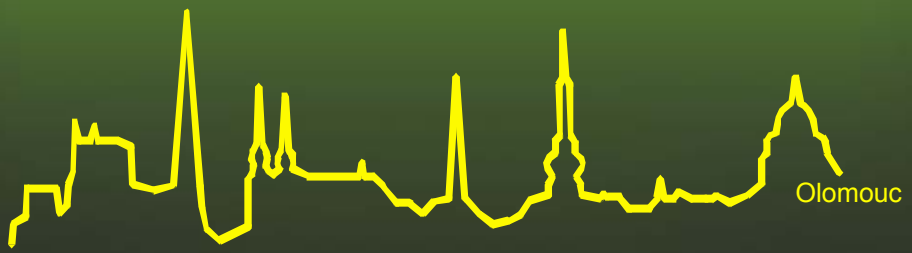
Laboratory of Growth Regulators

Miroslav Strnad

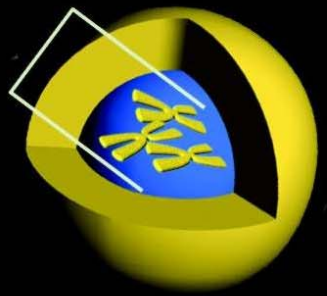
NEW ANTICANCER DRUGS DERIVED FROM PLANT HORMONES (Olomoucines)



Palacky University & Institute of Experimental Botany AS CR
Olomouc, Czech Republic

Landmarks in Biology and Genetics

Gregor Mendel
laws of genetics
1865



Human Genome Project

James Watson & Francis Crick
double-helical structure of DNA
1953

Institute of Experimental Botany

Academy of Sciences of the Czech Republic

UNIVERSITAS PALACKIANA OLOMUCENSIS

Co je Laboratoř růstových regulátorů?

1. **Laboratoř růstových regulátorů je společným pracovištěm Ústavu experimentální botaniky AVČR a Přírodovědecké fakulty Univerzity Palackého.**

2. **Byla založena v září 1996 jako výzkumné pracoviště interdisciplinárního charakteru.**

3. **Účelem pracoviště je integrovat kapacity PřF UP a ÚEB AV ČR pro společné řešení vědecko-výzkumných projektů v oblasti molekulárních a fyziologických mechanismů účinků růstových regulátorů u živých organismů.**

Co je Laboratoř růstových regulátorů?

Pracoviště se zabývá vědecko-výzkumnou a pedagogickou činností v oboru experimentální biologie, zejména:

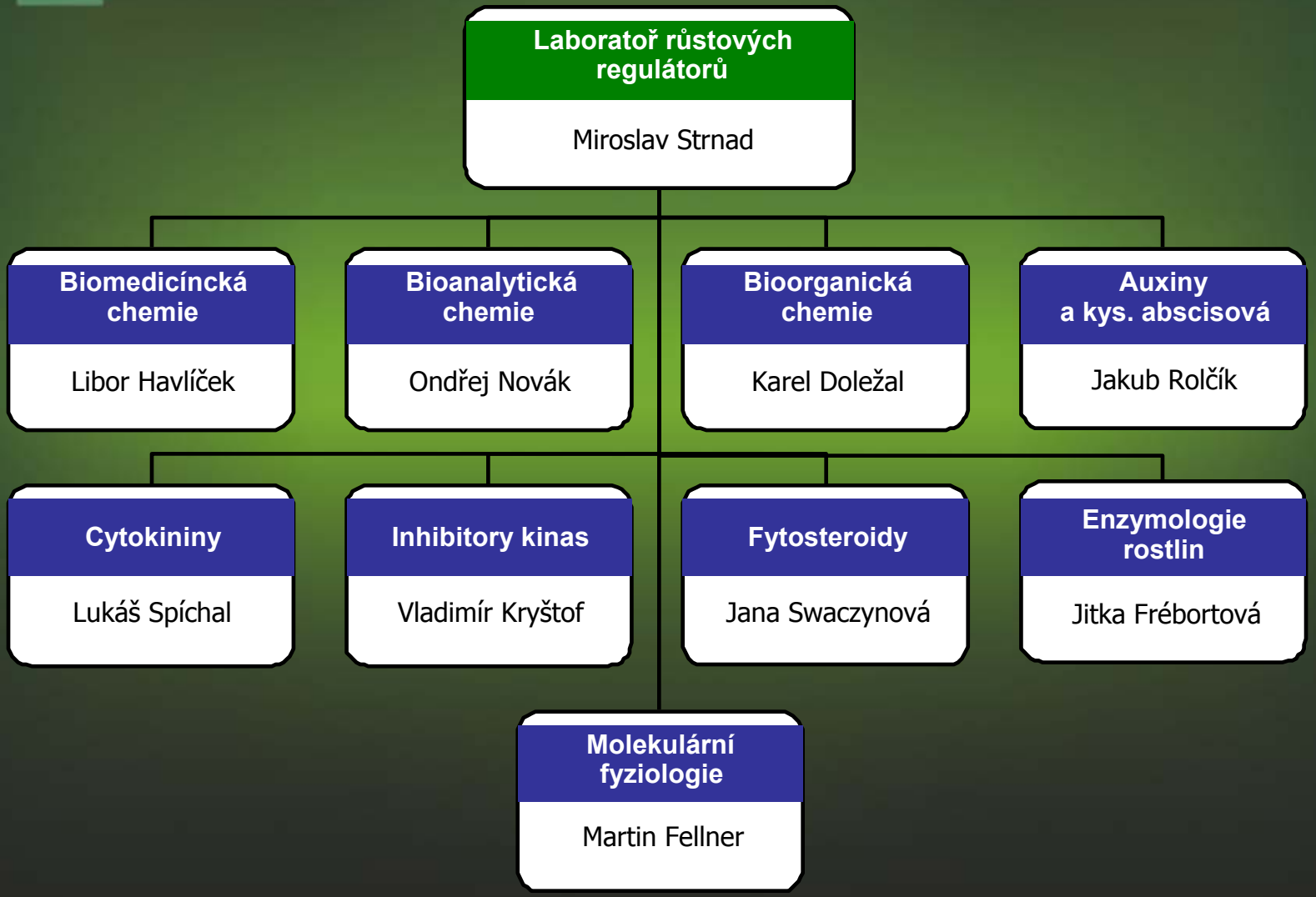
a) **pak přípravou nových, vysoce biologicky účinných růstových regulátorů na bázi purinu,**

b) **vývojem metod jejich analýzy,**

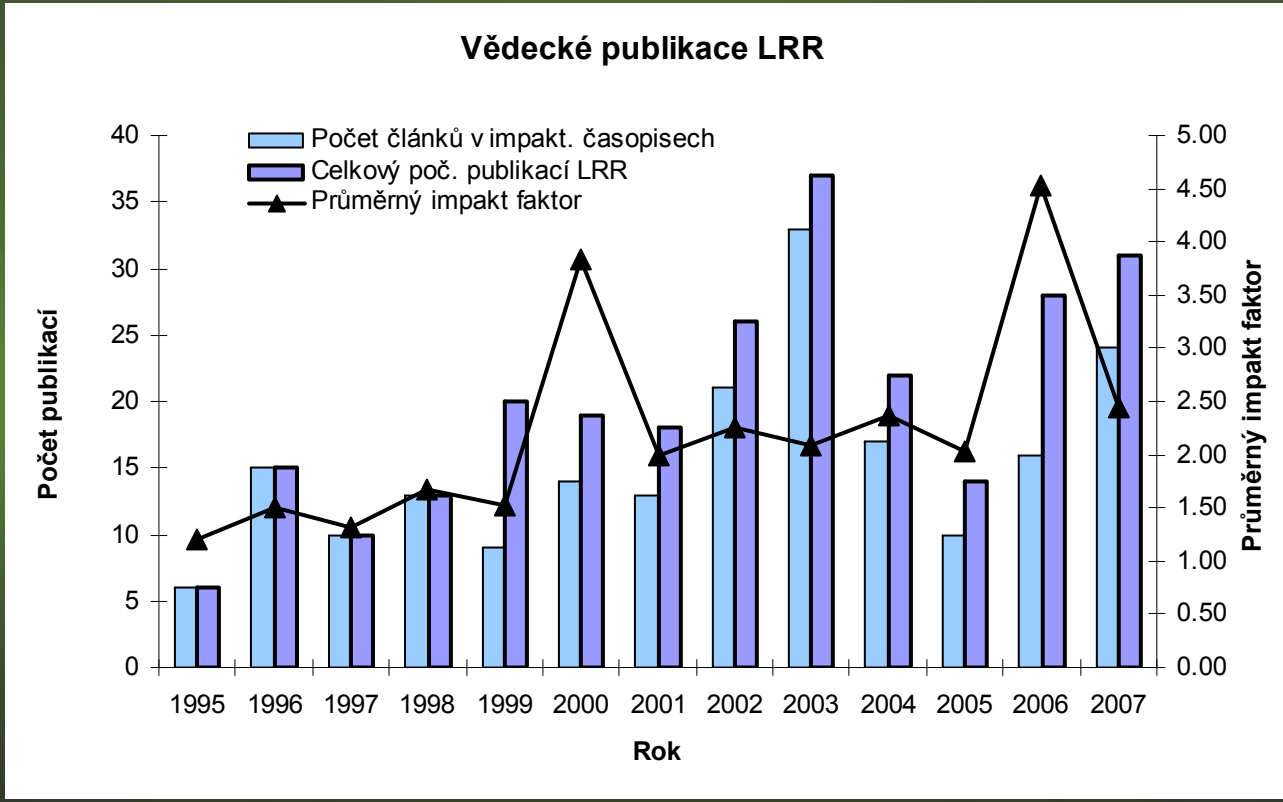
c) **studiem jejich funkcí a účinků v růstových a vývojových procesech normální a nádorové buňky, včetně vývoje protinádorových látek odvozených od rostlinných hormonů.**

d) **studiem onkogenů a nádorových supresorových genů, mechanismů regulace jejich exprese, včetně vývoje transgenních organismů kontrolovaně exprimujících různé geny zapojené v růstově-regulačních a obranných funkcích organismů.**

