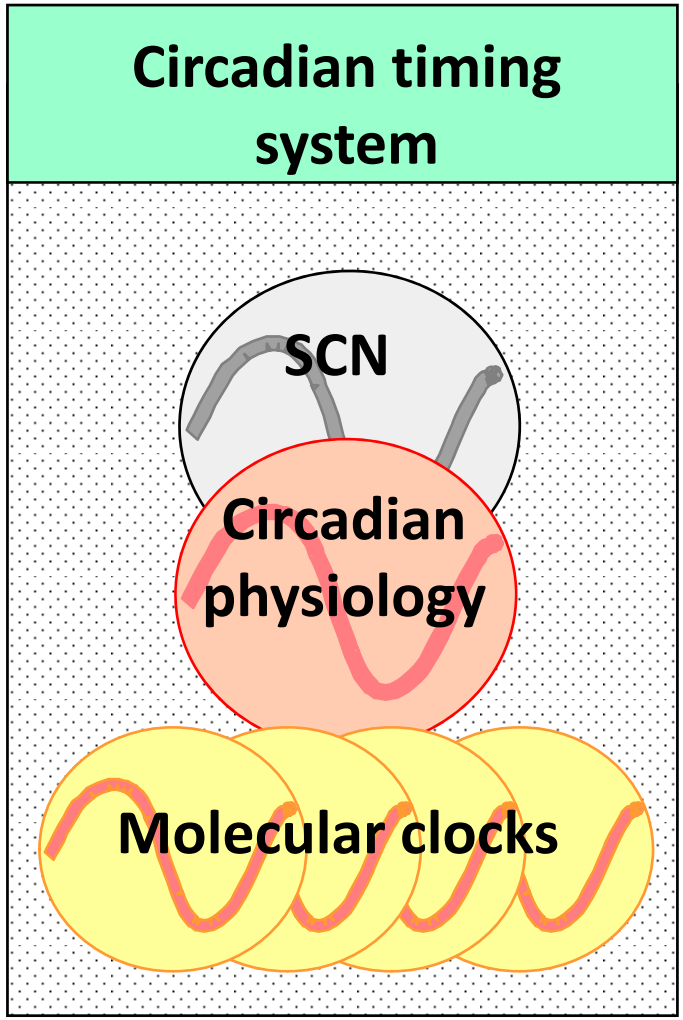
Circadian timing system in health and diseases

- Rhythms in behavioral, cognitive, sensory, muscular, cardiovascular, respiratory, immune, renal, hepatic, GI, hormonal,… functions
- Circadian/circannual disease patterns
- Treatment timing for cancer, CNS, cardiovascular, respiratory, rheumatologic, psychiatric,…diseases

→Relevance for all medical specialties

The Circadian Timing System

The temporal coordination
of metabolism and proliferation
along the 24 hours
represent a major task of
the mammalian circadian system,
that is achieved through
its organization
in a hierarchical manner.

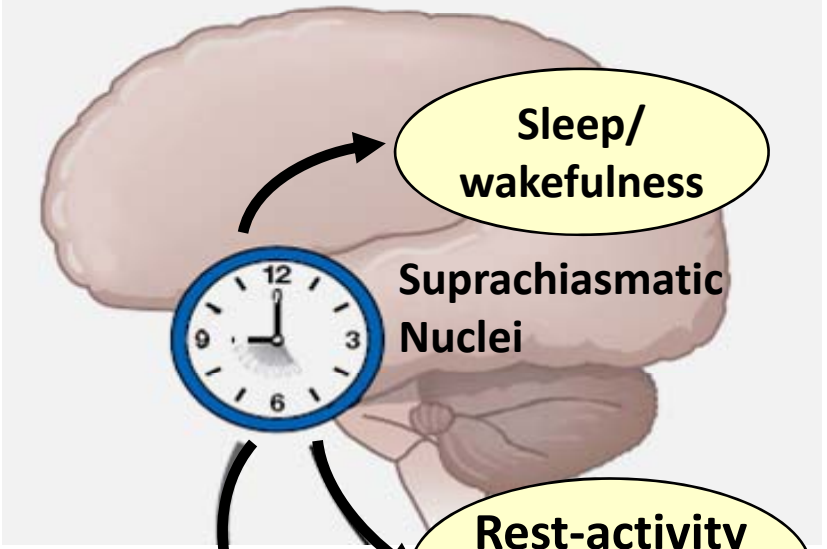


The Circadian Timing System, a coordinator of life processes

Circadian Timing system

Environment

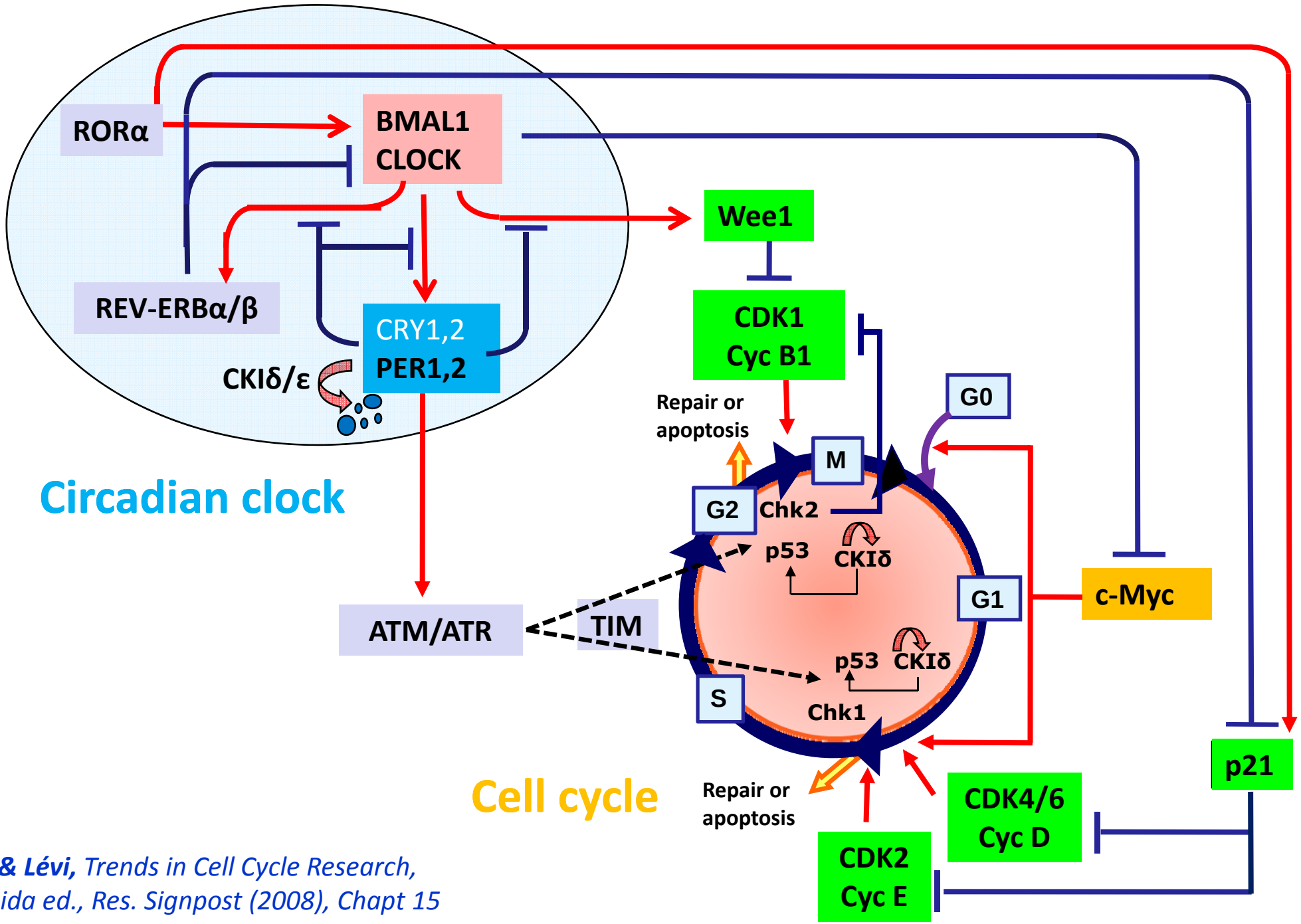
Day/night
Social
Familial
Meals



Circadian clocks in peripheral organs

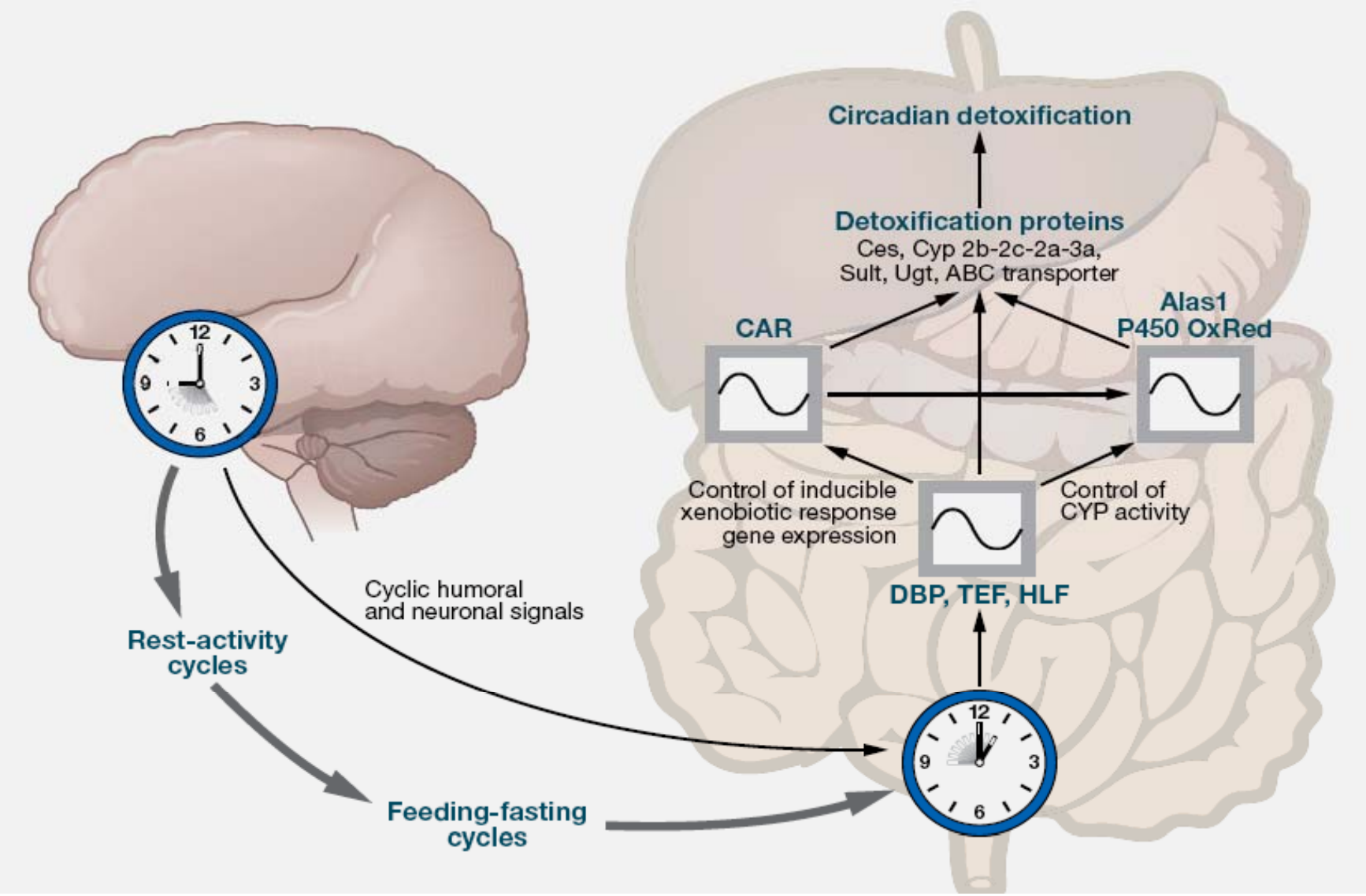
Molecular circadian clocks

- 12 specific clock genes
- transcription/translation feedback loops
- all mammalian cells
- controls
  - cell division cycle, DNA repair, apoptosis
  - metabolism, drug detoxification



Okyar & Lévi, *Trends in Cell Cycle Research,*
K. Yoshida ed., Res. Signpost (2008), Chapt 15

Coordination of detoxification by the circadian timing system



Lévi & Schibler, Annu Rev Pharm Toxicol 2007

- **The circadian timing system**

  (circadian biomarkers and molecular clocks)

- **Circadian disruption**

  (cancer processes and treatments)

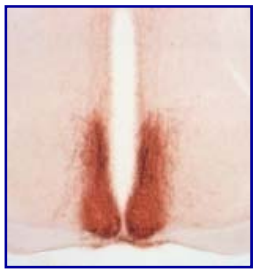
- **Circadian induction**

  (cancer and host clocks)