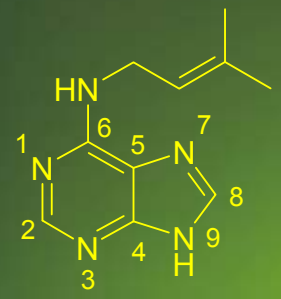
Organizační schéma LRR



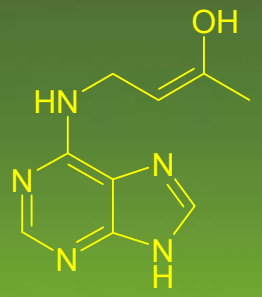
Publikační aktivita LRR



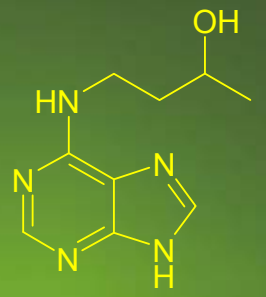
Isoprenoid and Aromatic Cytokinins



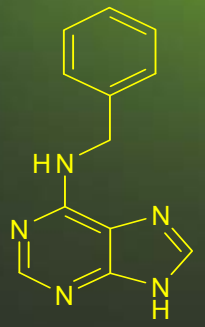
N^6-isopentenyladenine



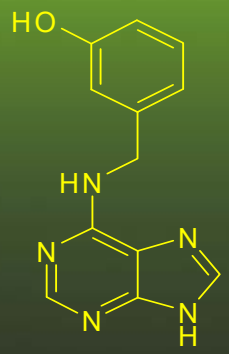
***trans*-zeatin**



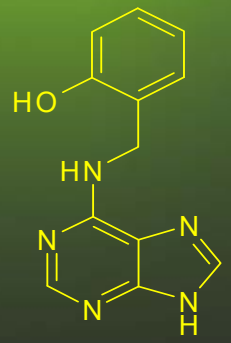
dihydrozeatin



N^6-benzyladenine



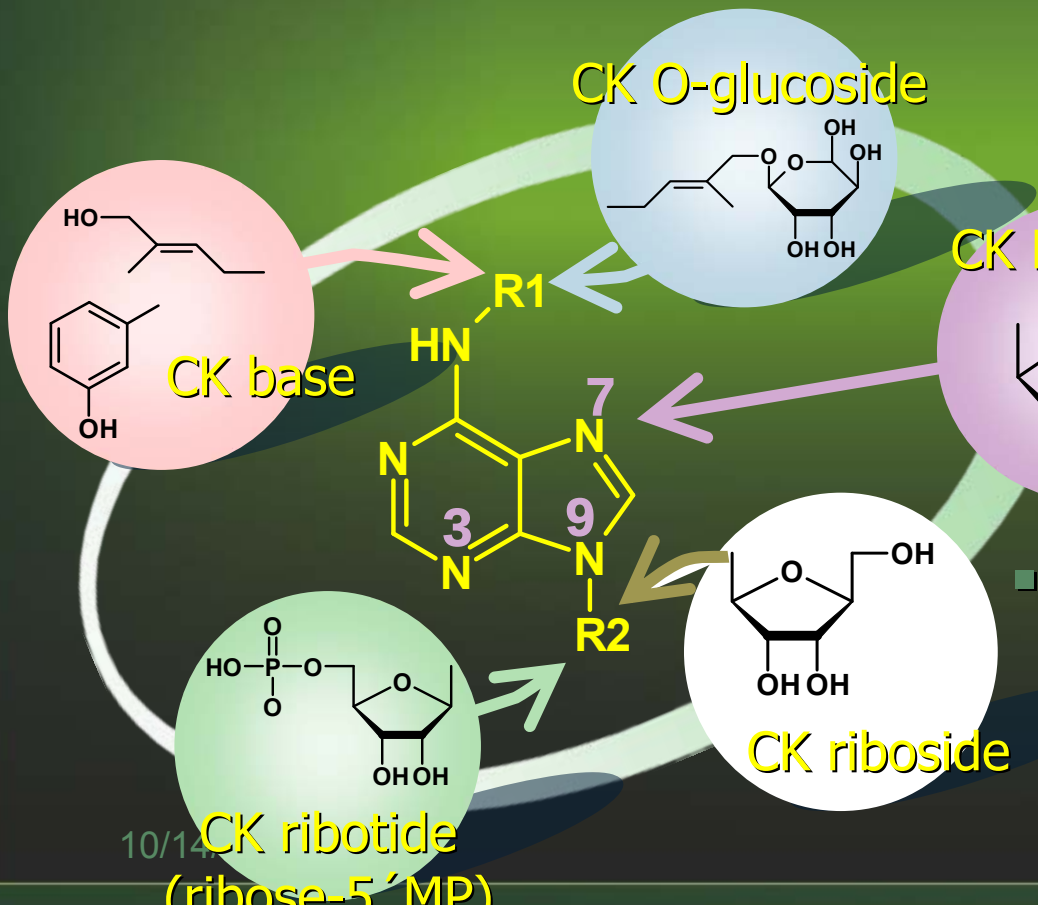
***meta*-topolin**



***ortho*-topolin**

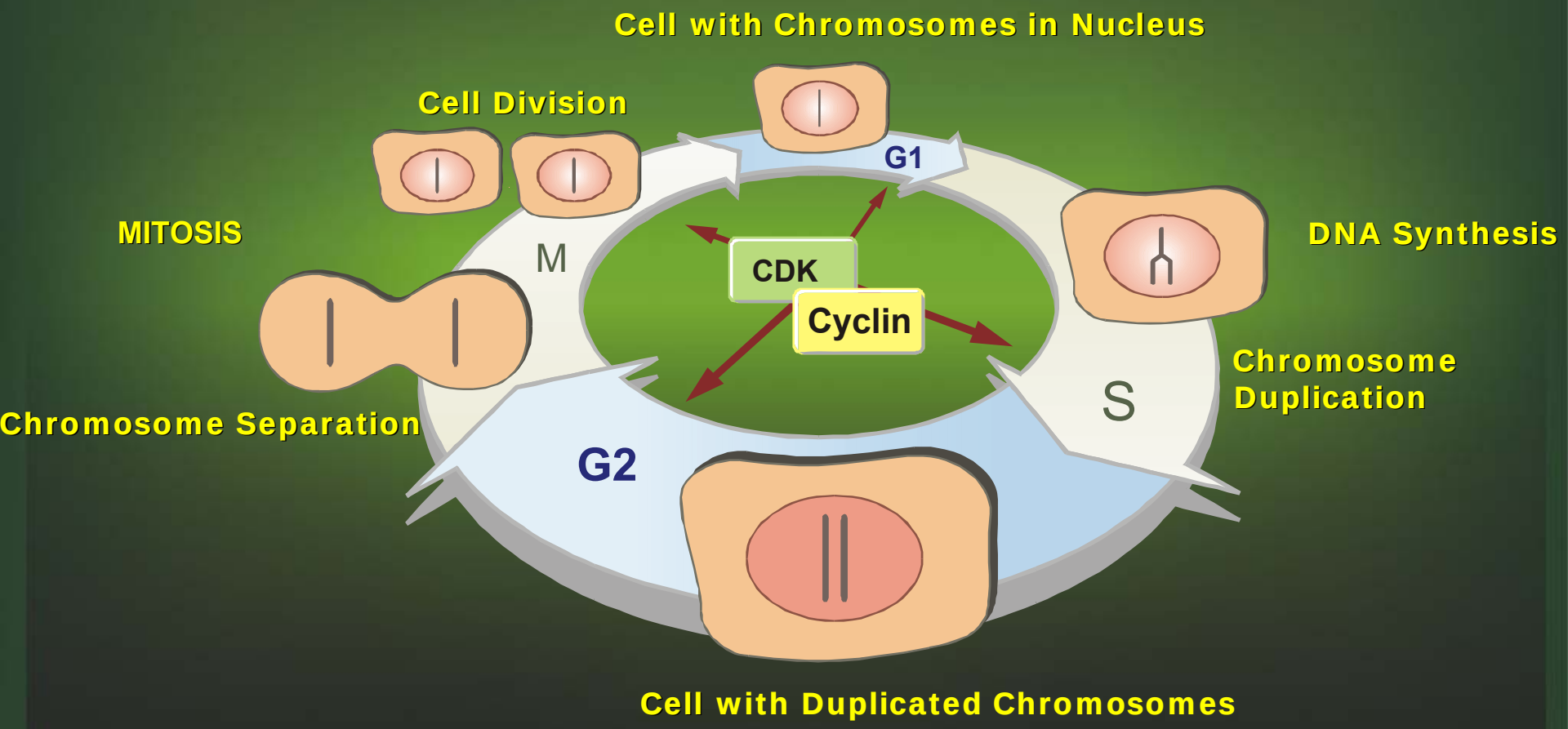
Cytokinins (CK)

- CKs regulate cell division in shoots and roots, growth of stems and roots and specific components of the cell cycle.

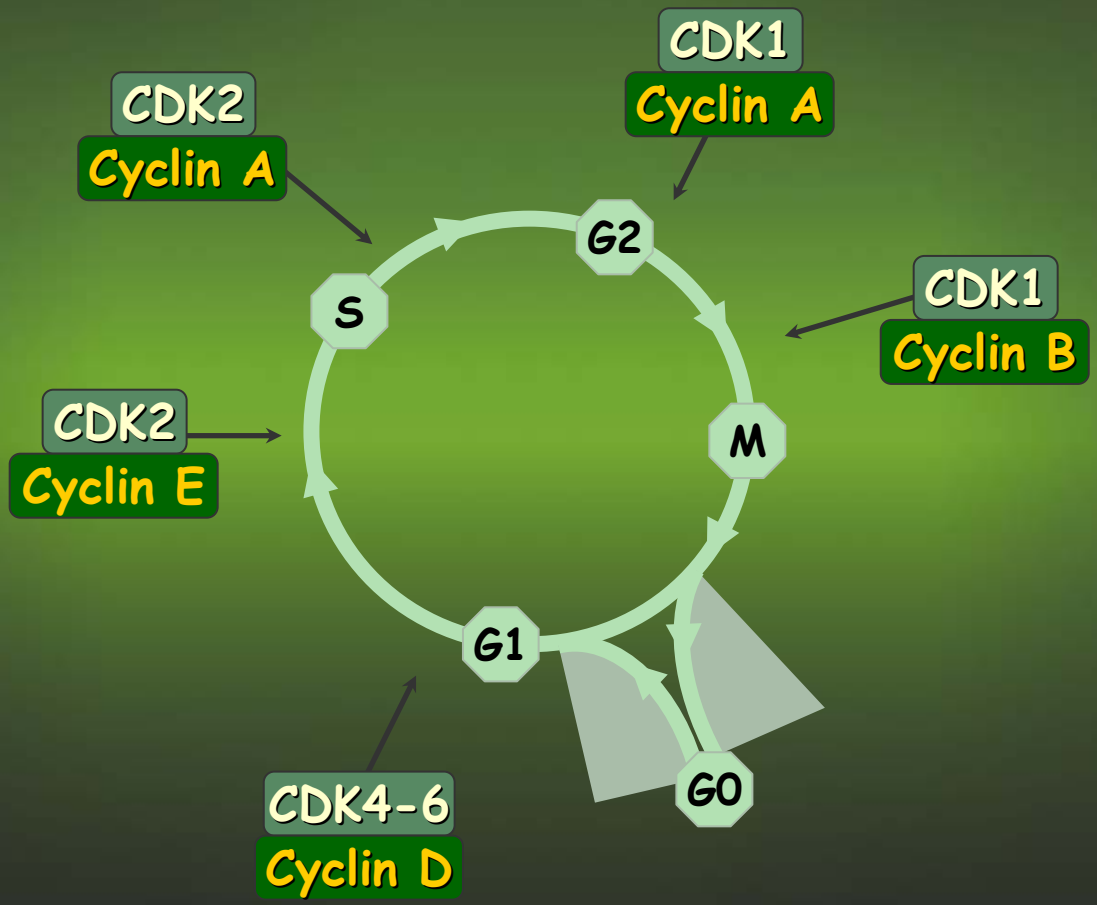


- CKs overproduction is implicated in plant tumour development.
- CKs delay leaf senescence.
- CKs promote chloroplast development and cell extension in leaves and cotyledons.

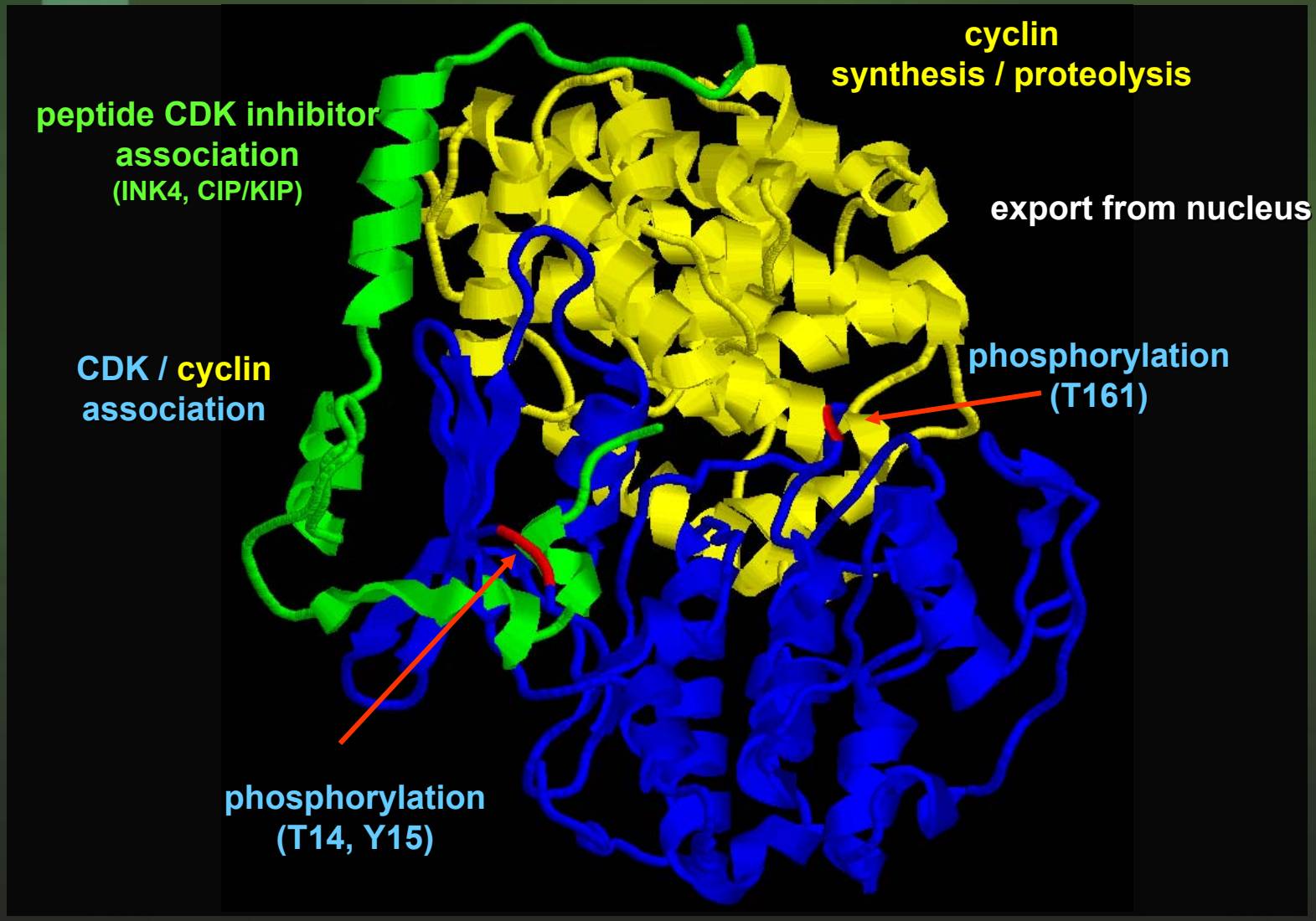
Cell Cycle


Cell with Chromosomes in Nucleus
Cell Division
G1
MITOSIS
M
CDK
Cyclin
DNA Synthesis
Chromosome Duplication
S
Chromosome Separation
G2
Cell with Duplicated Chromosomes