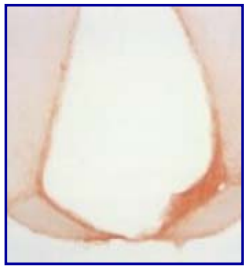
- **Conclusions**

Circadian disruption in mice

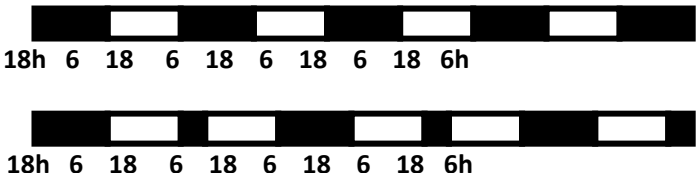
Model



Intact SCN

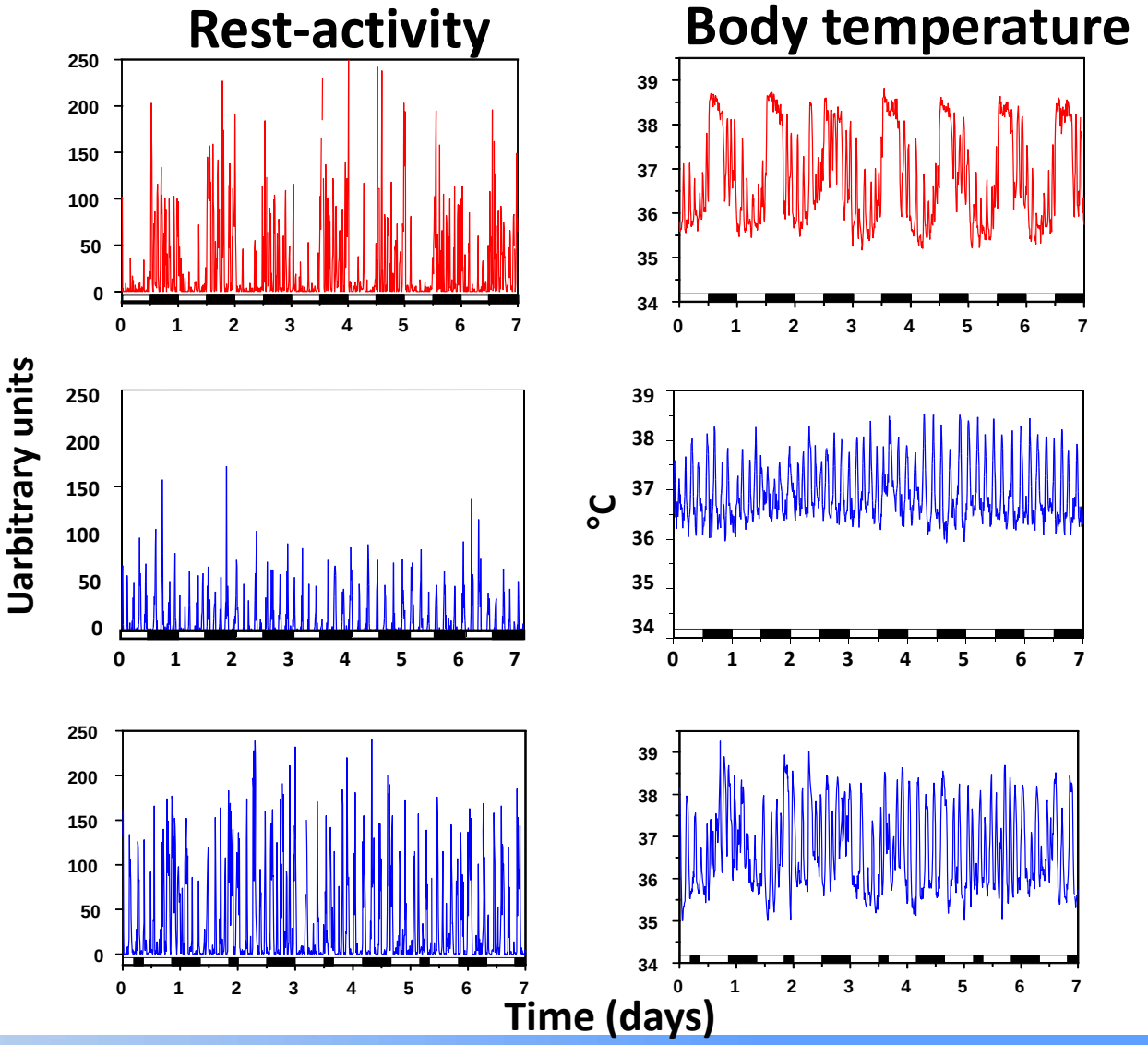


Ablated SCN



Chronic jet lag

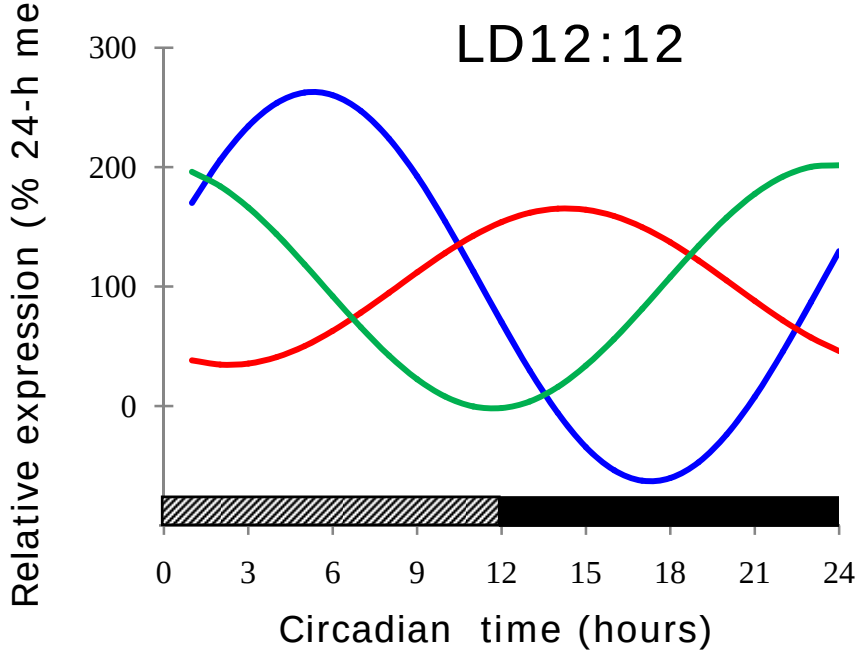
Biomarkers



Circadian disruption in mice

24-h cosine model of liver molecular clock

Rev-erbα → Per2 → Bmal1

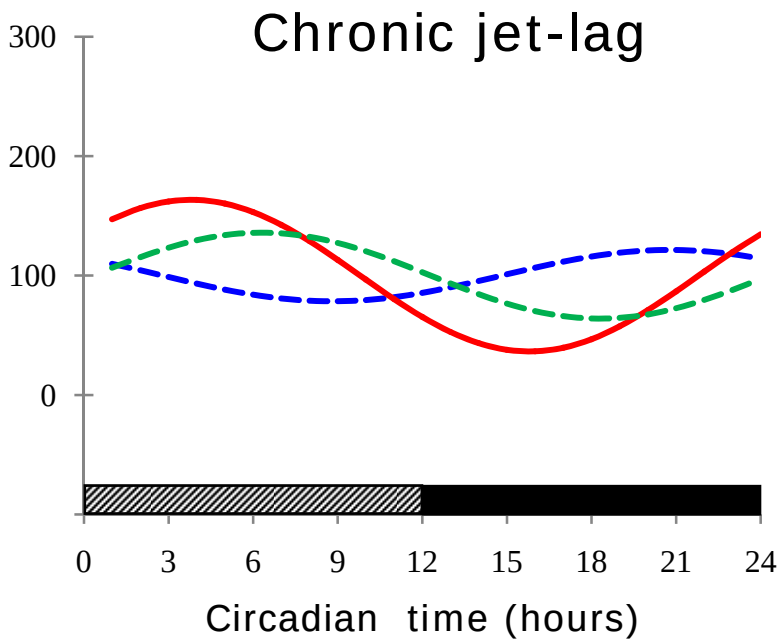


Acrophase

Reverbα 5^{20}

Per2 14^{20}

Bmal1 23^{40}



Acrophase

Reverbα ns

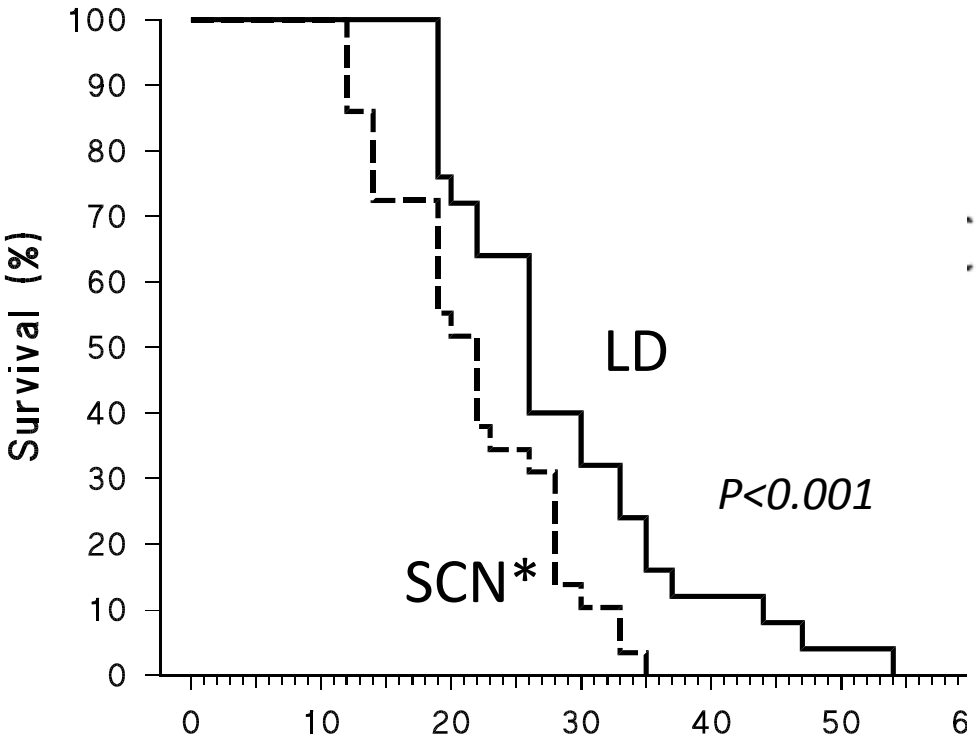
Per2 3^{50}

Bmal1 ns

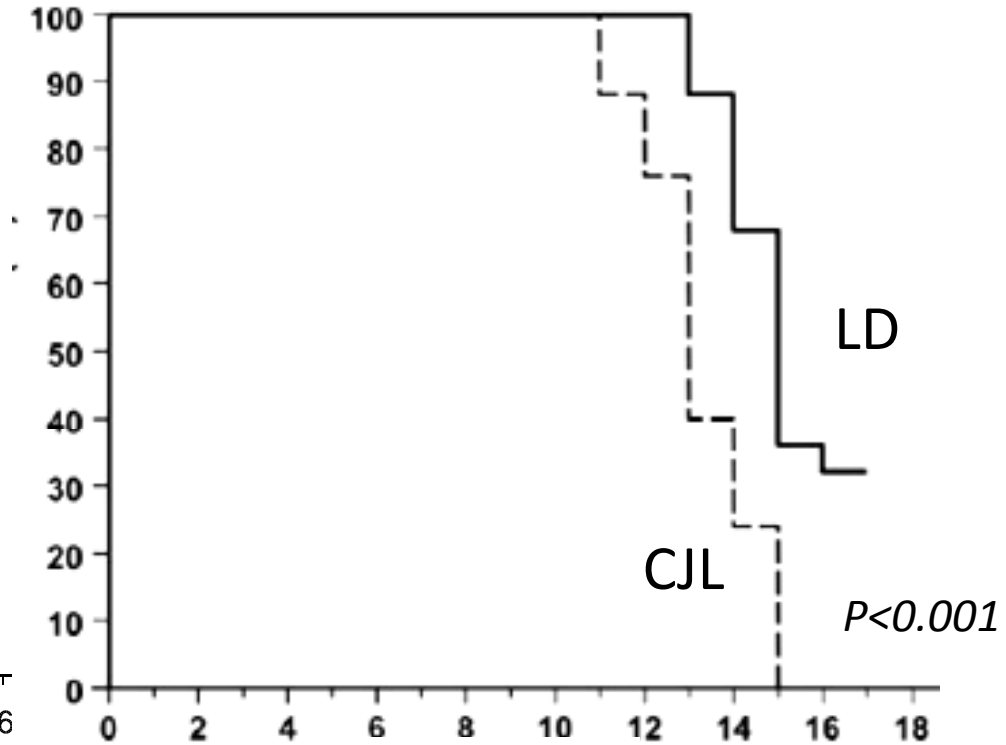
Circadian disruption on cancer progression in mice

Survival

SCN ablation vs sham



CJL vs LD12:12

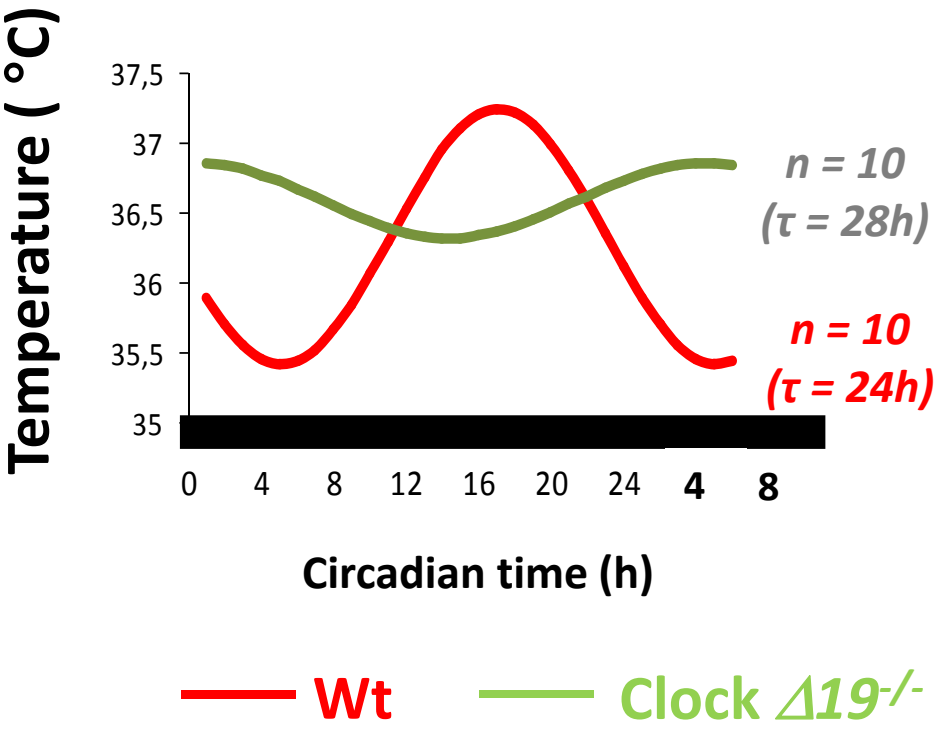


Time (days after tumor inoculation)

Circadian disruption : *Clock* gene mutation in DD

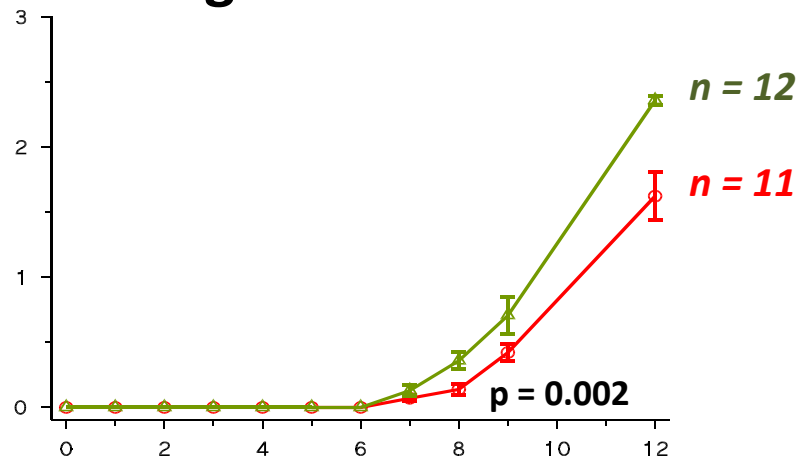
Circadian physiology (DD)

Body temperature curve

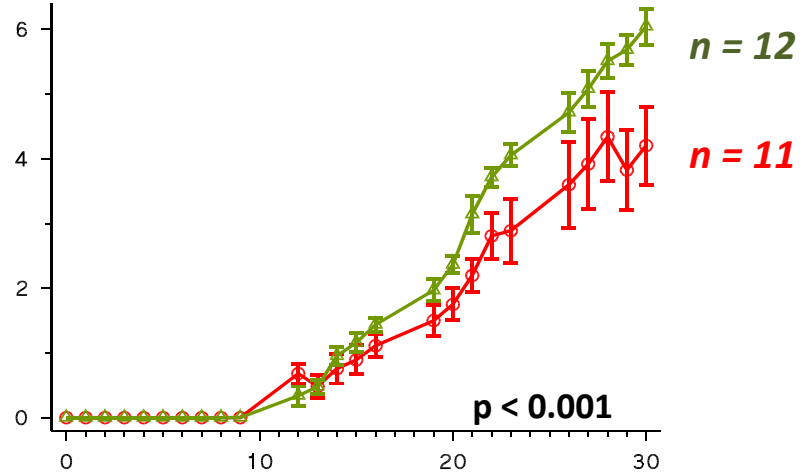


Tumor progression

Glasgow osteosarcoma



PO3 adenocarcinoma



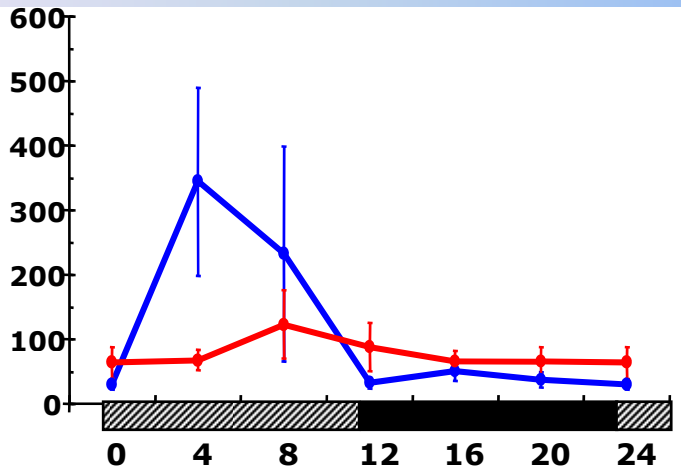
Tumor weight (g)

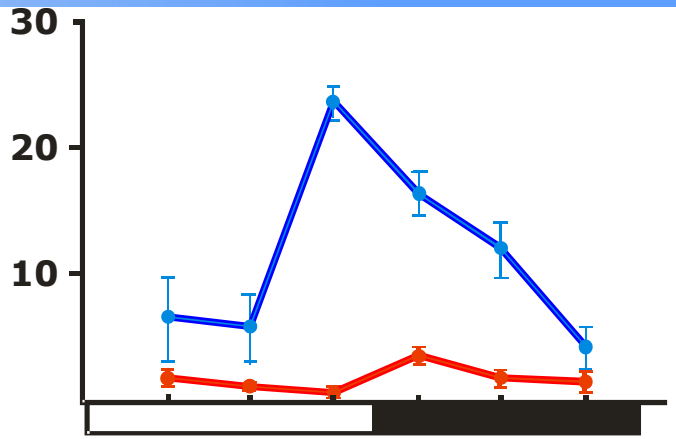
Time (days after tumor inoculation)

Li et al., Soumis

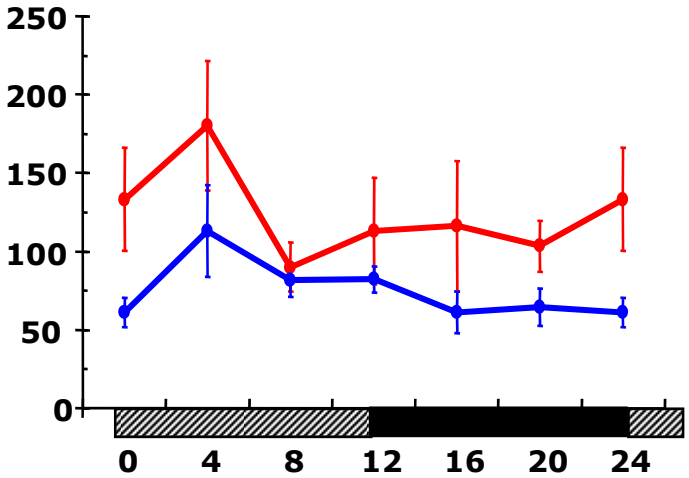
Circadian disruption: cell cycle genes in liver (mRNA)

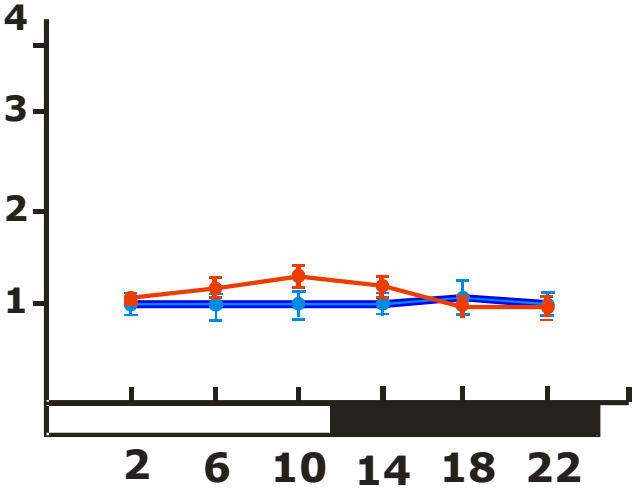
c-Myc





p53





LD12:12 DHC

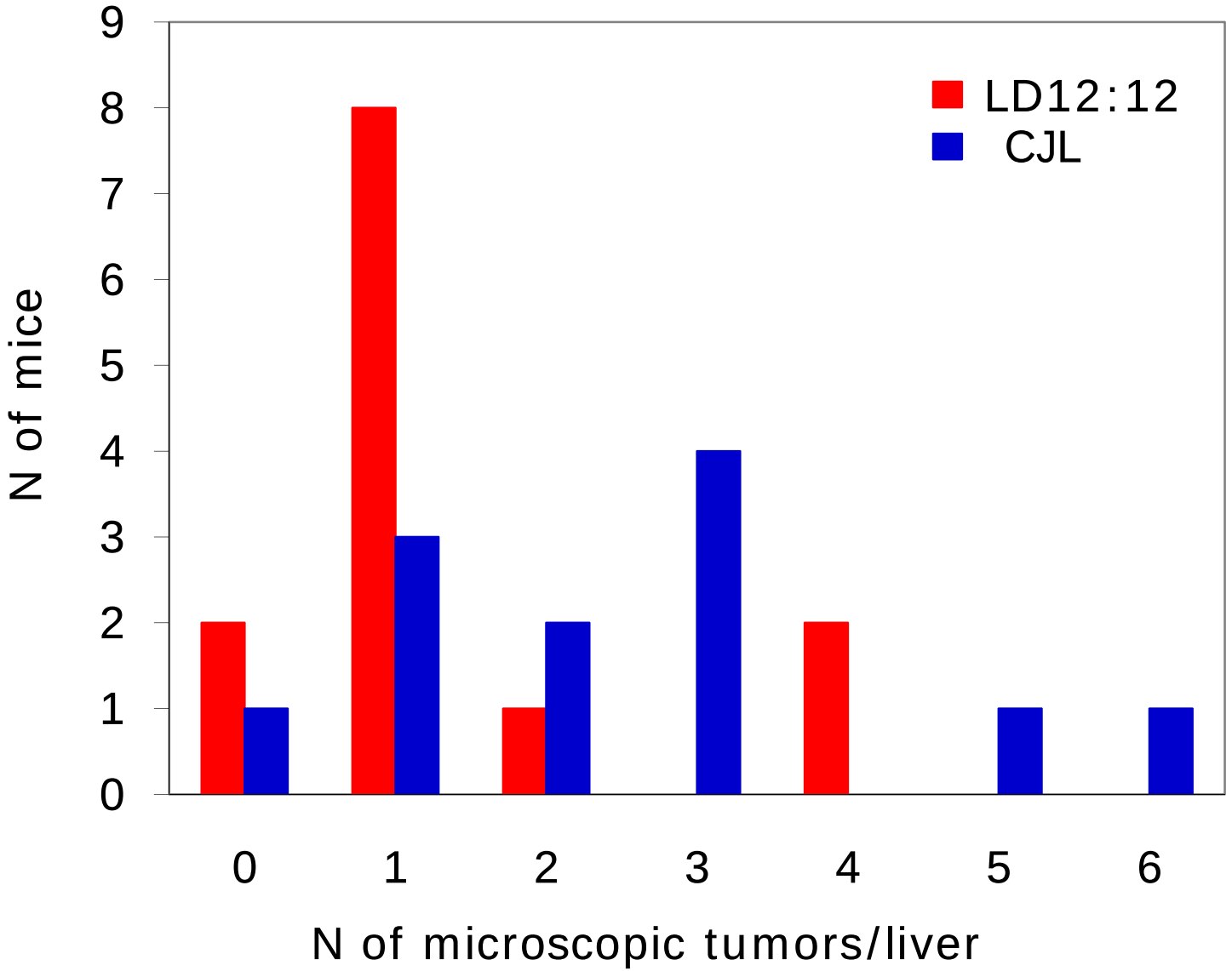
Wt mPer2$^{-/-}$

Filipski et al. J Natl Cancer Inst 2005

Fu et al. Cell 2002

http://rbc.vjf.inserm.fr

DEN hepatocarcinogenesis in mice on LD12:12 vs chronic jet lag



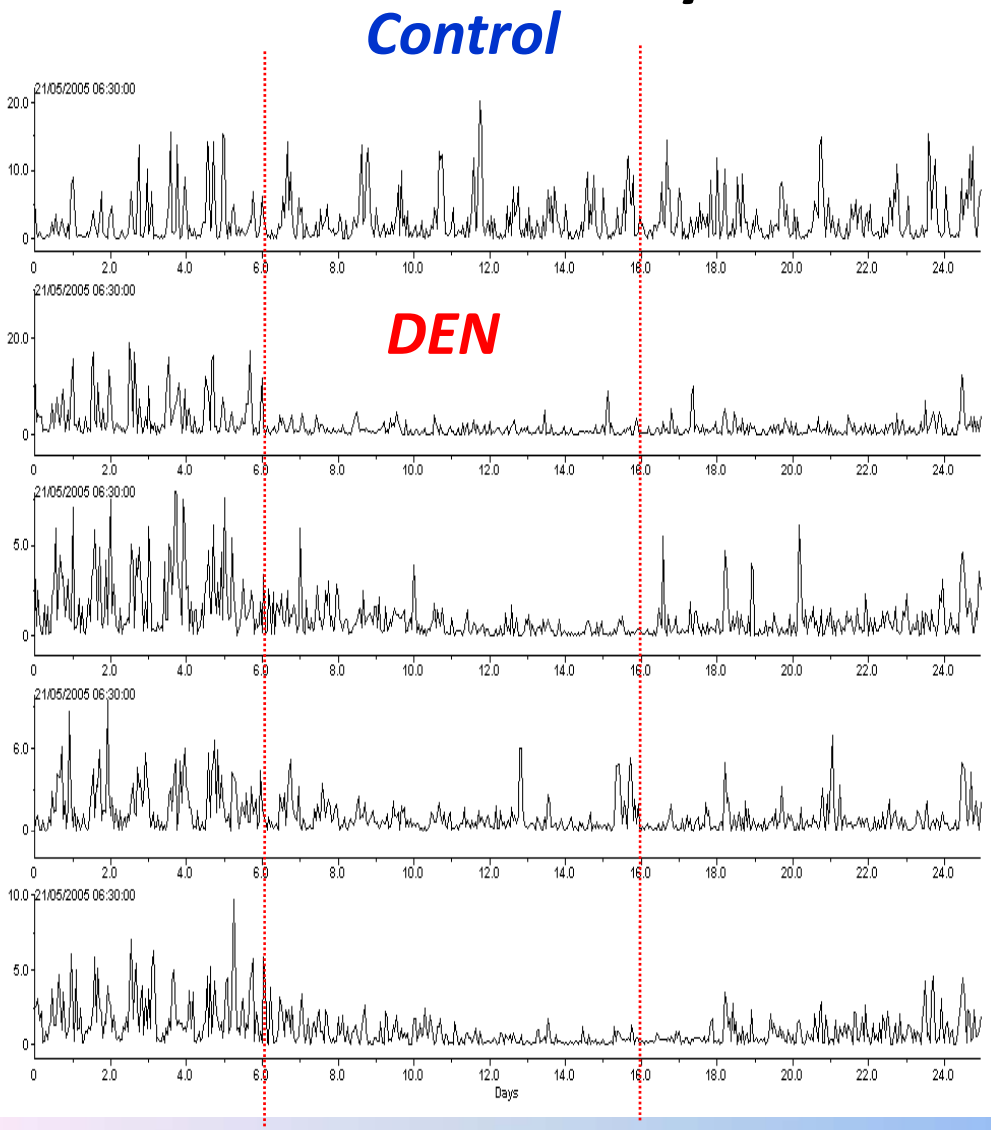
Mean diameter (largest tumor)

- LD12:12: 4 mm
- CJL : 8.5 mm

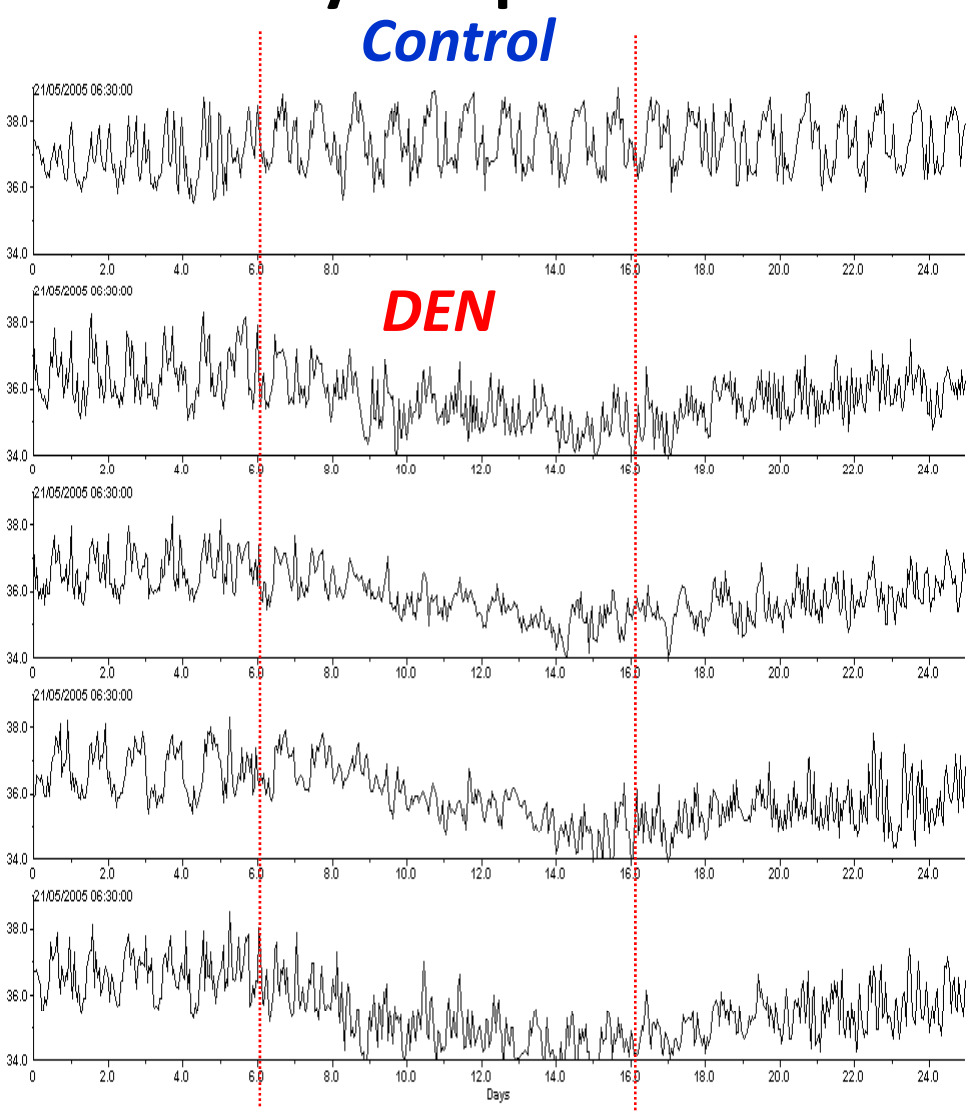
p=0.033

DEN effects on SCN biomarkers

Rest-activity



Body temperature



Relevance of experimental circadian disruption for malignant processes

SCN ablation
Chronic jet lag
Constant light
Per2, Clock mutations

Disruption of
- **circadian physiology**
- **host molecular clocks**

Genomic instability
Accelerated cell cycling

Accelerated cancer growth
Increased cancer incidence

Filipski et al. JNCI 2002, 2005; Cancer Res 2004; Fu et al. Cell 2002 ; Gery et al. Cancer Cell 2006

Breast, colorectal or prostate cancers: increased risk in shift workers

ELSEVIER

European Journal of Cancer 41 (2005) 2023–2032

European Journal of Cancer

www.ejconline.com

Night work and breast cancer risk: A systematic review and meta-analysis

Sarah P. Megdal [a], Candyce H. Kroenke [b,c], Francine Laden [b,c,d], Eero Pukkala [e], Eva S. Schernhammer [b,c,f,*]

- **13 studies (7 of airline cabin crew , 6 other night shift workers**
- **Aggregate estimated risk: 1.48 (95% CI, 1.36–1.61)**
- **Female airline cabin crew: SIR: 1.44 (95% CI, 1.26–1.65)**
- **Female night workers : RR: 1.51 (95% CI, 1.36–1.68)**

"Studies on night shift work and breast cancer risk collectively show an increased breast cancer risk among women. Publication bias is unlikely to have influenced the results."

Carcinogenicity of shift-work, painting, and fire-fighting

Kurt Straif, Robert Baan, Yann Grosse, Béatrice Secretan, Fatiha El Ghissassi, Véronique Bouvard, Andrea Altieri, Lamia Benbrahim-Tallaa, Vincent Cogliano, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group

…About 15–20% of the working population in Europe and the USA is engaged in shift-work that involves night work, which is most prevalent (above 30%) in the health-care, industrial manufacturing, mining, transport, communication, leisure, and hospitality sectors.

Among the many different patterns of shift-work, those including nightwork are the most disruptive for the circadian clock….