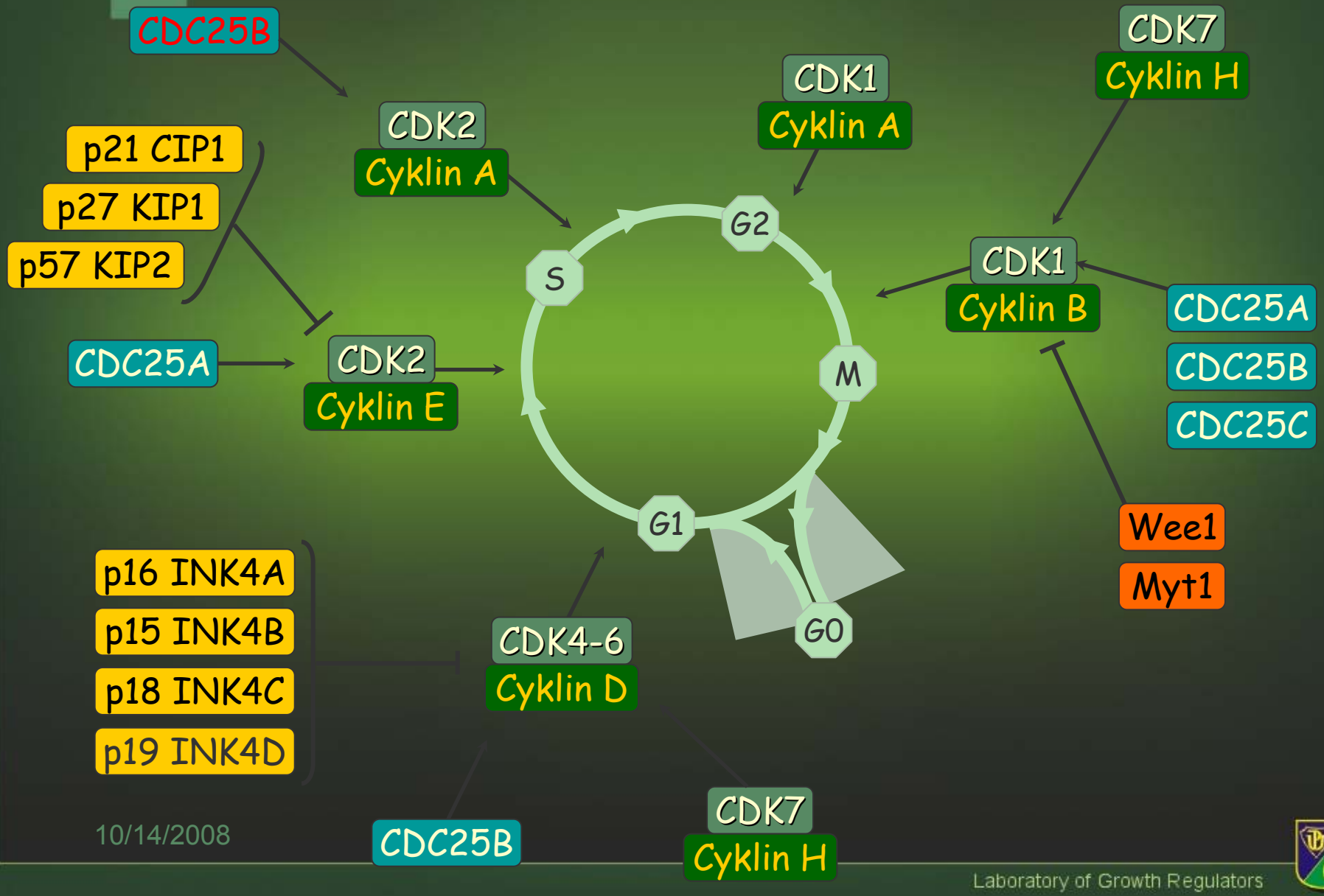
Cell Cycle and CDK



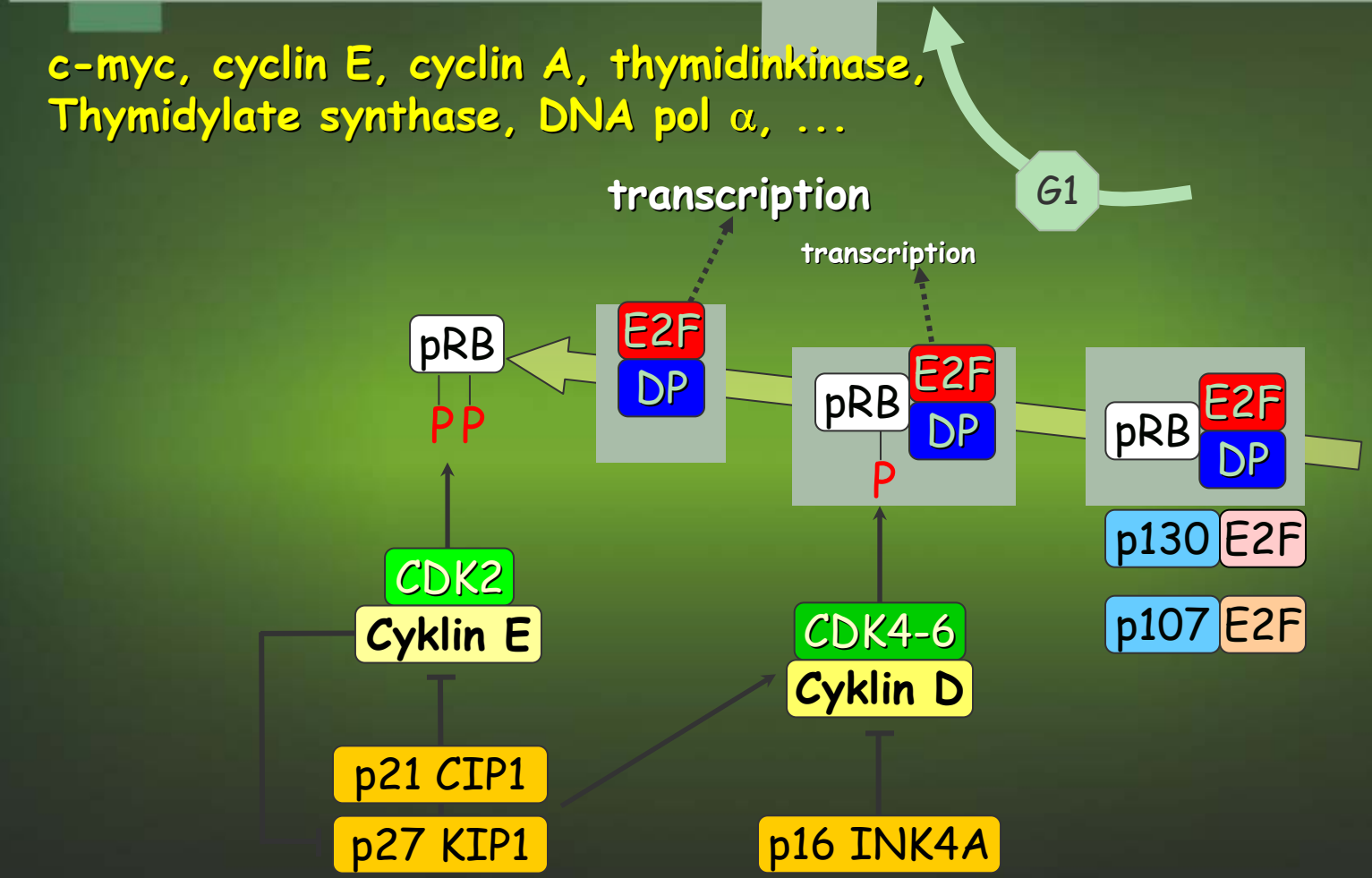
Regulation of CDK Activity



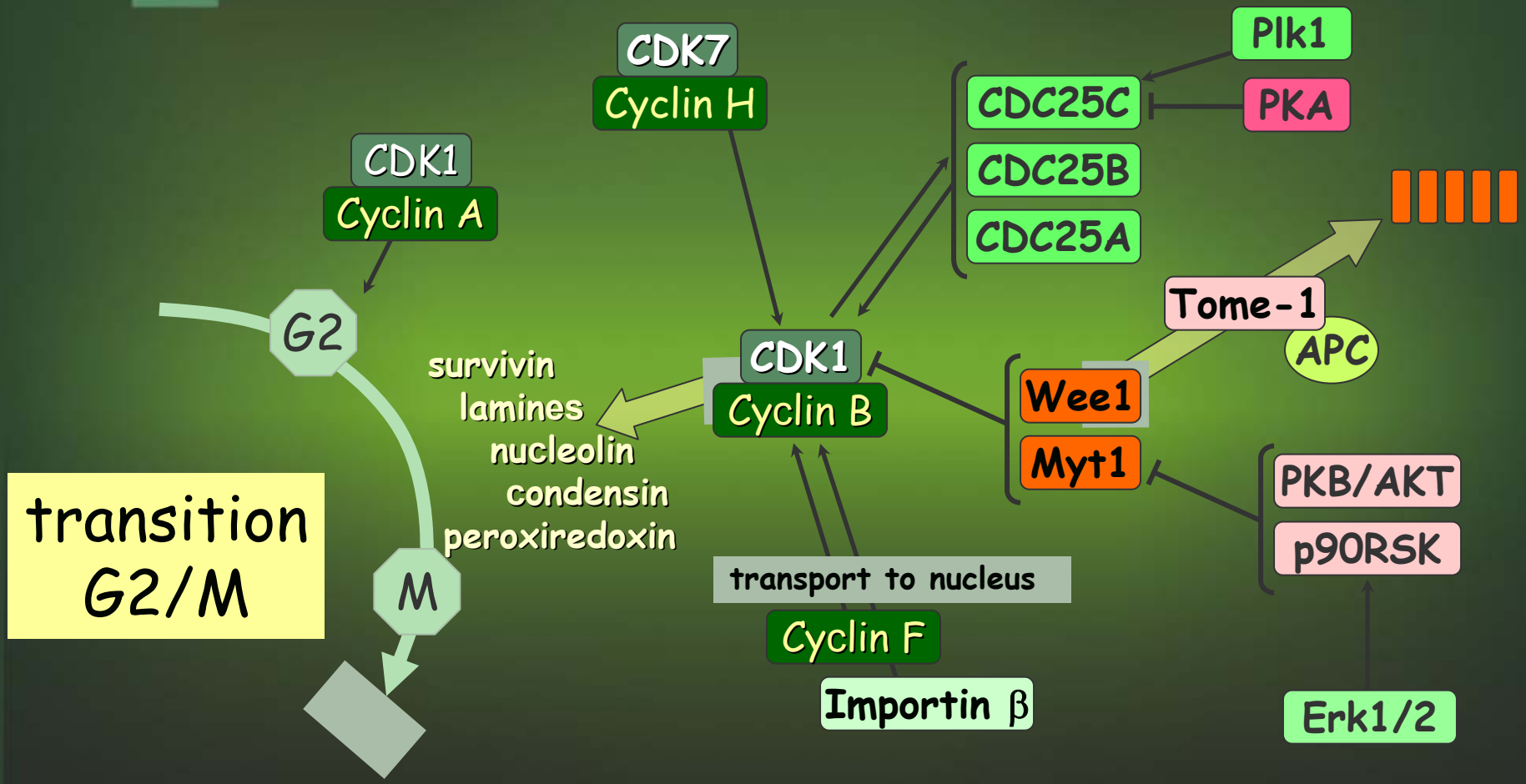
CDK Regulation



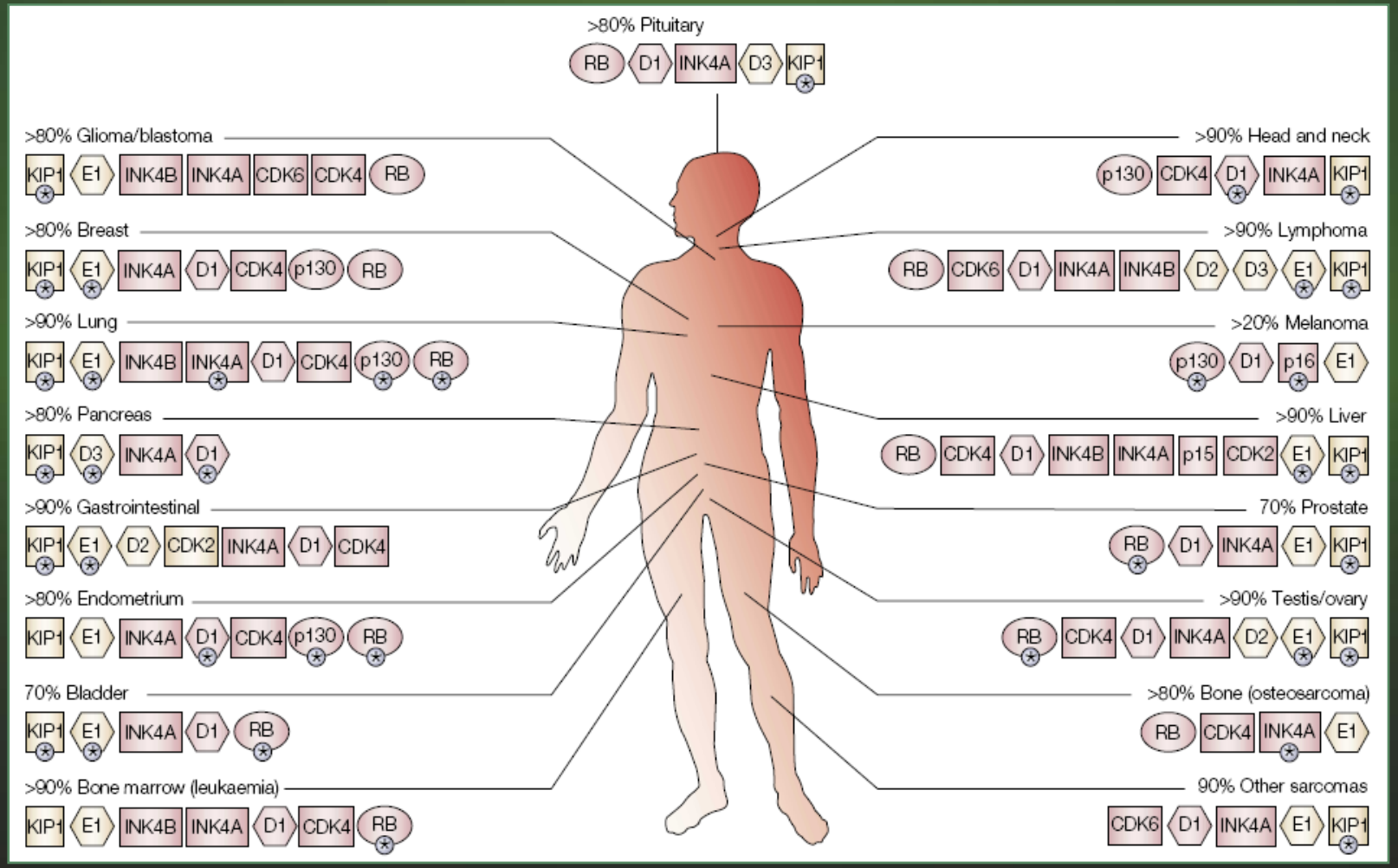
CDK and Cell Cycle Regulation during G1/S Transition



CDK and G2/M Transition



Alterations of G1/S regulators

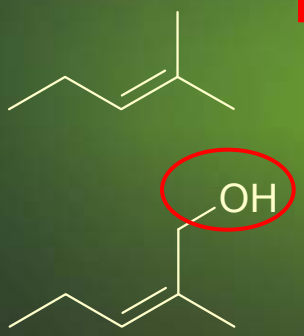


Nature Reviews Cancer 1, 222-231 (2001)