…the Working Group concluded that "shift-work that involves circadian disruption is probably carcinogenic to humans" (Risk level 2A)

http://oncology.thelancet.com Vol 8 December 2007

http://monographs.iarc.fr/

Performance status (WHO)

0	Able to carry out normal activity without restriction
I	Restricted in physically strenuous activity but ambulatory and able to do light work.
II	Ambulatory and capable of self-care but unable to carry out any work. Up and about >50% of waking hours
III	Capable of only limited self-care, confined to bed or chair > 50% of waking hours
IV	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

Circadian disruption in cancer patients

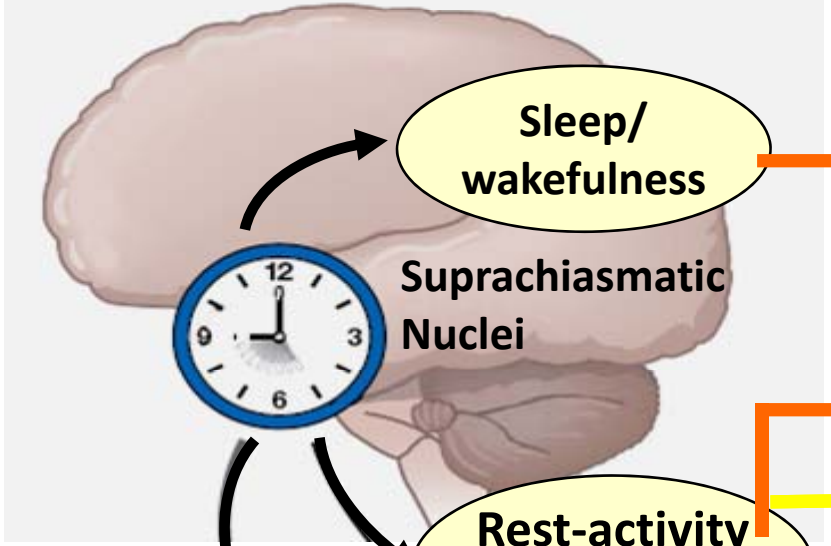
- **Performance status (PS) : the main prognostic variable of survival across all cancers.**
- **PS is based on a subjective rating of the patient's daily activities by the physician.**
- **Health-related Quality of Life through patient-rated questionnaires also appears as an independent prognostic factor of survival.**

Circadian disruption in cancer patients: relations with symptoms and quality of life

Environment

Day/night
Social
Familial
Meals

Circadian Timing system



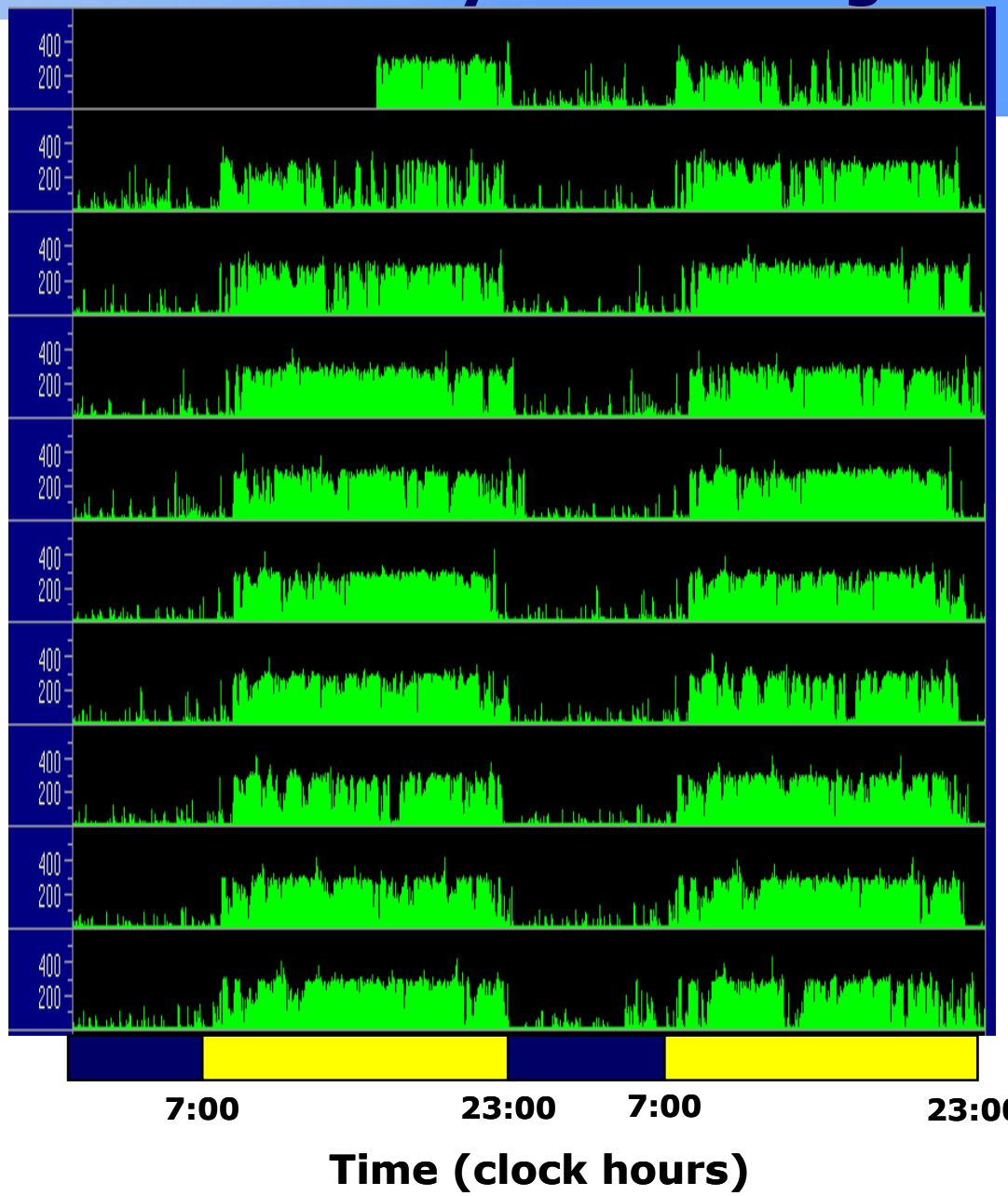
HR-QoL

Sleep disorders
Fatigue
PS
Anorexia

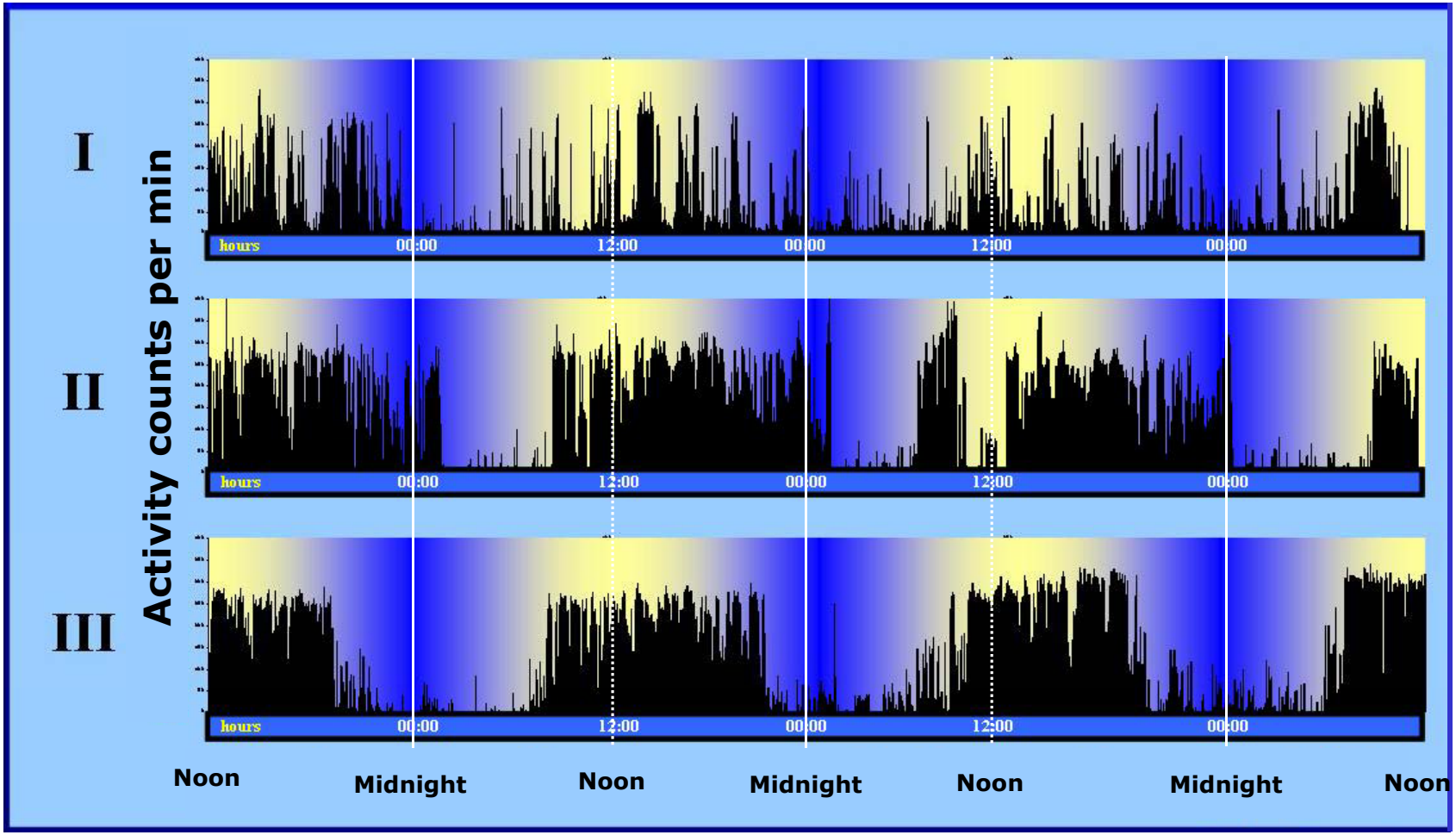
Circadian clocks in peripheral organs

Rest-activity monitoring

- Piezo-electric accelerometer
- Count of accelerations per min
- « Continuous » recording for days or weeks
- Zero-crossing vs PIM mode
- Quantified parameters (r24, I<O)



Variable interindividual patterns in rest-activity rhythm in cancer patients (metastatic colorectal cancer)



Relation between rest-activity cycle and QoL (EORTC)
192 patients with metastatic colorectal cancer

Table 3. Differences in Mean Quality of Life Scores According to Patients' $I < O$

	$I < O$ Quartile Group[a]				
QoL Parameter	1	2	3	4	p (Kruskal–Wallis)
Global QoL	53	54.2	63.2	68.4	0.001
Physical functioning	62.5	72.4	82.2	89.5	<0.0001
Social functioning	60.2	63	74.4	80.7	0.006
Fatigue	50.9	40.7	36	26.8	0.001
Appetite loss	37	24.6	18	11.7	0.01
Constipation	37.1	21.4	20.5	12.8	0.03
Pain	35.7	19.6	21.1	13.7	0.05
Depression	6.9	6.1	5	4	0.02

Only statistically validated differences appear in the table.

[a] $I < O$ quartile groups—1: $I < O < 25\%$ quartile, 2: $I < O > 25\%$ and $<50\%$ quartile, 3: $I < O > 50\%$ and $<75\%$ quartile, 4: $I < O > 75\%$ quartile.

British Journal of Cancer (2005), 1 – 7
© 2005 Cancer Research UK All rights reserved 0007 – 0920/05 $30.00
www.bjcancer.com

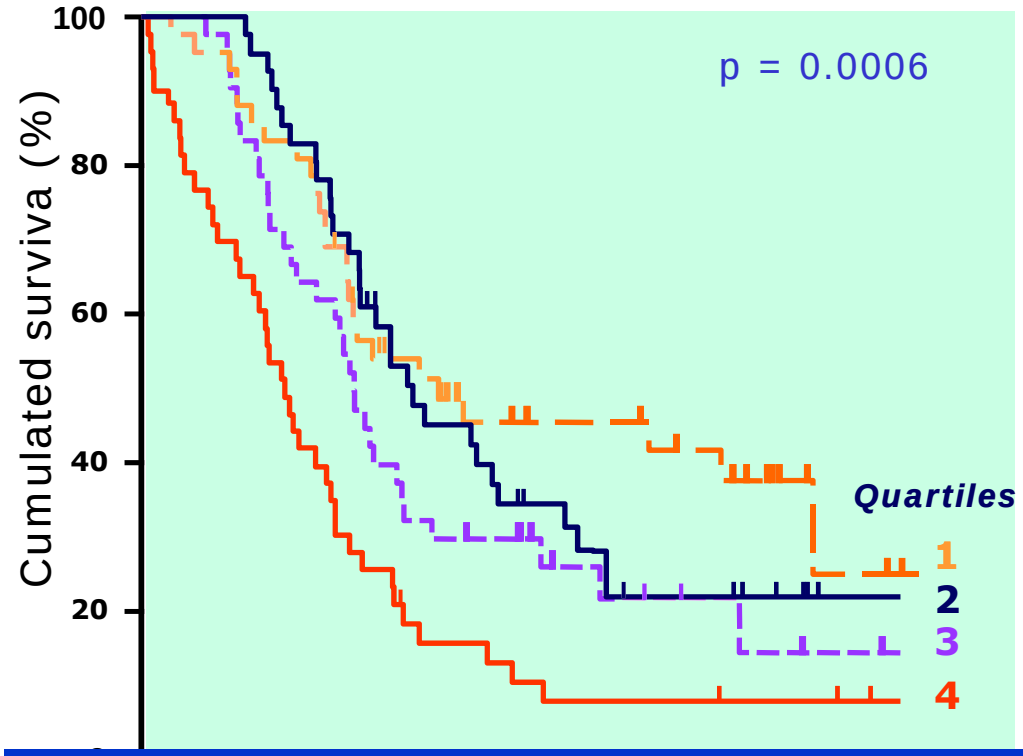
Full Paper

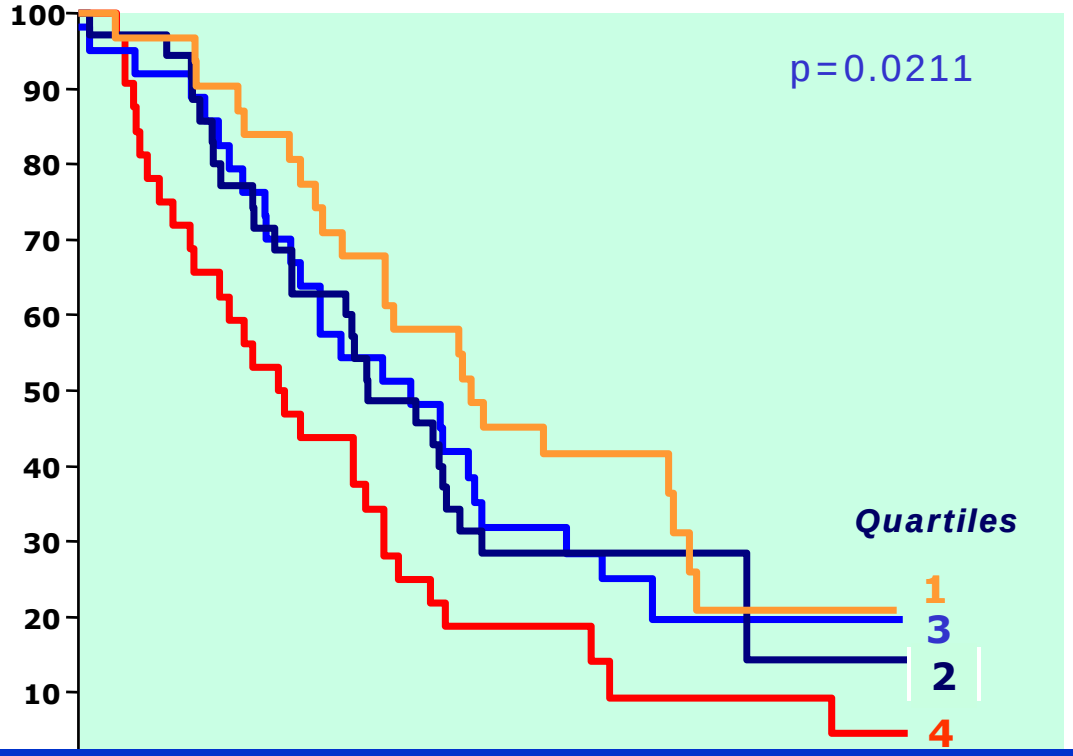
Circadian function in patients with advanced non-small-cell lung cancer