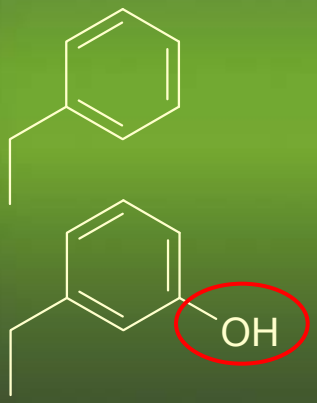
Chemical structure…

N^6-substituted derivatives of adenine

isoprenoid



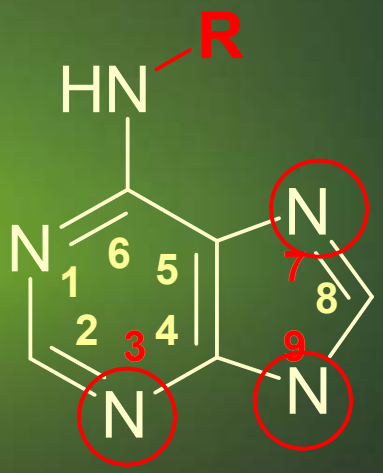
aromatic



free basis

3/7/9 N-glucosides
9 ribosides
9 ribotides

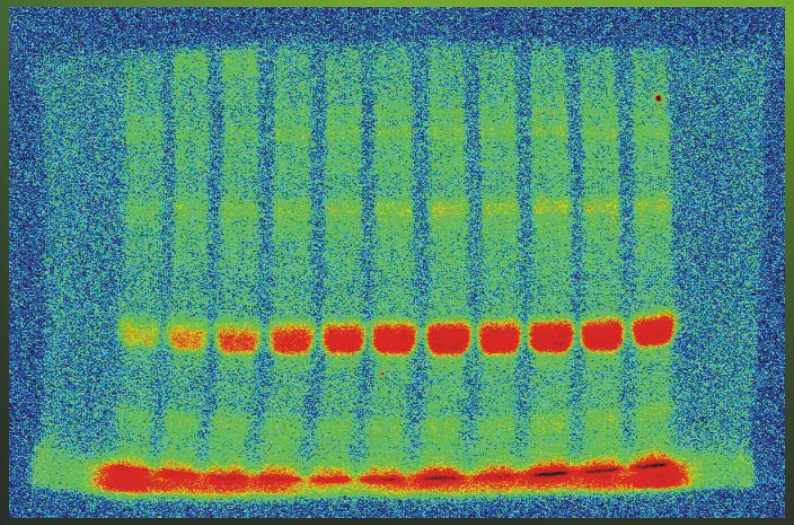
O-glycosides



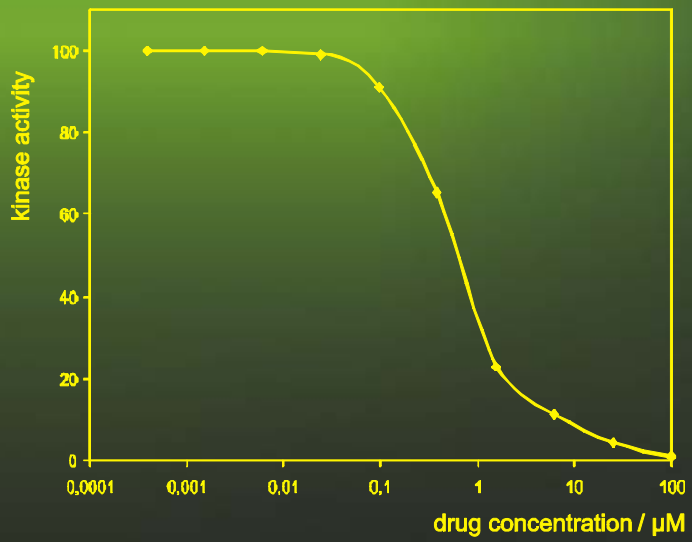
Which of the biologically active cytokinins and cytokinin activate/inhibits cyclin-depedent kinases?

CDK Inhibition Assays

- ✓ human cyclin B / CDK1 complex produced via baculoviral expression system
- ✓ enzyme purification on Ni-NTA affinity column.
- ✓ assay in the presence of histone H1, ATP + [γ-^{33}P] ATP and tested drug
- ✓ SDS gel electrophoresis, digital image analyser BAS-1800
- ✓ graphic analysis (IC_{50})



SDS gel with reaction mixtures containing decreasing concentrations of CDK inhibitor (BAS-1800 scan)



Dose-response curve of CDK inhibition by purine inhibitor

IC_{50} Values for Various Purines Added to Purified CDC2, CDK5 and CDK4 Kinases

Compound		IC_{50} (μM)		
		CDK1	CDK5	CDK4
6-substituted purines	6-aminopurine (adenine)	200	-	-
	6-dimethylaminopurine	120	120	-
	6-allylaminopurine	50	100	>500
	isopentenyladenine	55	70	200
	6-benzylaminopurine	200	80	-
	6-furfurylaminopurine (kinetin)	180	-	-
	trans-zeatin	70	150	>1000
	dihydrozeatin	80	-	-
	cis-zeatin	150	100	-
	meta -topolin	70	-	-
	ortho-topolin	200	-	-
	6-(o-β-D-glucopyranosyl)-zeatin	850	-	-

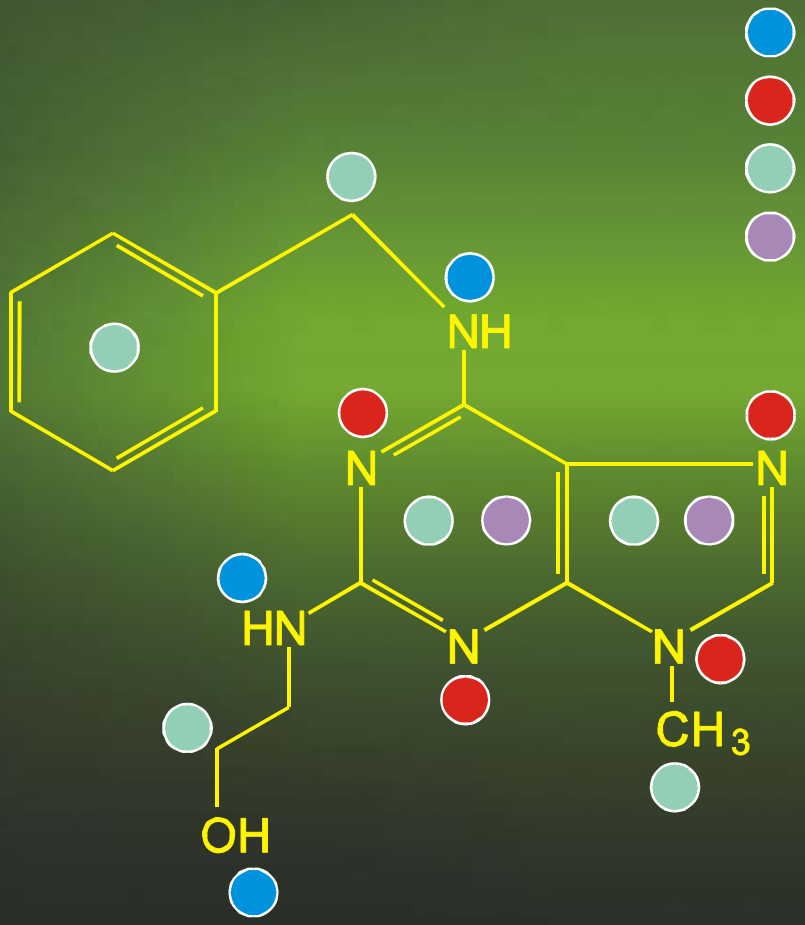
IC_{50} Values for Various Purines Added to Purified CDC2, CDK5 and CDK4 Kinases

Compound		IC_{50} (μM)		
		CDK1	CDK5	CDK4
6,9-substituted purines	adenosine	55	-	-
	6-benzylamino-9-(2-tetrahydropyranyl)purine(BPA)	200	160	-
	dihydrozeatin riboside	>500	>500	-
	benzyladenosine	>500	-	-
	meta-topolin riboside	>500	-	-
	ortho-topolin riboside	>500	-	-
	zeatin riboside	>500	>1000	-
	zeatin-9-glucoside	>500	-	-
	zeatin riboside-5´-monophosphate	>500	-	-

IC_{50} values for various purines added to purified CDC2, CDK5 and CDK4 kinases

Compound		IC_{50} (μM) CDK1	CDK5	CDK4
2,6,9-substituted purines	2-amino-6-benzylamino-9-methylpurine	40	-	-
	2-chloro-6-benzylamino-9-methylpurine	70	-	-
	2-(2-hydroxyethylamino)-6-amino-9-methylpurine	50	-	-
	2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine (olomoucine)	7	3	>1000
	2-(2-hydroxyethylamino)-6-isopentenylamino-9-methylpurine	65	13	>1000
	2-(2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine	2	3	>1000
	2-(R)-(1-hydroxymethyl/propylamino/)-6-benzylamino-9-isopropylpurine (roscovitine)	0.2	0.1	>500