RD Levin[1], MA Daehler[1], JF Grutsch[1], J Quiton[2], CG Lis[*,1], C Peterson[1], D Gupta[1], K Watson[2], D Layer[2], S Huff-Adams[2], B Desai[2], P Sharma[2], M Wallam[2], M Delioukina[2], P Ball[2], M Bryant[2], M Ashford[2], D Copeland[2], M Ohmori[2], PA Wood[2] and WJM Hrushesky[2]

[1]Cancer Treatment Centers of America® (CTCA) at Midwestern Regional Medical Center, Zion, IL, USA; [2]WJB Dorn Veterans Affairs Medical Center, Columbia, SC, USA

24-h rest-activity rhythm, independent prognostic factor of survival in patients with metastatic colorectal cancer



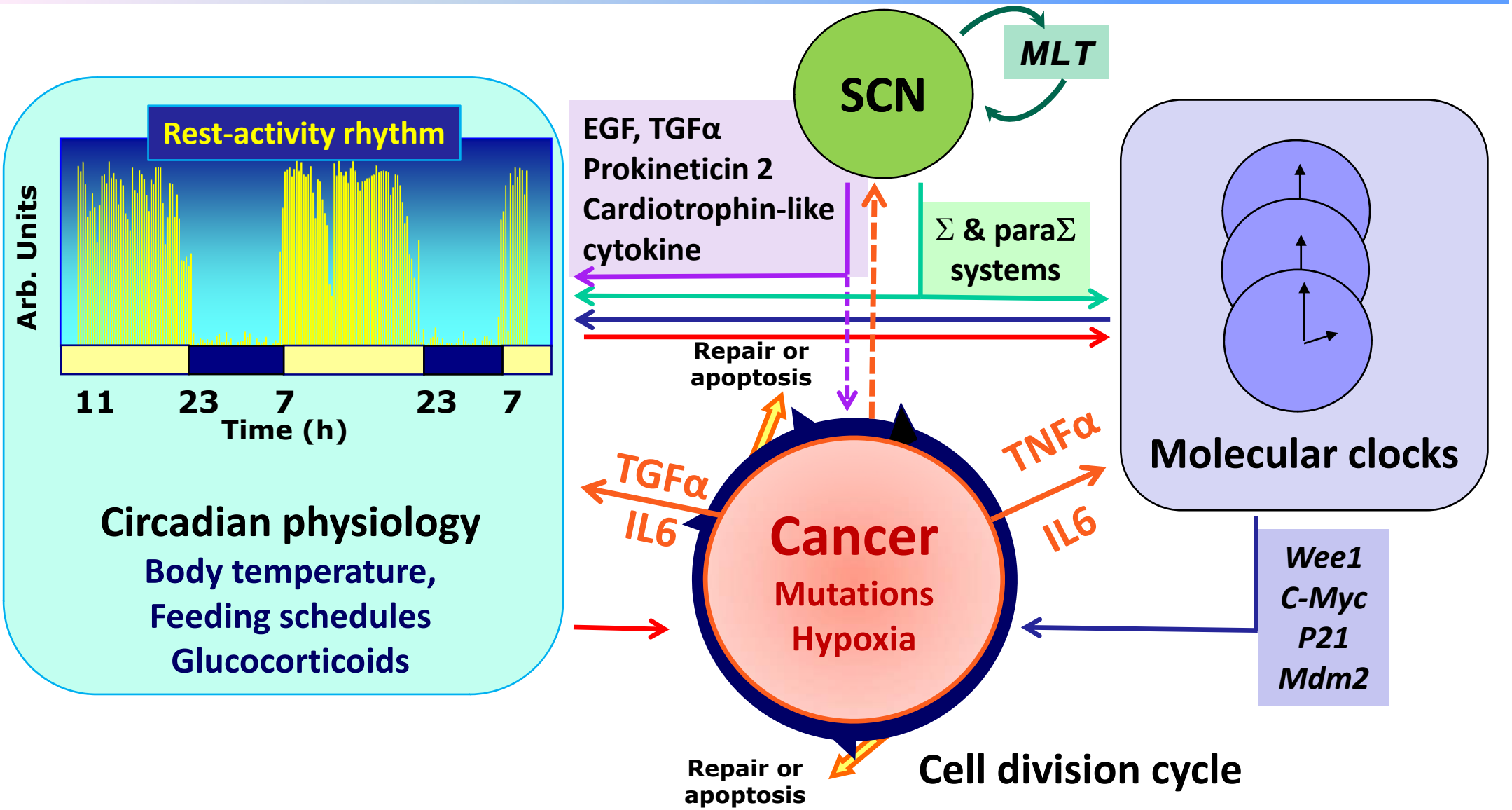


Relation between circadian rhythm and survival confirmed in advanced or metastatic lung, breast and GYN cancers and in metaanalysis in 500 pts with metastatic GI cancer

Mormont et al. Clin Cancer Res 2000; Rich et al . Clin Cancer Res 2005; Innominato et al. In prep.

http://rbc.vjf.inserm.fr

Potential mechanisms of cancer-associated circadian disruption

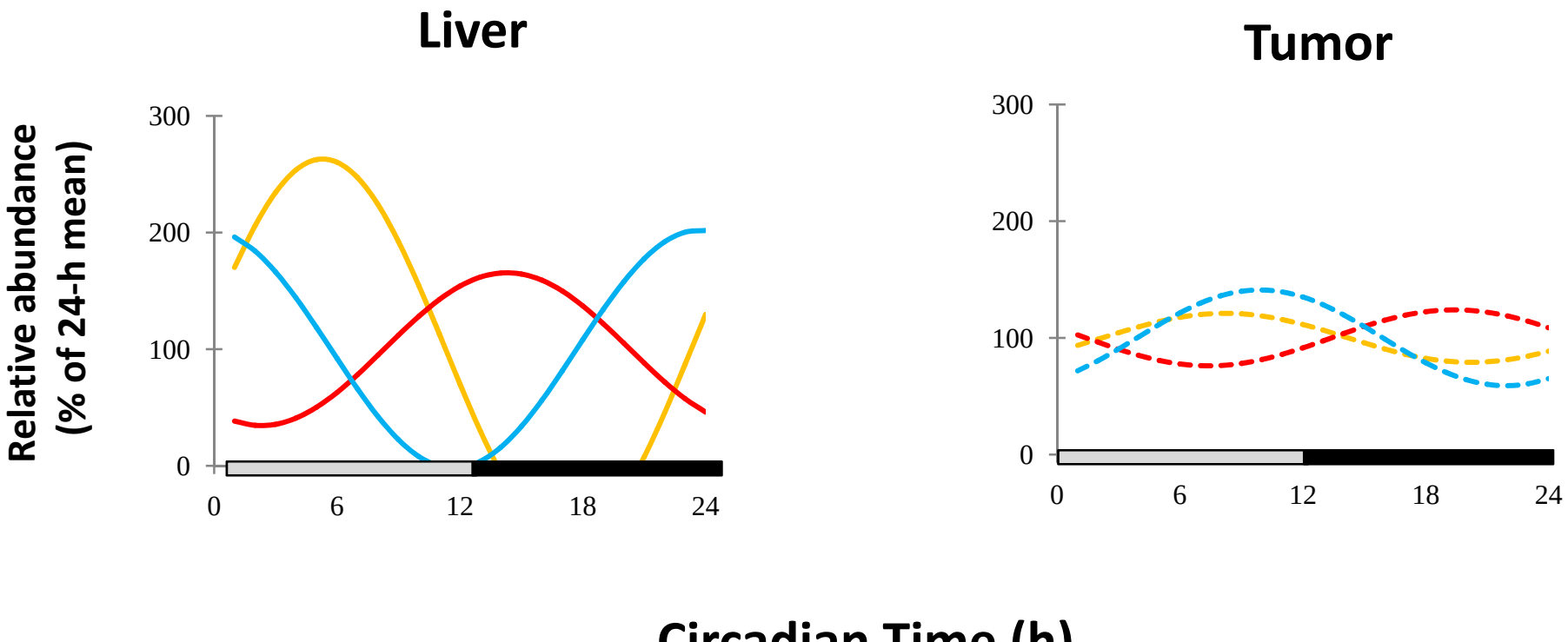


Levi et al. *Cold Spring Harb Symp Quant Biol. 2007, 72 : 465-475.*

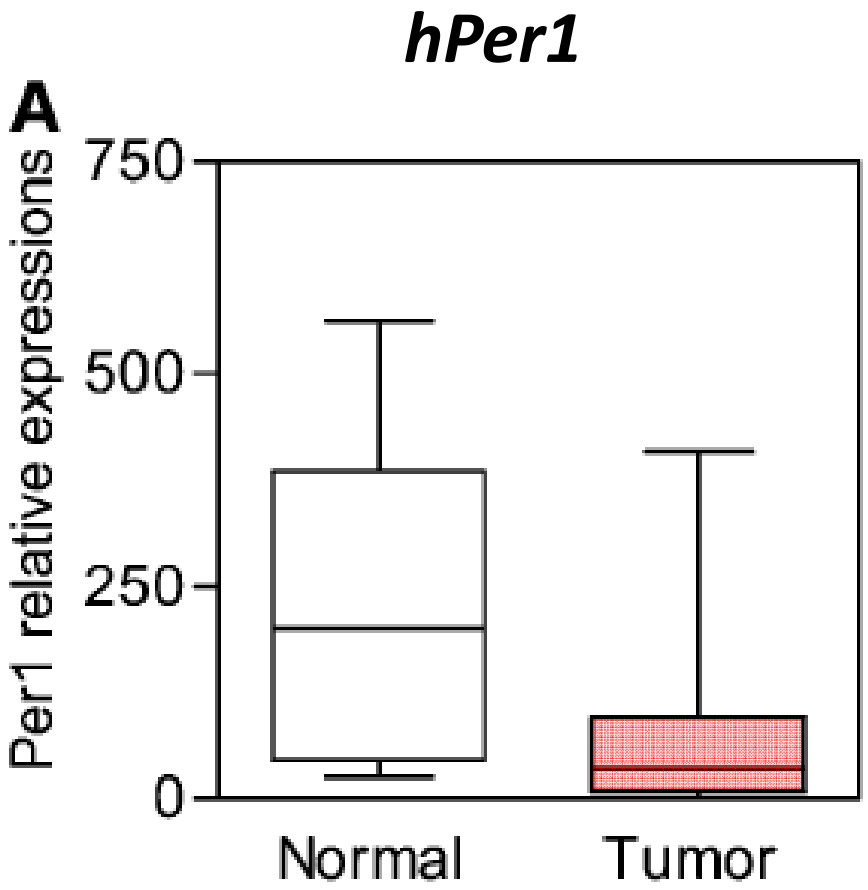
Circadian disruption in tumors

Transcriptional rhythms of clock genes

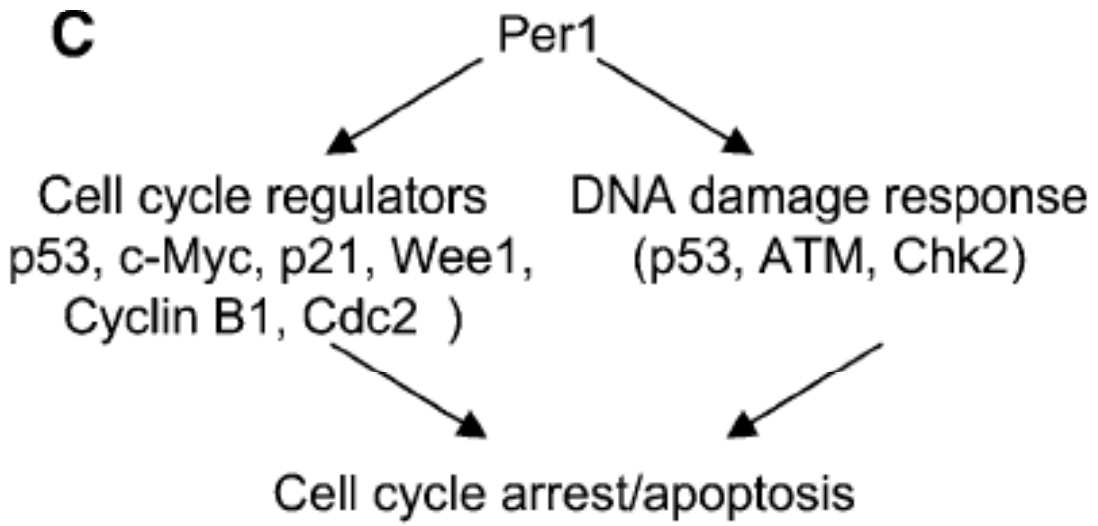
Rev-erbα, Per2 and Bmal1



Clock genes mRNA expression in human lung cancer



(N=33 patients)



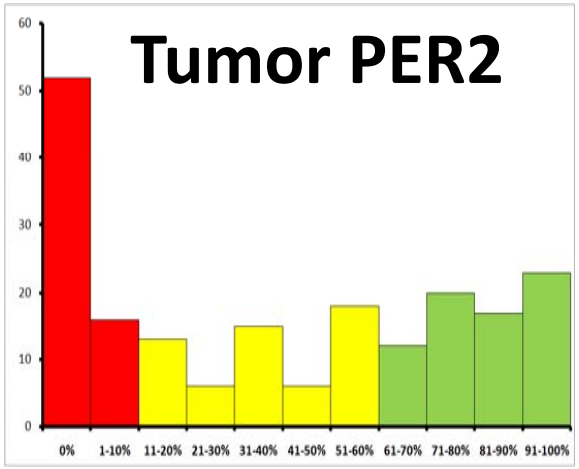
Clock genes expression in human cancers

Cancer type	Clock genes	mRNA expression	Ref.
Breast cancer	Per1	↓	[70]
Breast cancer*	Per1, Per2, Per3	↓	[89]
Familial-sporadic breast cancer	Per1, Per2	↓	[90]
Lung	Per1	↓	[5, 70]
Colon cancer	Per1	↓	[91]
Colon cancer	Per2 and Clock	= or ↓	[91]
Pancreatic cancer	Per1, Dec1	↓	[92]
Endometrial cancer	Per1	↓	[93, 94]
Myeloid leukemia			
chronic	Per1, Per2, Per3	↓	[95]
acute	Per2	↓	[96]
Non-Hodgkin lymphoma	NPas2	**	[97]

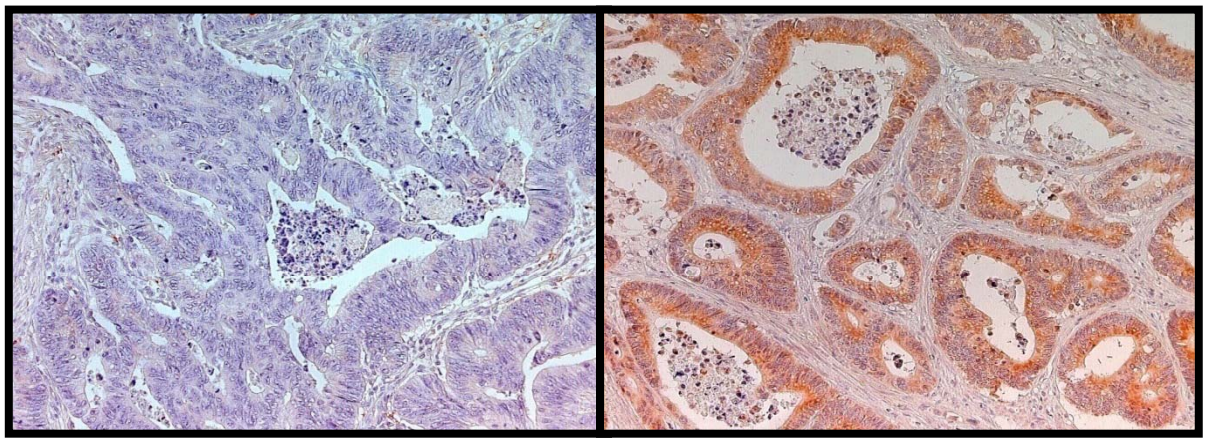
* Disturbances in the expression of the genes through promoter methylation in 95% of the specimens.
** Strong association between a functional polymorphism in the clock gene NPAS2 Ala 394 Thr and reduced risk for NHL.

PER2 protein expression in colorectal cancer

Patients with metastatic colorectal cancer
Multicenter trial
198 primary tumors before any chemo
IHC in triplicate (% labelled tumor cells)
Anti-PER1, anti-PER2, anti-PER3



0% → 100%



PER2 expression

16.7

17.8

19.5

P=0.013

Median survival (months)

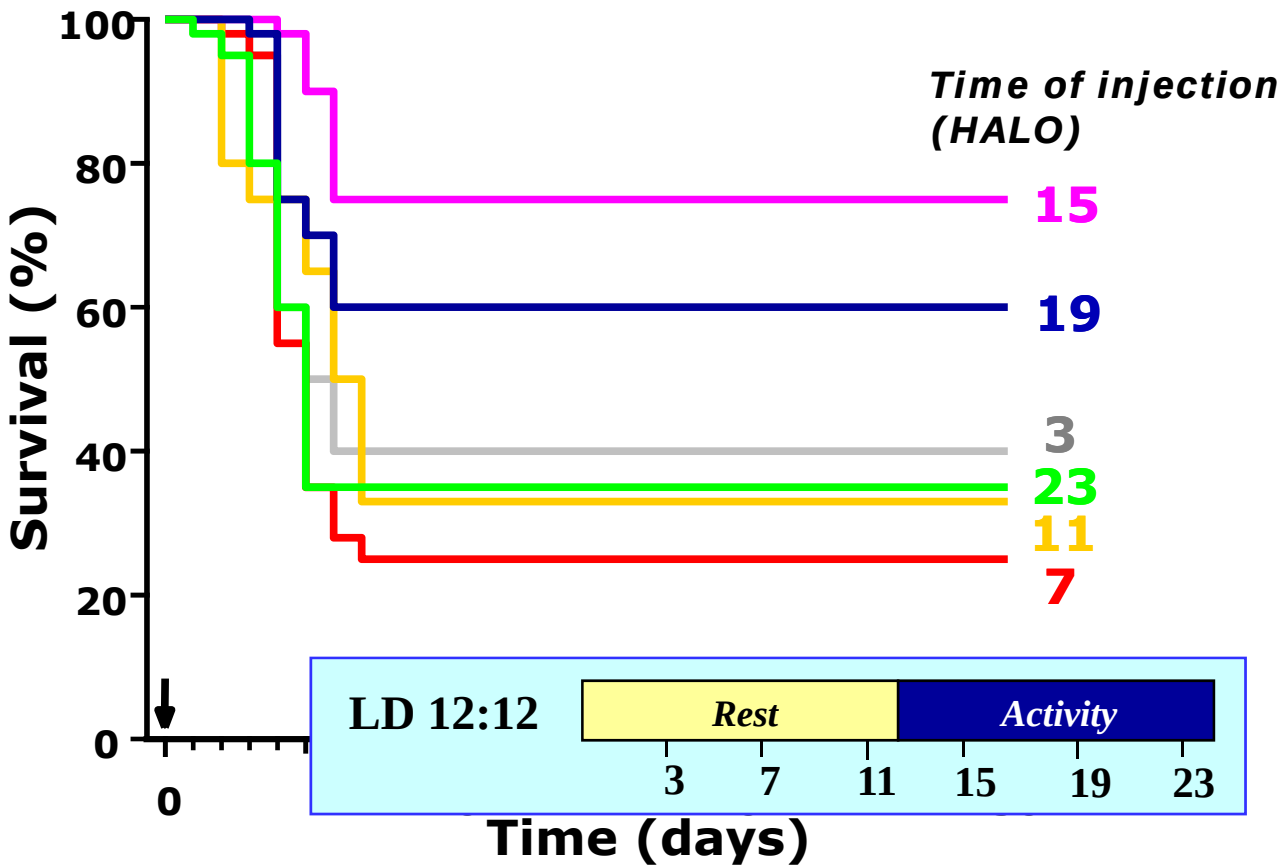
Iacobelli et al. Proc ASCO 2008

- **Toxicities and efficacy of anticancer treatments can be significantly reduced with appropriate timing**

- **Chronotherapeutics consist in the adaptation of anticancer drug delivery to circadian rhythms**
  - Timing
  - Chronomodulated delivery

Dosing time dependencies in experimental models

Oxaliplatin in B6D2F1 mice



Tolerability

42 anticancer drugs (all classes)

Efficacy

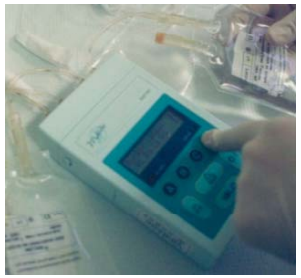
19 anticancer drugs

Best time

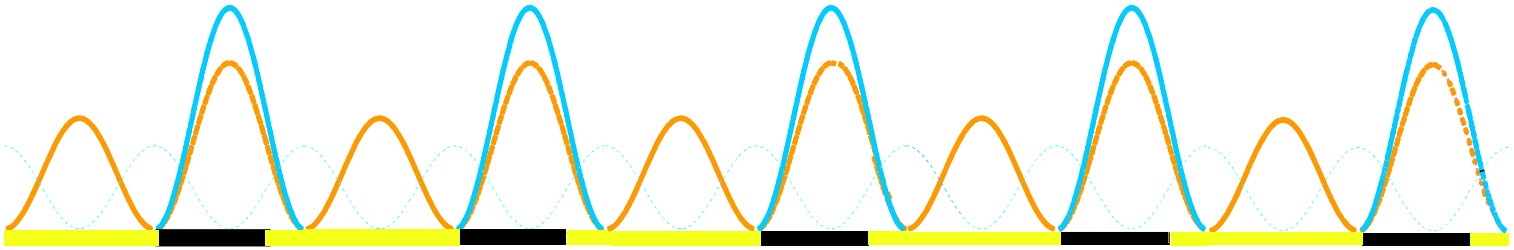
Similar for 90%

Chronomodulated chemotherapy

Dosing time dependency in
5-FU & oxaliplatin toxicities & efficacy



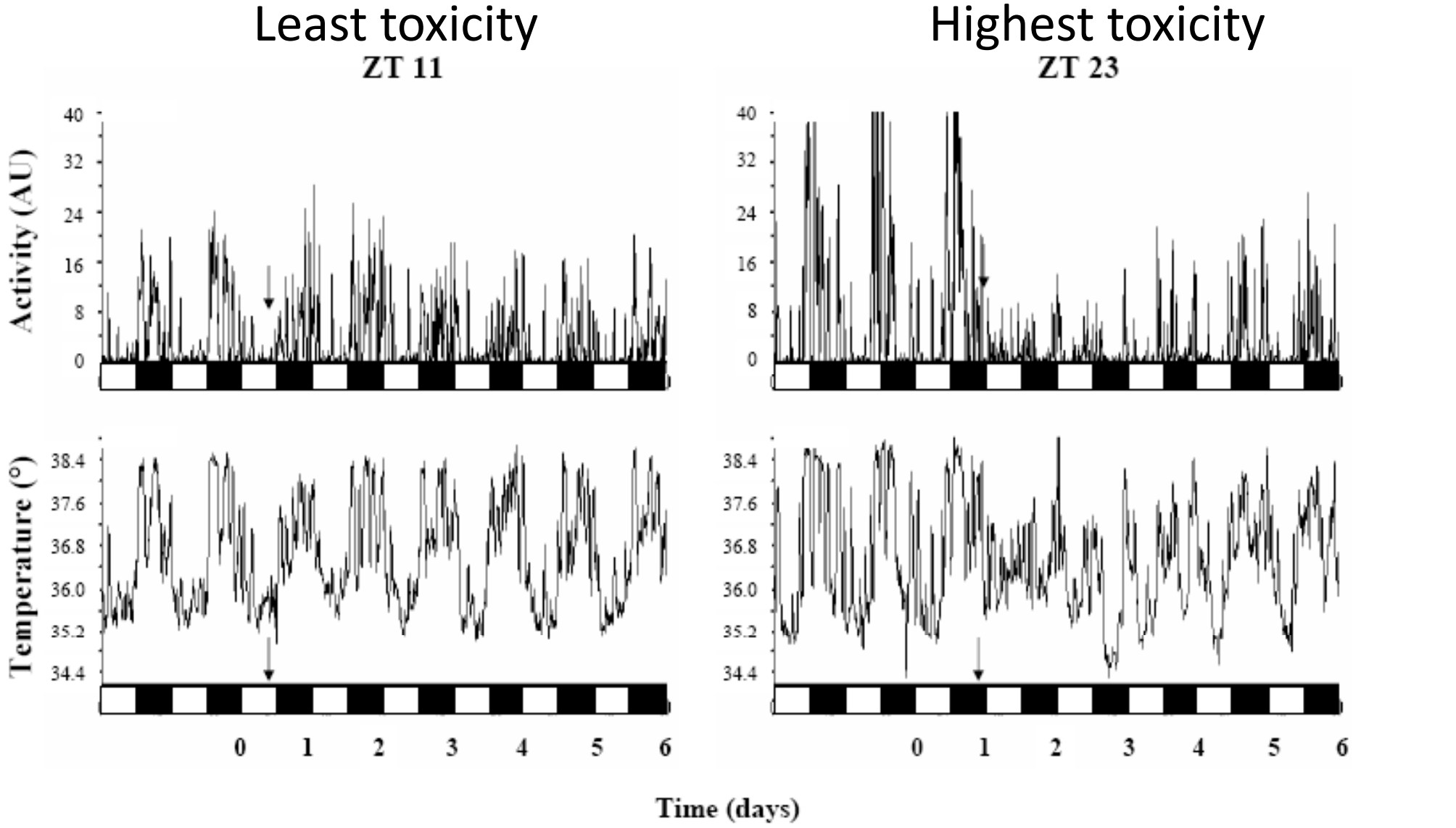
Chronomodulated 5-FU-LV-oxaliplatin (ChronoFLO)



	vs constant rate	*vs* opposite chronoFLO
	278 pts (no prior chemo)	114 pts (prior chemo)
Severe toxicity (gr 3-4)	***14% vs 76%***	***16% vs 80%***
Major tumor responses	***51% vs 30%***	***30% vs 12%***

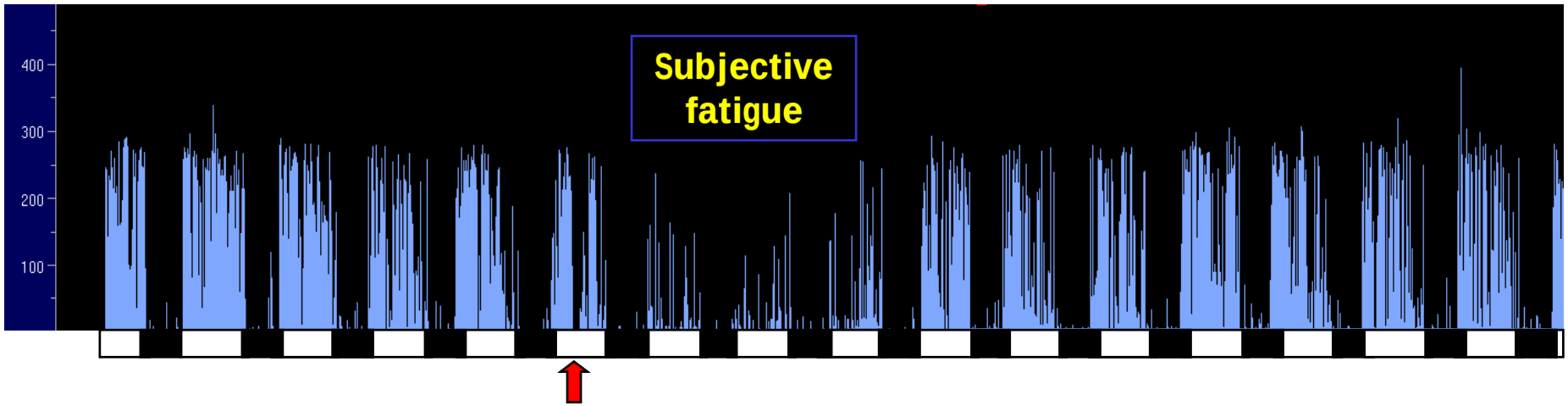
Lévi et al. Cancer 1992; JNCI 1994; Lancet 1997; Adv Drug Deliv Rev 2007; Phil Trans Royal Soc [A] 2008

Gemcitabine-induced circadian disruption according to drug timing (mice)

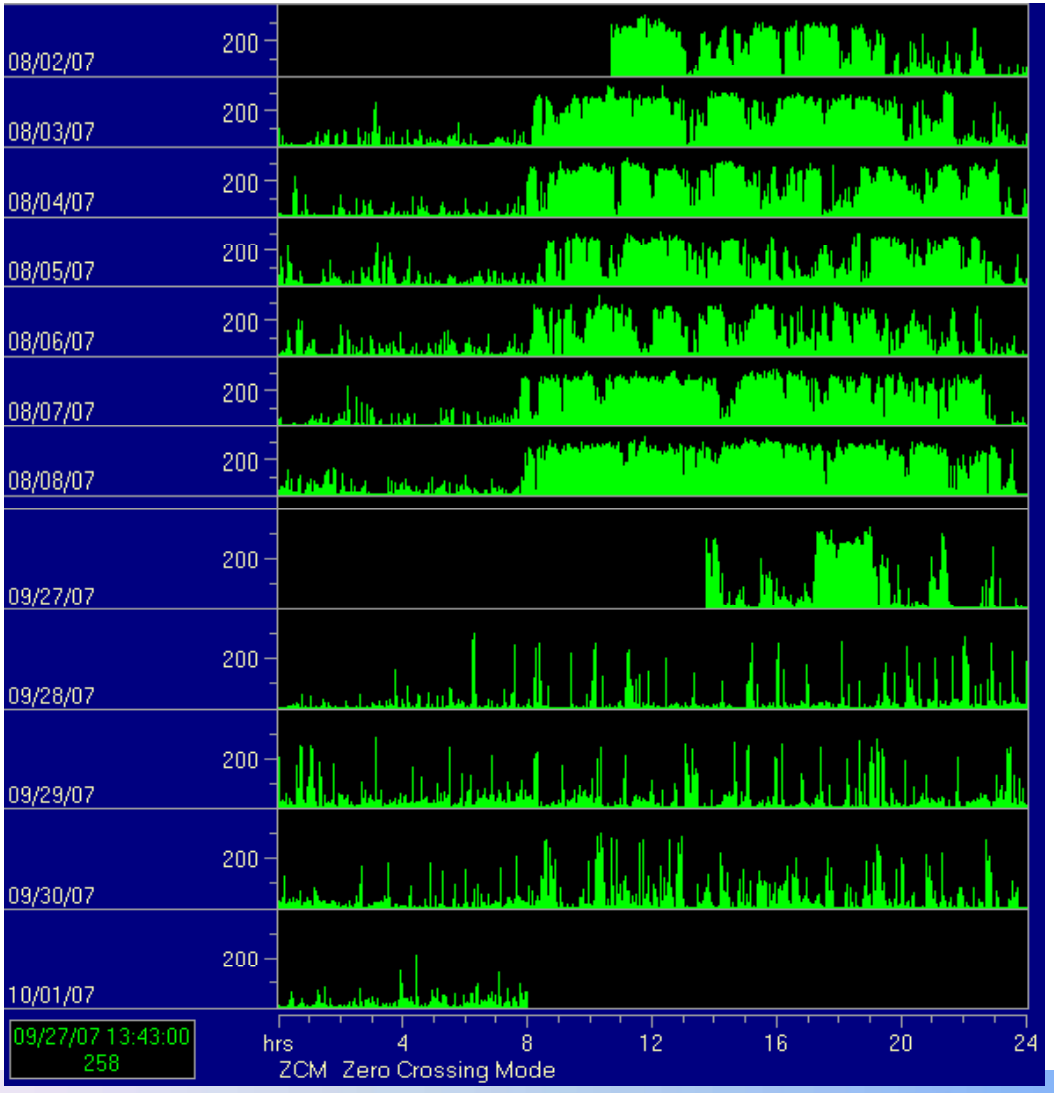


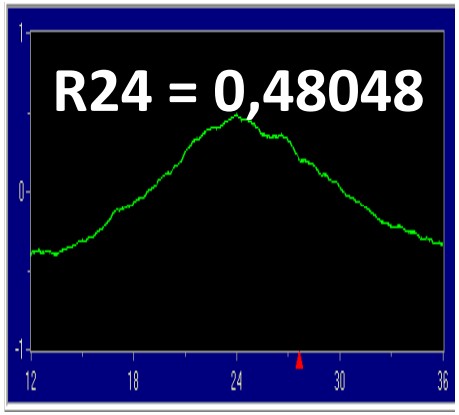
Li et al. J Biol Rhythms 2007

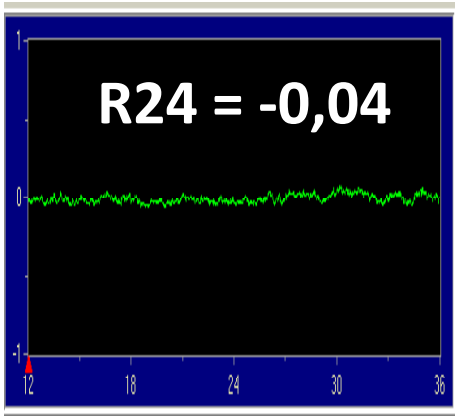
Circadian disruption (rest-activity rhyhtm) on docetaxel chemotherapy