Olomoucine and its Potential Interactions with a Binding Site



HYDROGEN BOND DONOR

HYDROGEN BOND ACCEPTOR

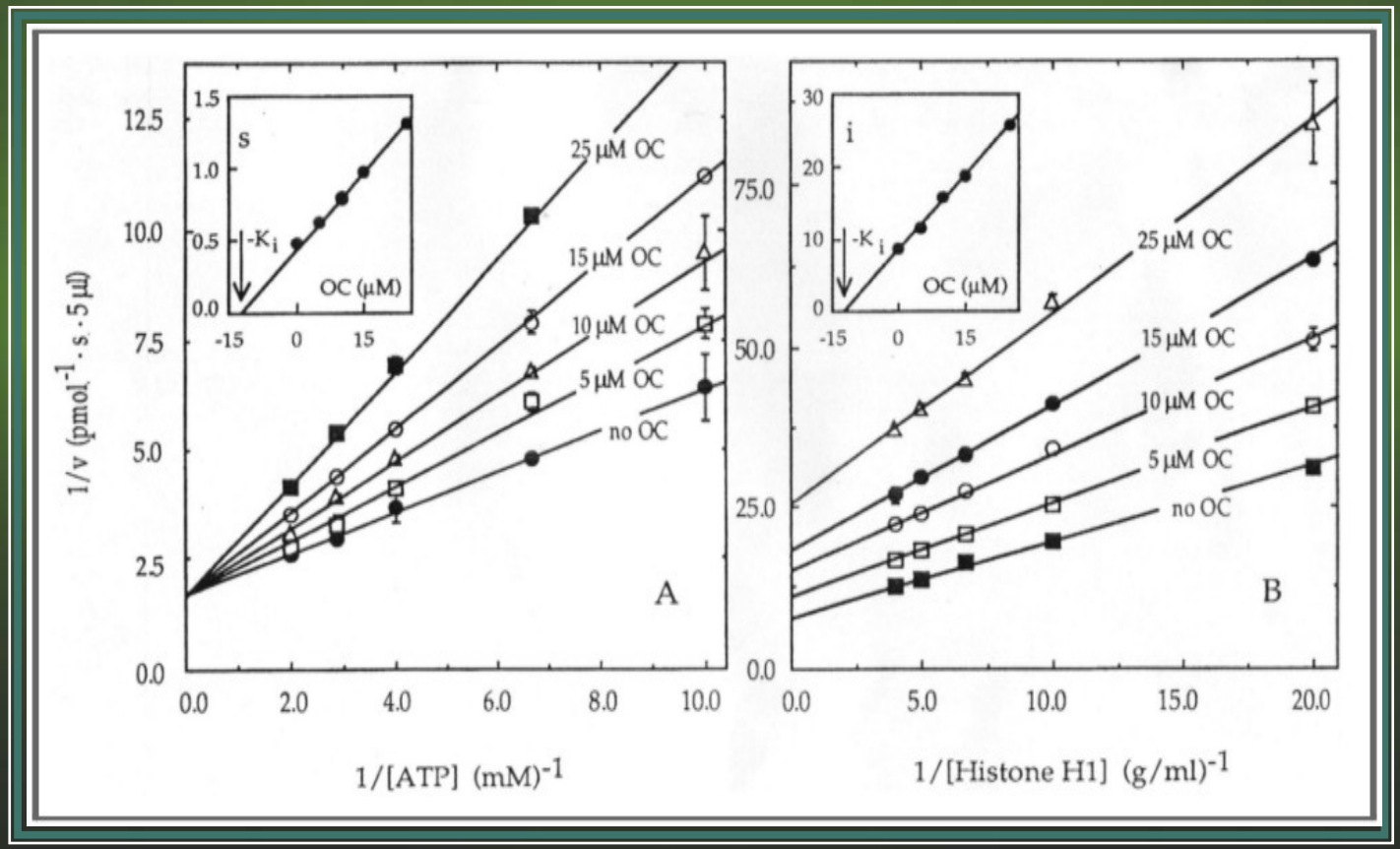
HYDROPHOBIC / LIPOPHILIC INTERACTIONS

CHARGE-TRANSFER INTERACTION

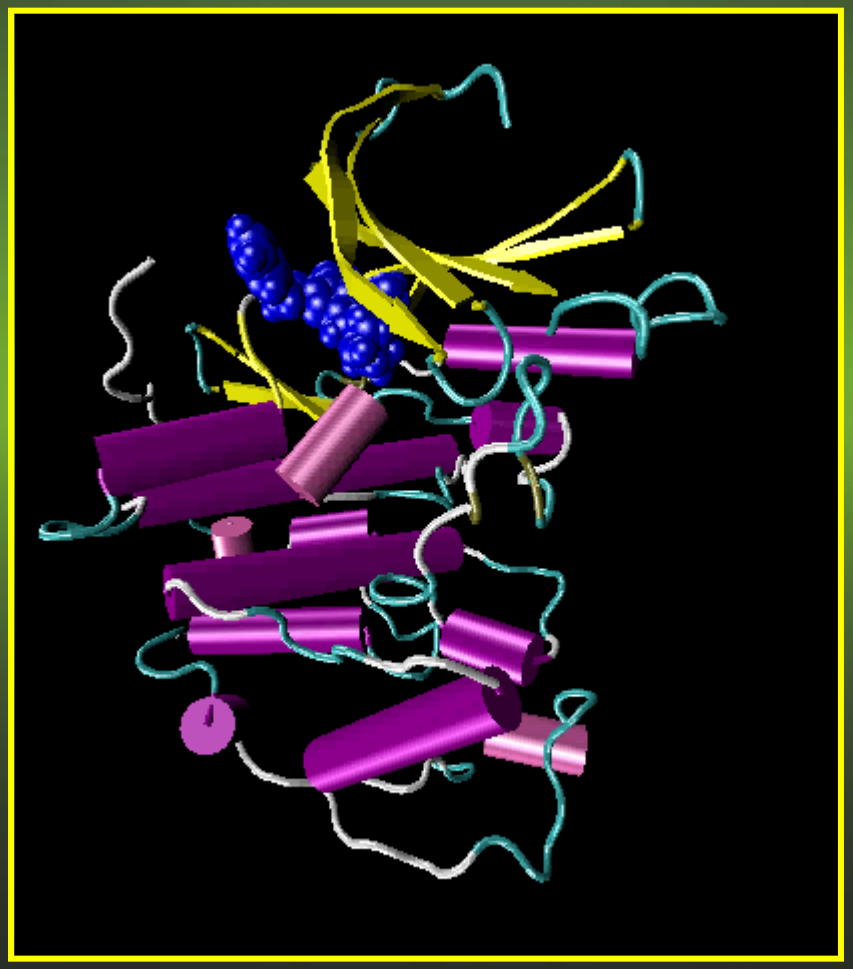
IC_{50} Values for Olomoucine and Isopentenyladenine Added to Various Purified Kinases

Enzyme	IC_{50} (μM)	
	olomoucine	isopentenyladenine
$p34^{cdc2}$ /cyclin A	50	-
$p34^{cdc2}$ /cyclin B	7	45
$p34^{cdc2}$ /cyclin E	10	-
$p33^{cdk2}$ /cyclin A	7	50
$p33^{cdk2}$ /cyclin E	7	-
$p34^{cdk4}$ /cyclin D	>1000	200
$p35^{cdk5}$ /35	3	80
$p40^{cdk6}$ /cyclin D3	>250	>100
GST- erk -1	30	90
c-protein kinase C α,β1,β2,γ	>1000	40-100
n-protein kinase C δ,ε,η,ζ	>1000	50-100
cAMP -dependent kinase	>1000	50
cGMP -dependent kinase	>1000	50
Calmodulin -dependent kinasell	>1000	>100
Myosin light -chain kinase	>1000	>1000
AMP- activated protein kinase	230	>1000
Insulin-receptor protein kinase	400	140
DNA topoisomerase I, II	250	-
DNA polymerase α,δ	500	-

Double reciprocal plots of kinetic data from assays of p^{34cdc2}/cyclin B protein kinase activity at different concentrations of olomoucine

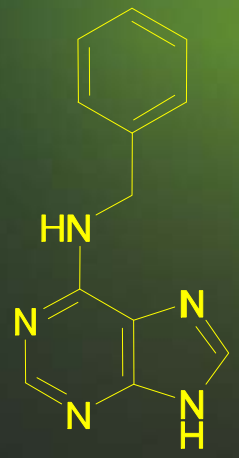


Schematic Drawing of CDK2 with the Inhibitor Roscovitine Superimposed in the Binding Pocket

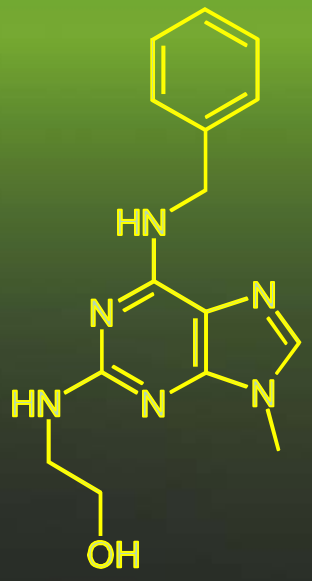


Development of Purine CDK2 Inhibitors

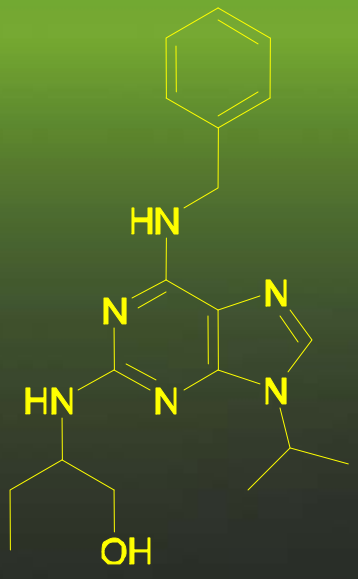
Compound	IC_{50} / μM
6-benzylaminopurine	200
olomoucine	7
roscovitine	0.2
olomoucine II	0.02



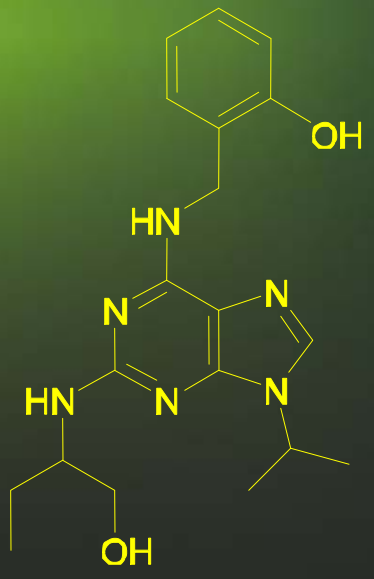
6-benzylaminopurine



Olomoucine



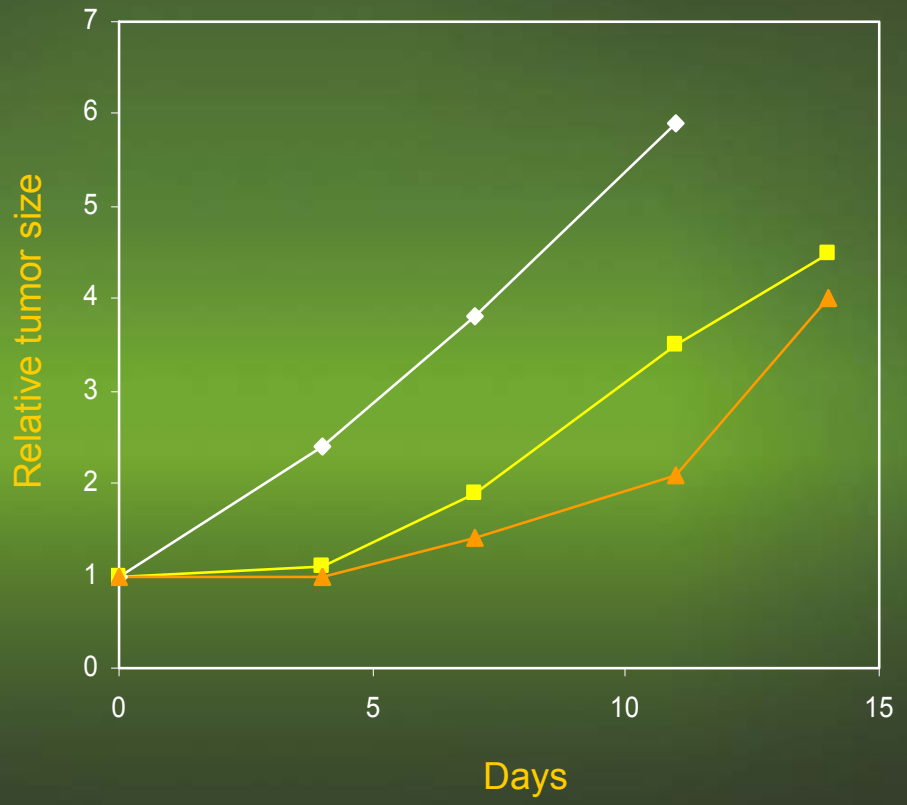
Roscovitine



Olomoucine II

Therapeutical Effects of CDK Inhibitors

Roscovitine (Cyc202)



Control

Roscovitine 200 mg / kg

Roscovitine 500 mg / kg

MOUSE XENOGRAFTS: LOVO AND MESSA-DX5 CARCINOMAS

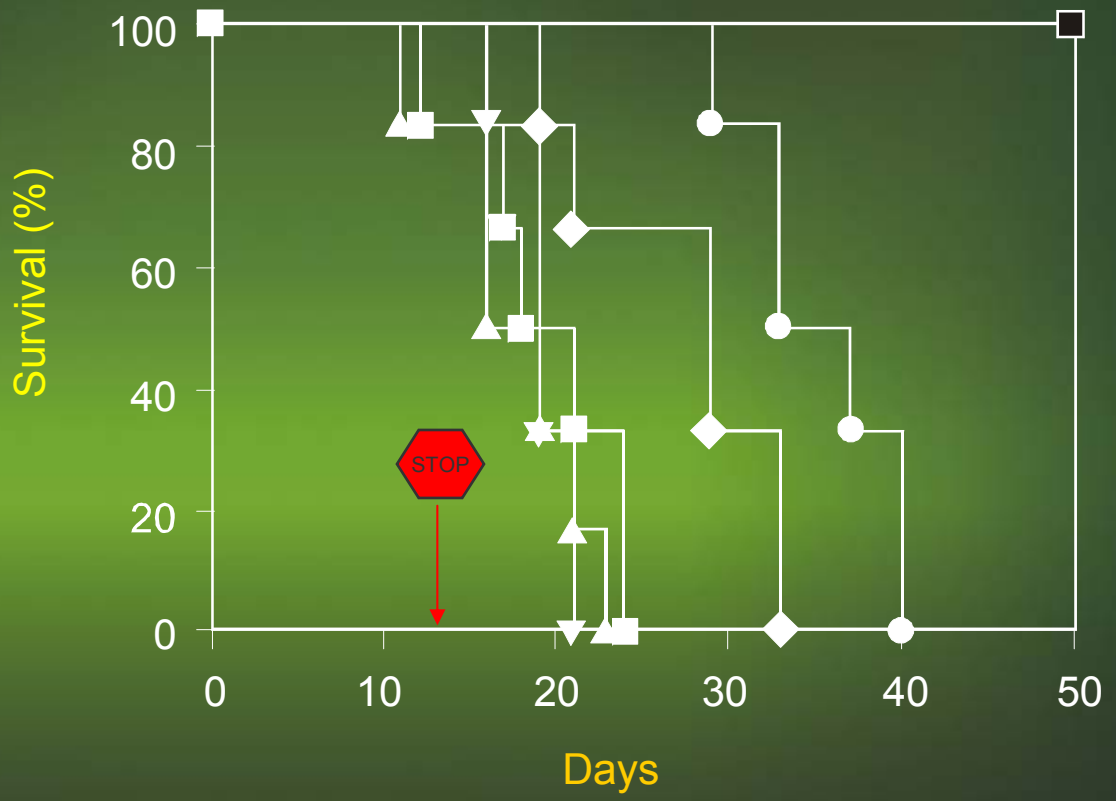
Int. J. Cancer 102, 463-468, 2002

10/14/2008

Therapeutical Effects of CDK Inhibitors

Sequential combination:

1. Taxol
2. Purvalanol A



Cancer Cell 2, 43-54, 2002

Intensive apoptoses

Reduction of tumor

Low toxicity

Unlimited survival

Carcinoma MCF7

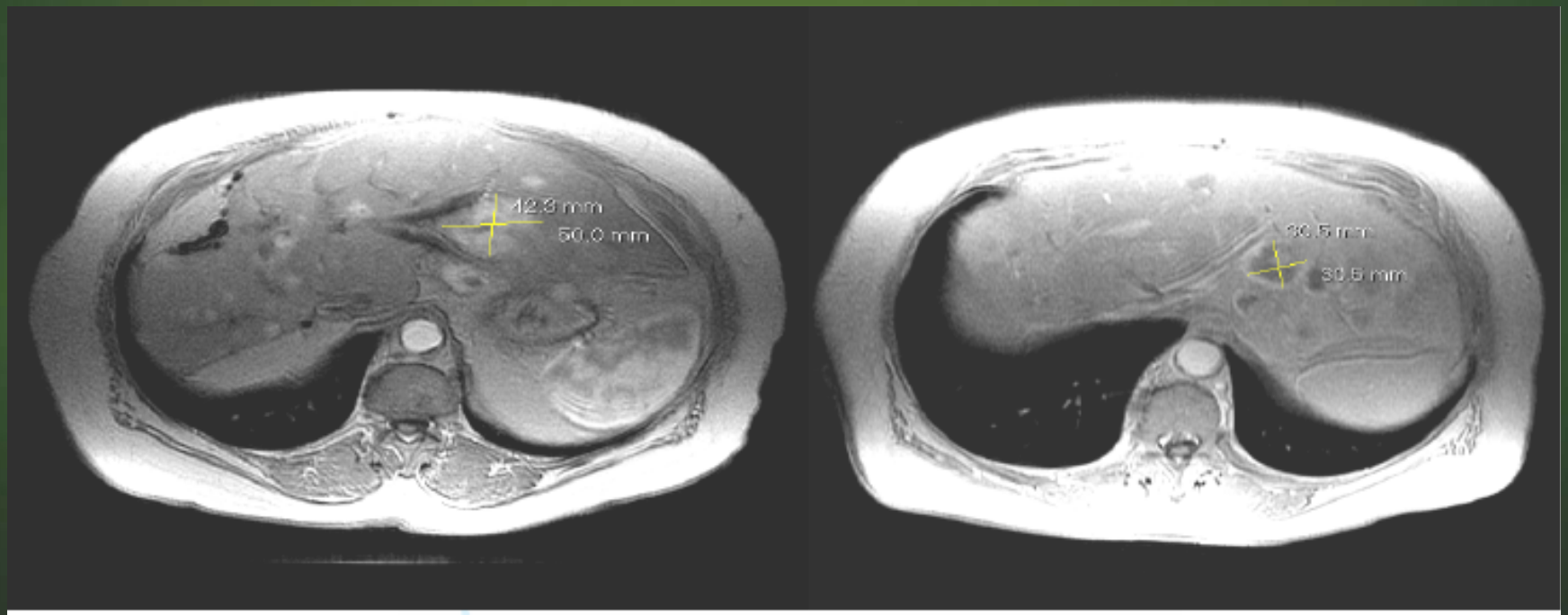
Roscovitine in Clinical Trials

R-roscovitine (CYC202, Seliciclib)

Licenced to Cyclacel Ltd.

Seliciclib is currently in Phase II clinical trials as a single therapy in multiple myeloma as well as two other B-cell hematological malignancies: B-cell Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma.
An additional Phase II clinical trial is in progress investigating the effects of Seliciclib in patients with Non-Small Cell Lung Cancer in combination with gemcitabine and cisplatin.

Roscovitine (Seliciclib)^{R} – Clinical Results

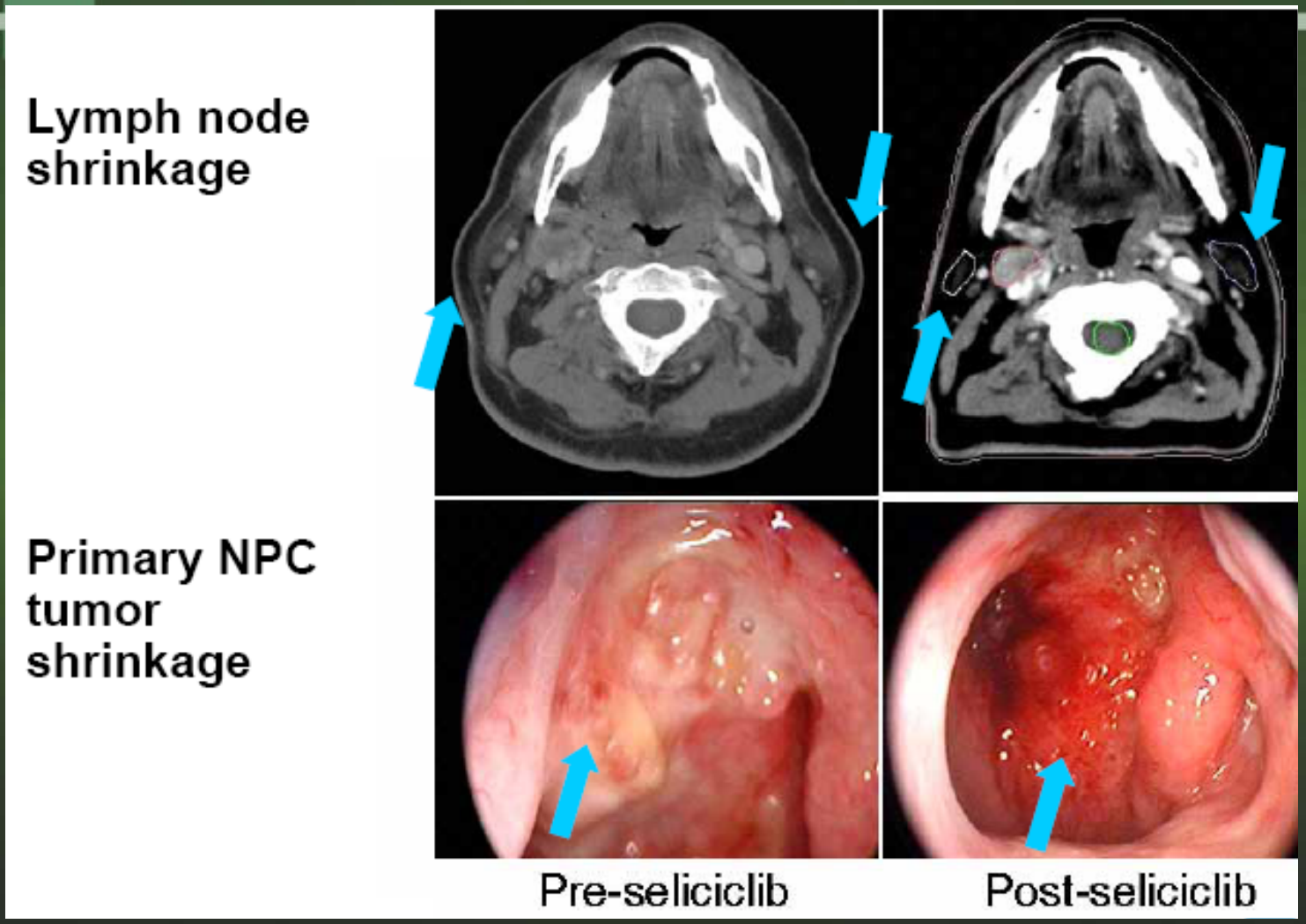


Baseline
Jan. 20, 2003

July 7, 2003

Seliciclib: Monotherapy of hepatocelullar liver carcinoma - reduction 46%, data from clinical examinations of Cyclacel, Scotland

Roscovitine (Seliciclib)^{R} – Clinical Results

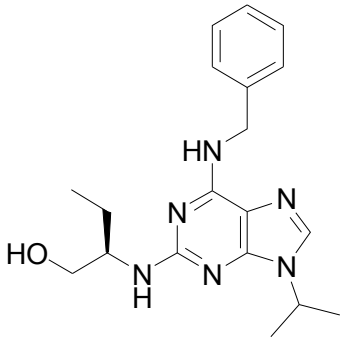


Seliciclib: Monotherapy of nazopharingal carcinoma associated with EBV infection (Epstein-Barr virus), no drug available – reduction > 50%, regression of neck metastasis lymph nodes

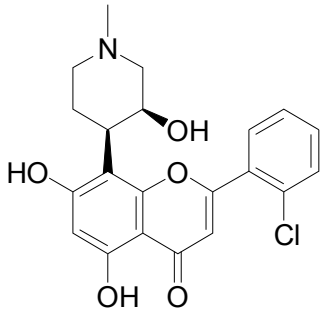
List of CDK inhibitors in clinical development