Circadian disruption (rest-activity rhythm) on irinotecan chemotherapy (cancer patient)

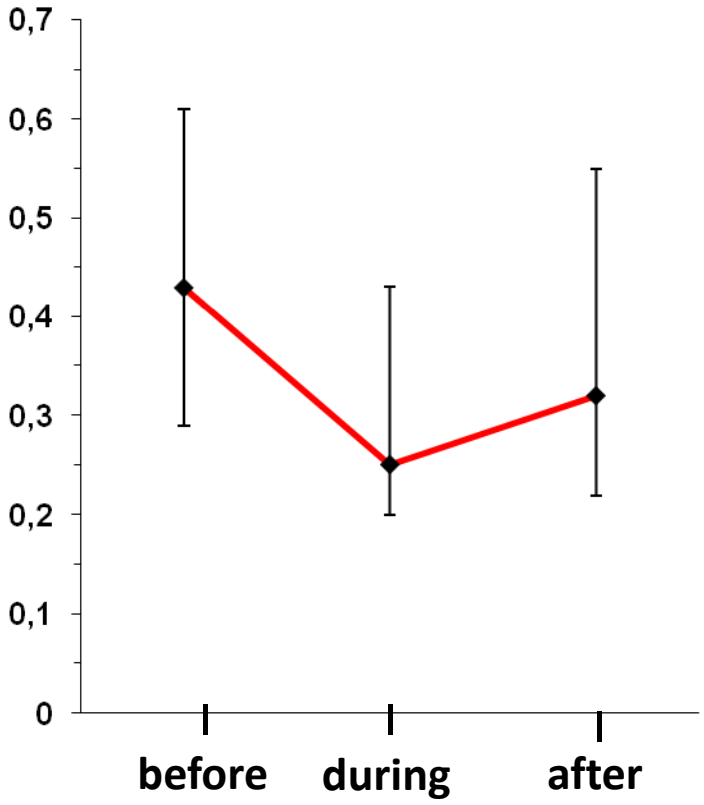




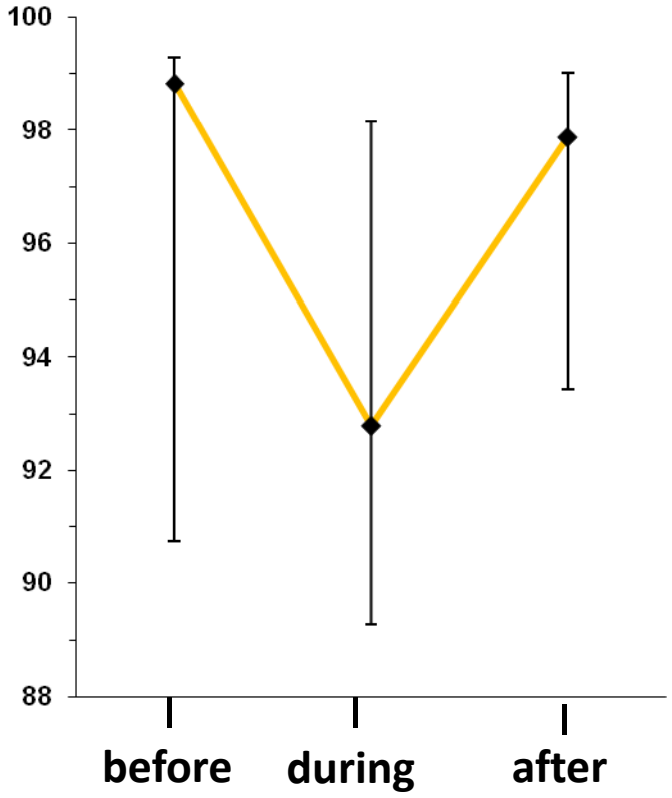


Circadian disruption on chemotherapy (rest-activity rhythm in 20 cancer patients)

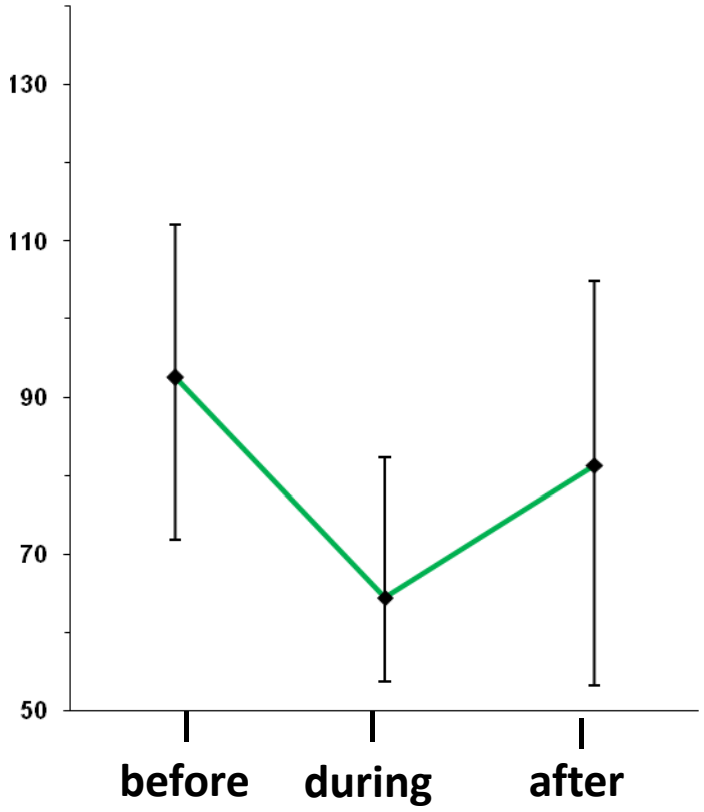
Autocorrelation r24



Dichotomy I<O



Circadian amplitude



Median and 25th - 75th quartile of the parameters distributions;
p from Wilcoxon for treatment effect <0.001 for each circadian parameter

Iurisci et al. In preparation

- **The circadian timing system**

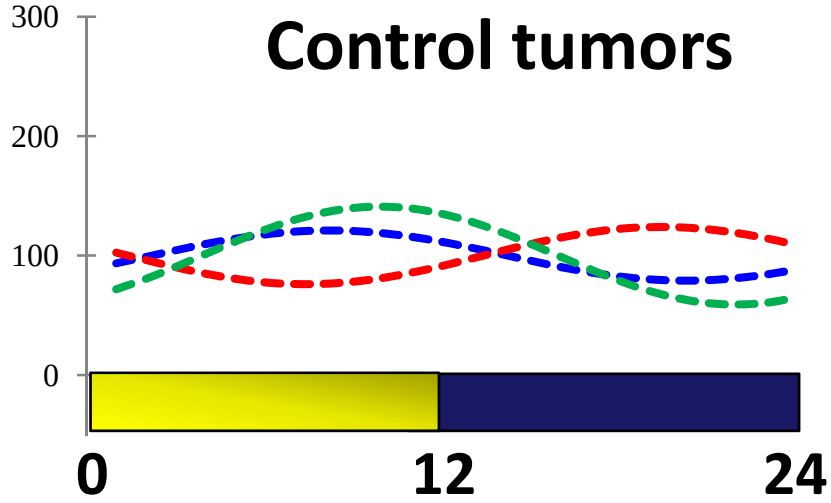
  (circadian biomarkers and molecular clocks)
- **Circadian disruption**

  (cancer processes and treatments)
- **Circadian induction**

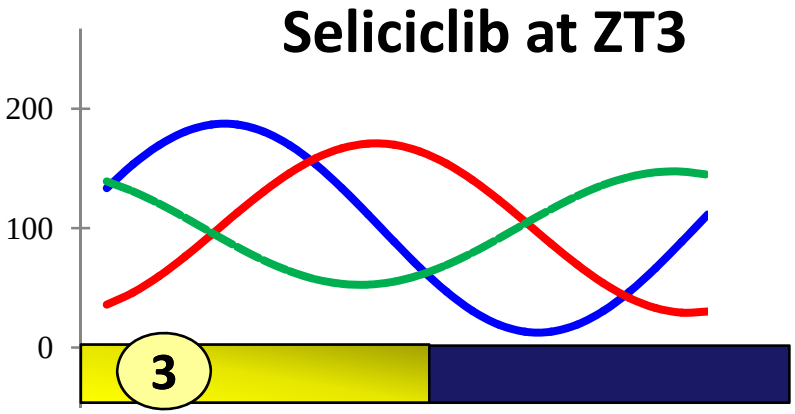
  (cancer and host clocks)
- **Conclusions**

Seliciclib induction of molecular clock in tumor (GOS)

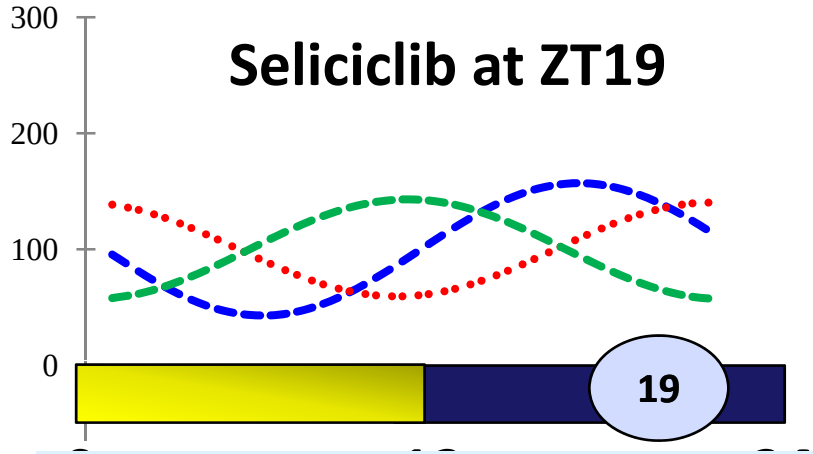
Reverbα → Per2 → Bmal1



In GOS tumor,
Seliciclib inhibits
activities of
CDK1, CDK2, CDK7,
CDK9, cyclin H & CKIδ/ε



60% tumor inhibition

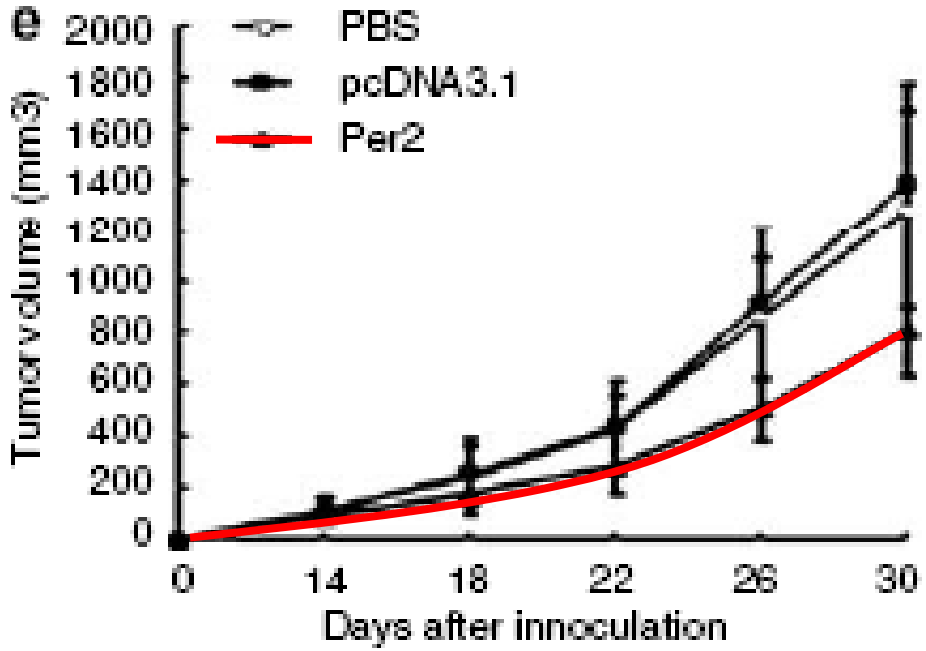


30% tumor inhibition

Circadian time (h)

Per2 gene delivery as a novel therapeutic intervention for treatment of cancer

Intratumor delivery of *Per2* had significant antitumor effects in C57BL/6 mice transplanted with Lewis lung carcinoma. It inhibited PCNA expression and induced apoptosis. (*Hua et al Cancer Gene Ther, 2007 June 22*).



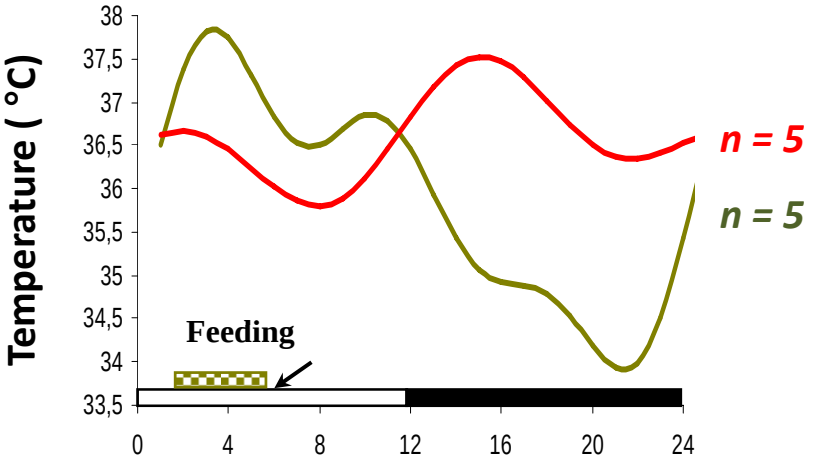
$p<0.01$

Re-inforcement of circadian timing system (meal timing at light –MTL)

Circadian Timing system

Physiology

Body temperature curve

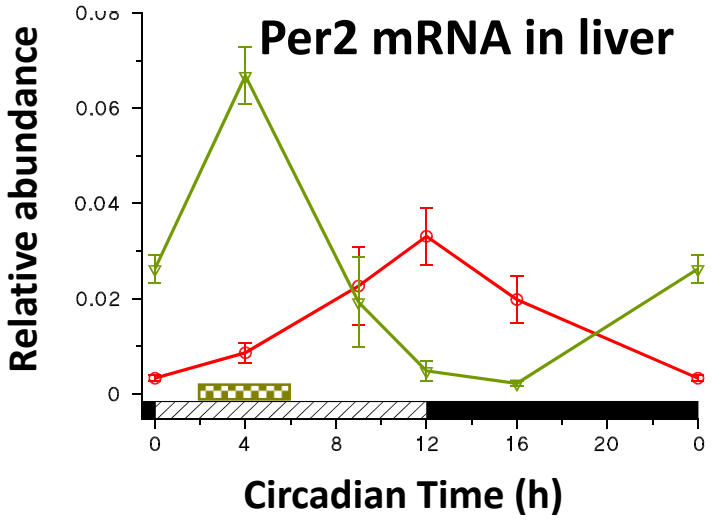


Ad lib

MTL

Molecular clock

Per2 mRNA in liver

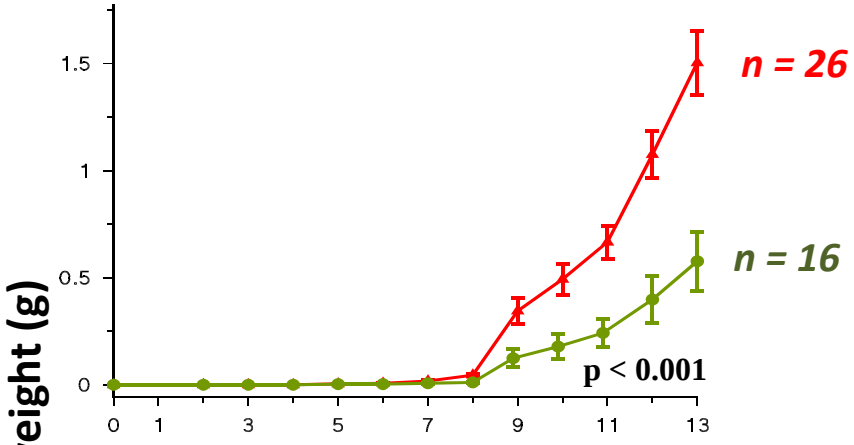


n = 2-4/point

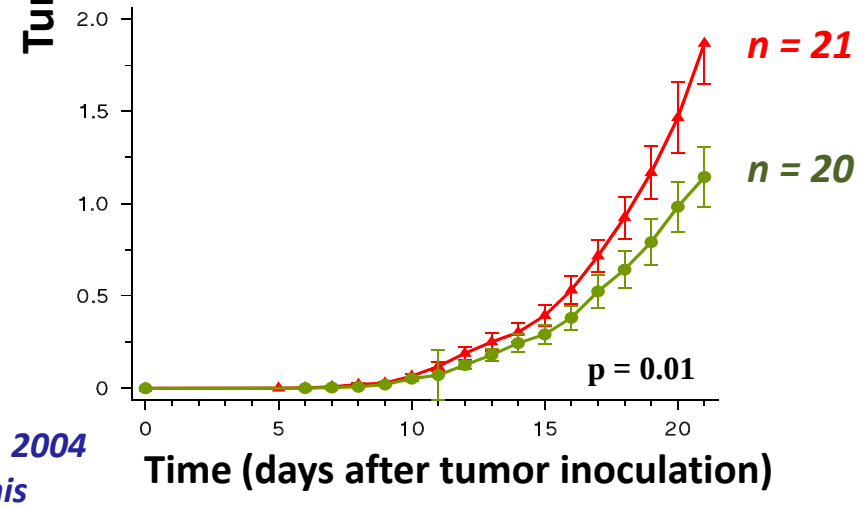
Wu et al., Life Sci;. 2004
Li et al.. Soumis

Tumor progression

Glasgow osteosarcoma



PO3 adenocarcinoma



Pharmacologic treatment of circadian disruption

Morning glucocorticoids in NSCLC patients

Parameters	Corticotherapy (6)		No corticotherapy (17)		*p*
MOY	104.8	± 25.5	>	93.8 ± 38.2	0.26
R24	**0.388**	**± 0.181**	**>**	**0.287 ± 0.149**	**0.089**
MOI	**91.77**	**± 6.45**	**>**	**78.76 ± 28.36**	**0.14**
MIO	92.88	± 5.49	>	82.45 ± 29.56	0.205

C. Focan, personal communication

Pharmacologic treatment of circadian disruption

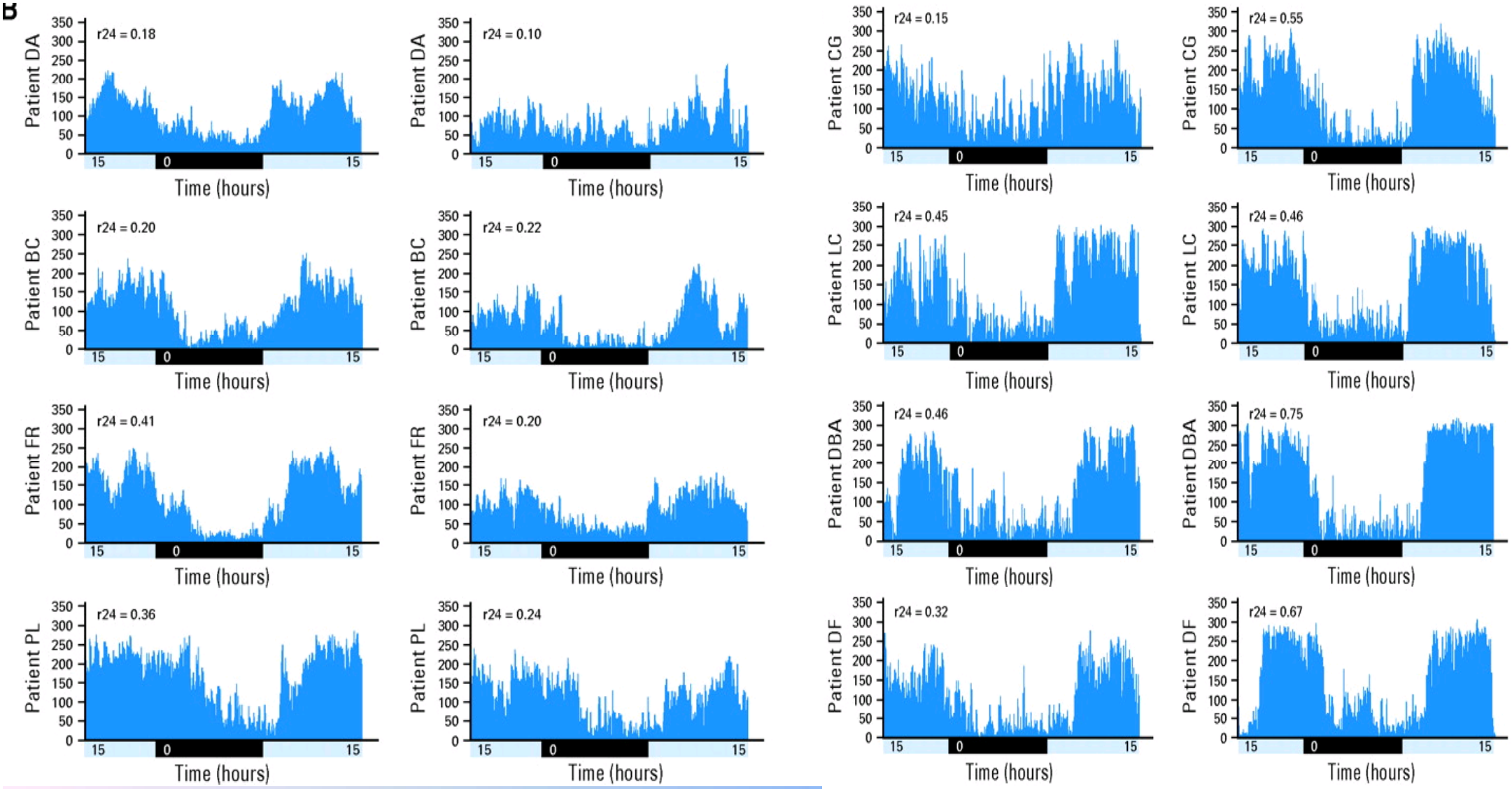
Gefitinib (TKI) in NSCLC patients

- 10 patients treated with gefitinib (250 mg daily)
- 4 patients treated with chemotherapy as controls

Circadian disruption or induction in cancer therapy

Chemotherapy (4 patients)

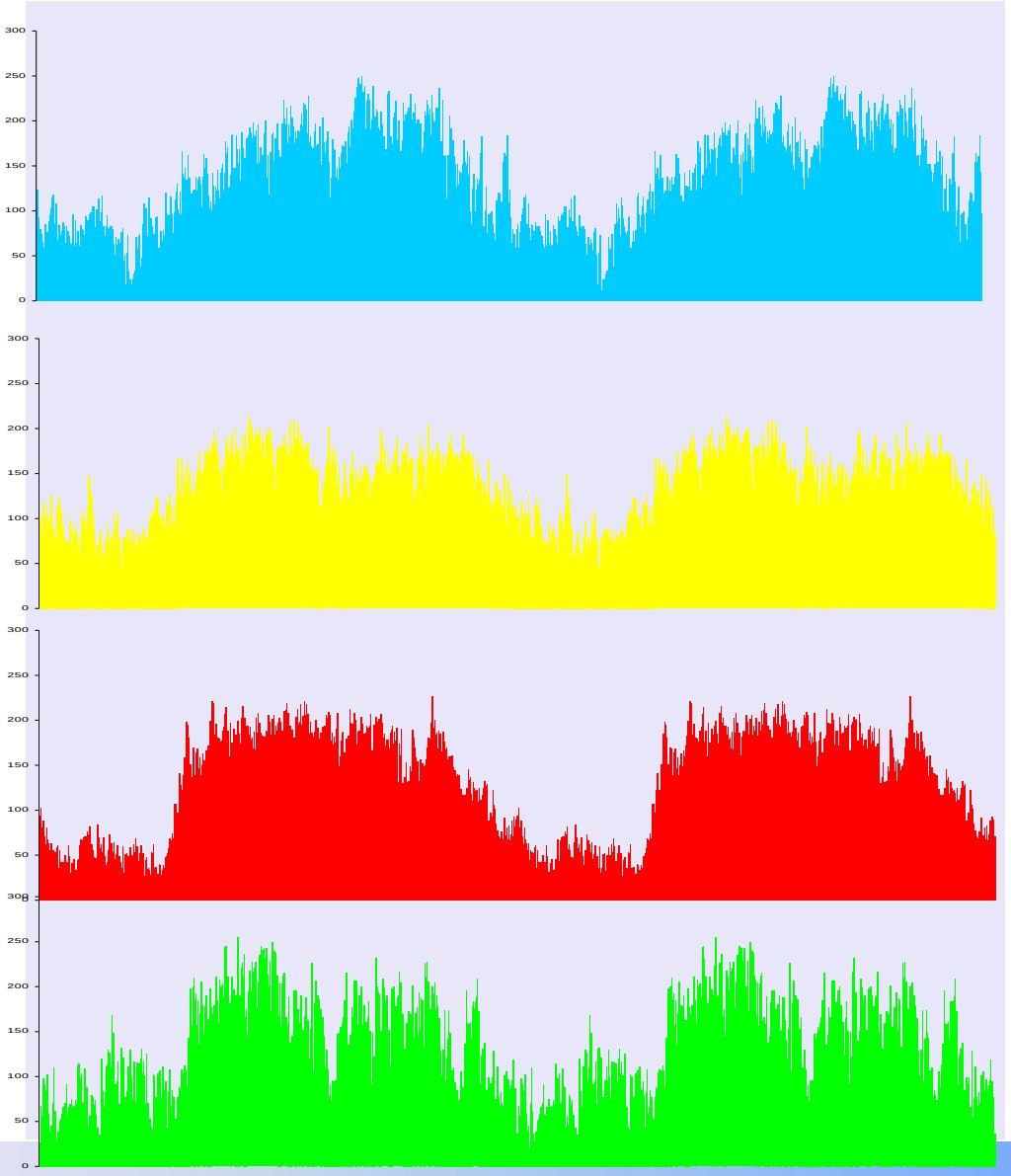
Gefitinib (10 patients)



Iurisci et al, JCO 2007

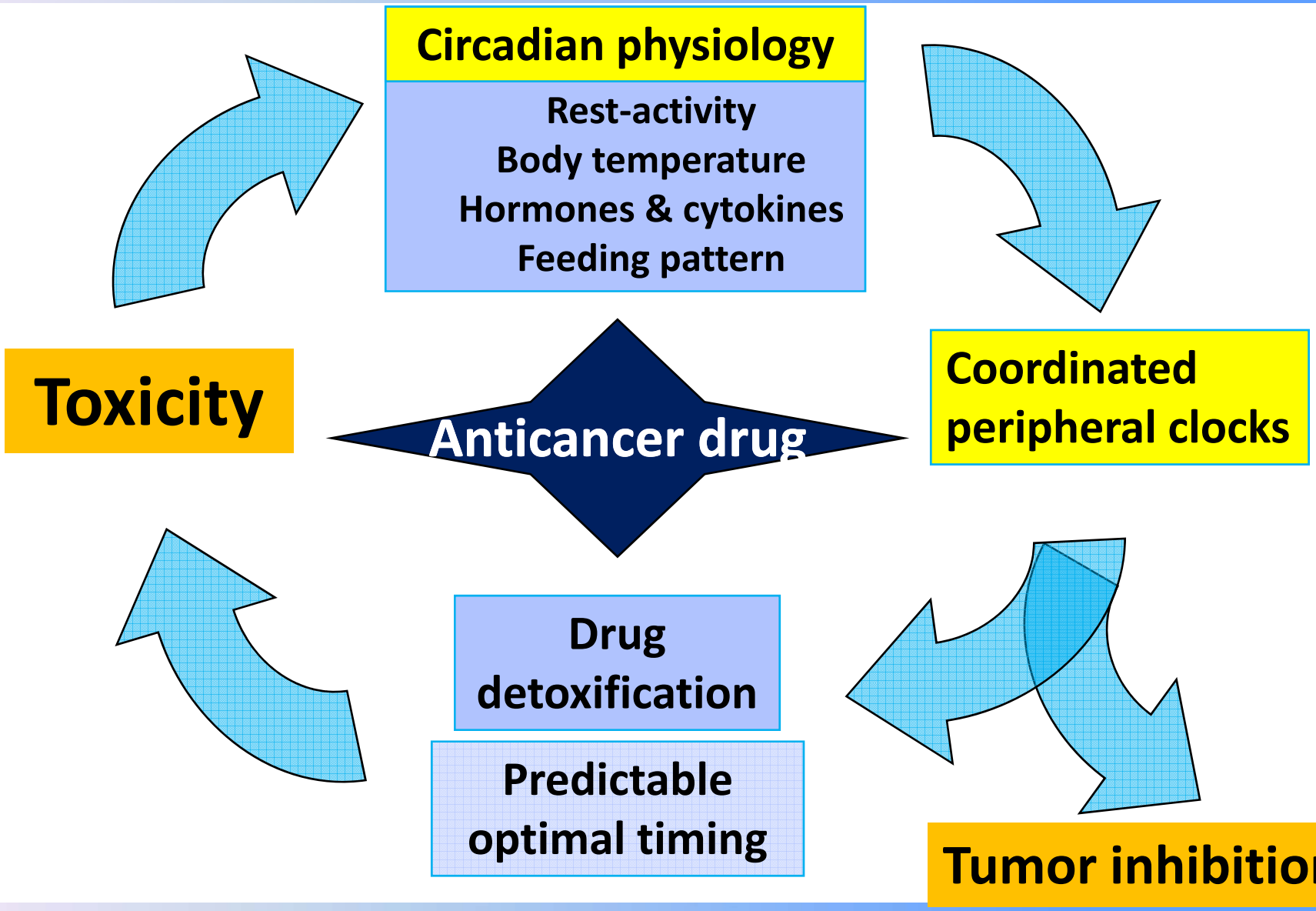
http://rbc.vjf.inserm.fr

Erlotinib (TKI) on the rest-activity rhyhtm of a NSCLC patient



	R24	I<0
Before	0.27	94.6
Morning erlotinib	0.17	89.9
Evening erlotinib	0.40	98.5
After	0.29	95.5

Hypothetical relations between circadian timing system, toxicity and efficacy of anticancer treatments



- **The circadian timing system**

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  (cancer processes and treatments)
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➡ **Conclusions**

Conclusions & perspectives

- Down regulation of tumor growth by the circadian timing system

- Implications for cancer prevention and treatments

- The circadian timing system: a potential target to be shielded or reinforced in cancer therapy

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