Compound	Structure	Sponsor	Comments	Phase	Route
Seliciclib (CYC202, *R*-roscovitine)	**1**	Cyclacel	Selective CDK2-CDK7-CDK9 inhibitor	II	*p.o.*
Alvocidib (flavopiridol, HMR1275)	**2**	Sanofi-Aventis	Promiscuous kinase inhibitor with potent CDK-inhibitory activity	II	*i.v.*
UCN-01	**3**	Kyowa Hakko Kogyo	Promiscuous kinase inhibitor with CDK-inhibitory activity	II	*i.v.*
E7070 (indisulam)	**4**	Eisai	G1/S cell cycle agent with indirect effects on CDK function	I/II	*i.v.*
SNS-032 (formerly BMS-387032)	**5**	Sunesis	Selective CDK2-CDK7-CDK9 inhibitor	I	*i.v.*
ON 01910.Na	**6**	Onconova	Dual specificity non-ATP-competitive CDK1/PLK1 inhibitor	I	*i.v.*
AZD-5438	Not disclosed	AstraZeneca	Not known	I	?
ZK-CDK	Not disclosed	Schering AG	Dual-specificity CDK2/VEGF-/PDGF-RTK inhibitor	I	*p.o.*
PD 0332991	**7**	Pfizer	Highly CDK4-selective with G1/S activity	I	*p.o.*
PHA-690509	Not disclosed	Nerviano Medical Science	Not known	I	?
JNJ-7706621	**8**	Johnson & Johnson	Dual specificity CDK and ARK inhibitor	Preclinical	*p.o.*
Not disclosed	Not disclosed	Hoffmann-La Roche	Not known	Preclinical	*?*
GPC-286199	**9**	GPC Biotech	Pan-CDK inhibitor with antimitotic activity	Preclinical	*i.v.*

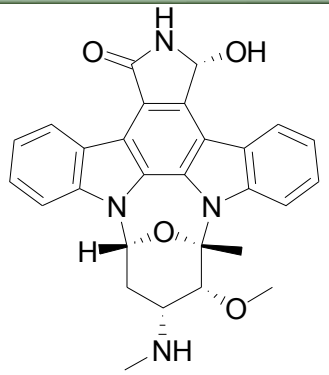
CDK inhibitors in clinical development



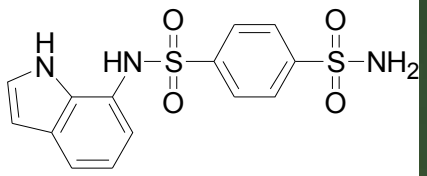
1: Roscovitine (Cyclacel)



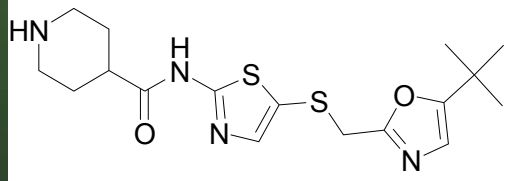
2: Flavopiridol (Aventis/NCI)



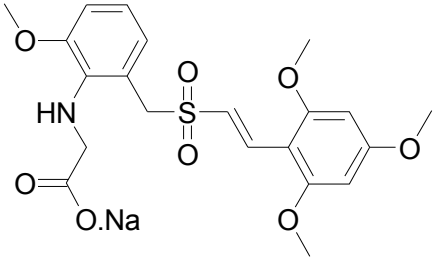
3: UCN-01 (Kyowa Hakko Kogyo)



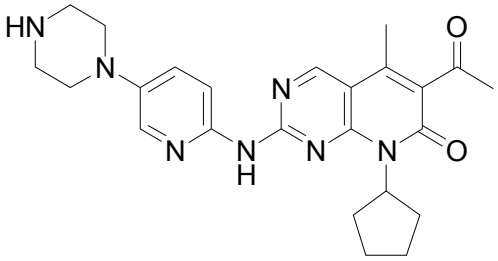
4: E7070 (Eisai)



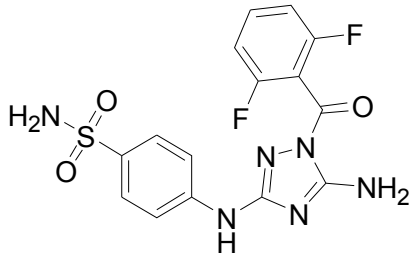
5: SNS-032 (Sunesis)



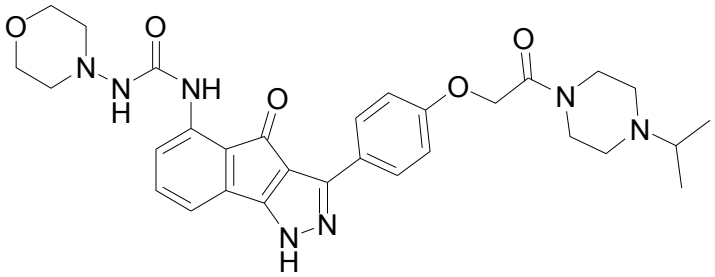
6: ON 01910Na (Onconova)



7: PD 0332991 (Pfizer)



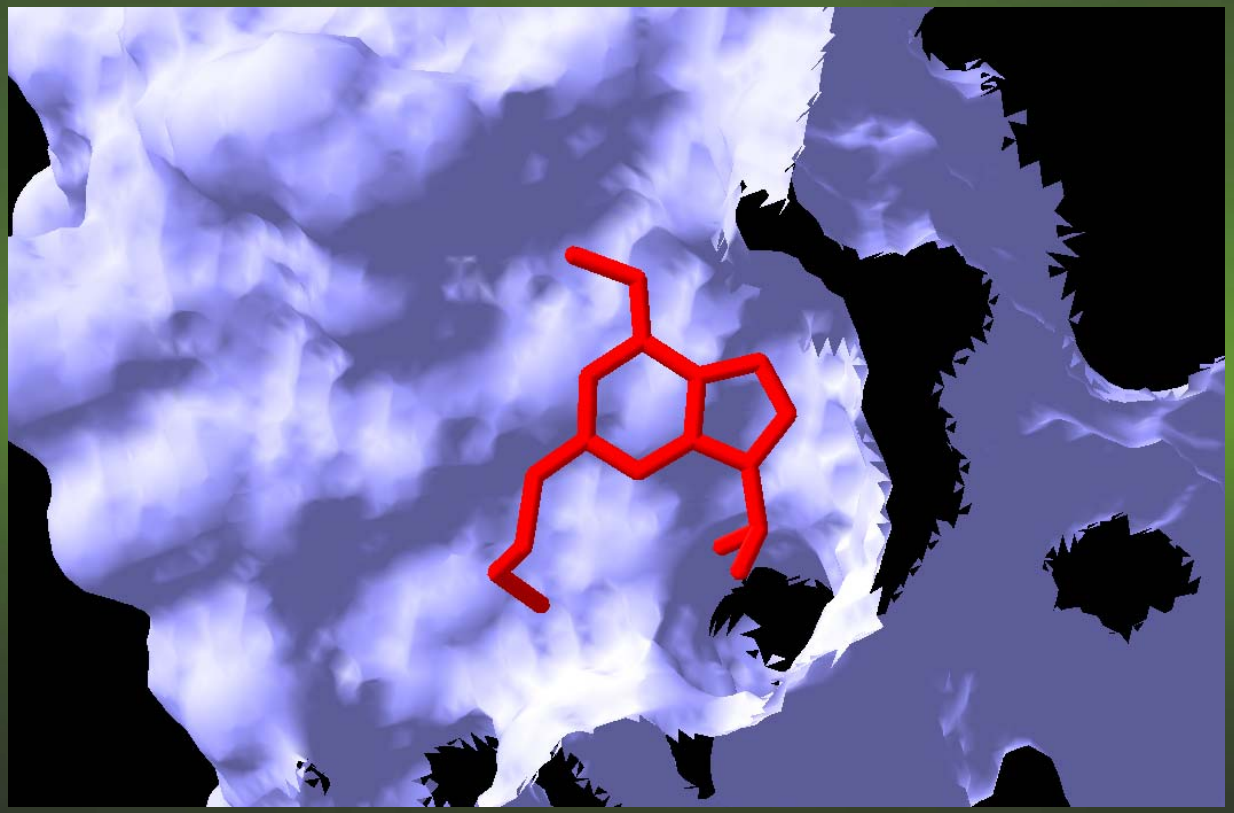
8: JNJ-7706621 (Johnson & Johnson)



9: GPC-286199 (GPC Biotech)

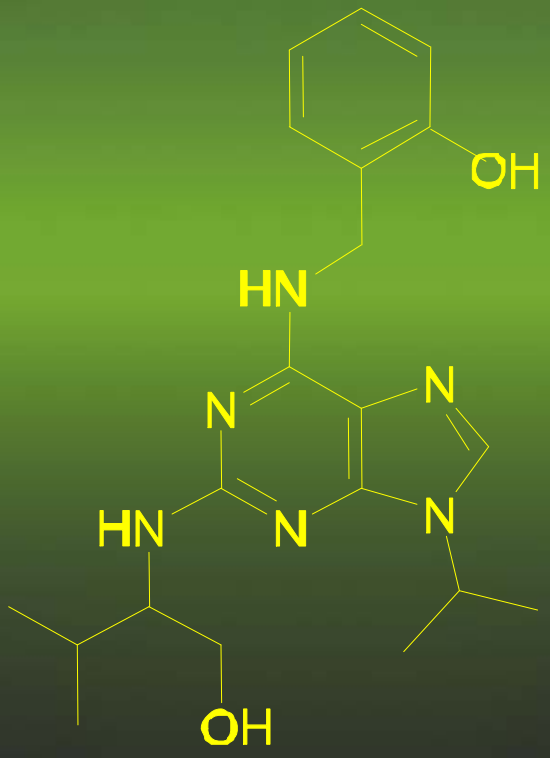
Cytokinin-Like Inhibitors of CDK9 (CDK7) = Olomoucine II

Molecular Docking



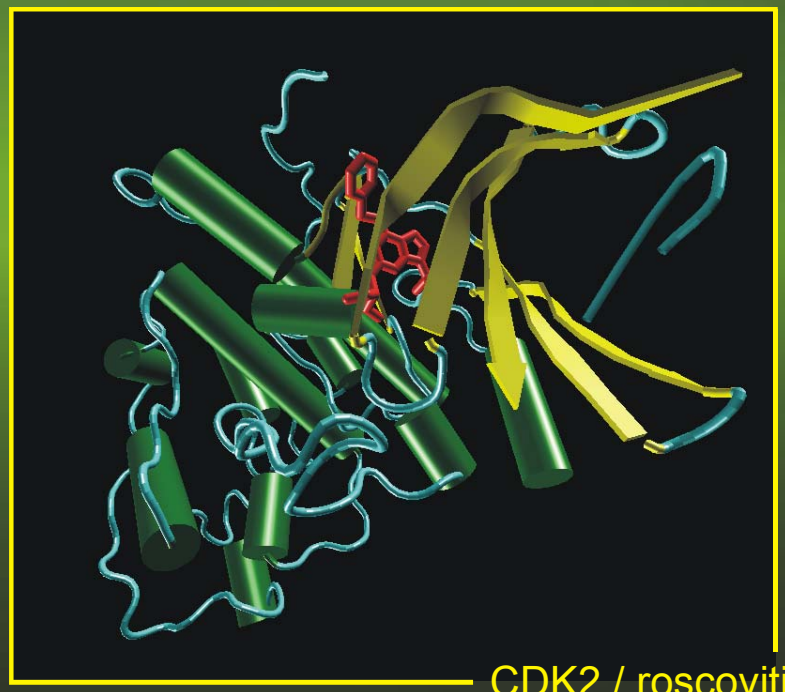
Insight into the active site of CDK2 with a purine bound.

Olomoucine II: *ortho*-Hydroxylated Roscovitine Derived from *ortho*-Topolin



Biochemical Aspects of Roscovitine

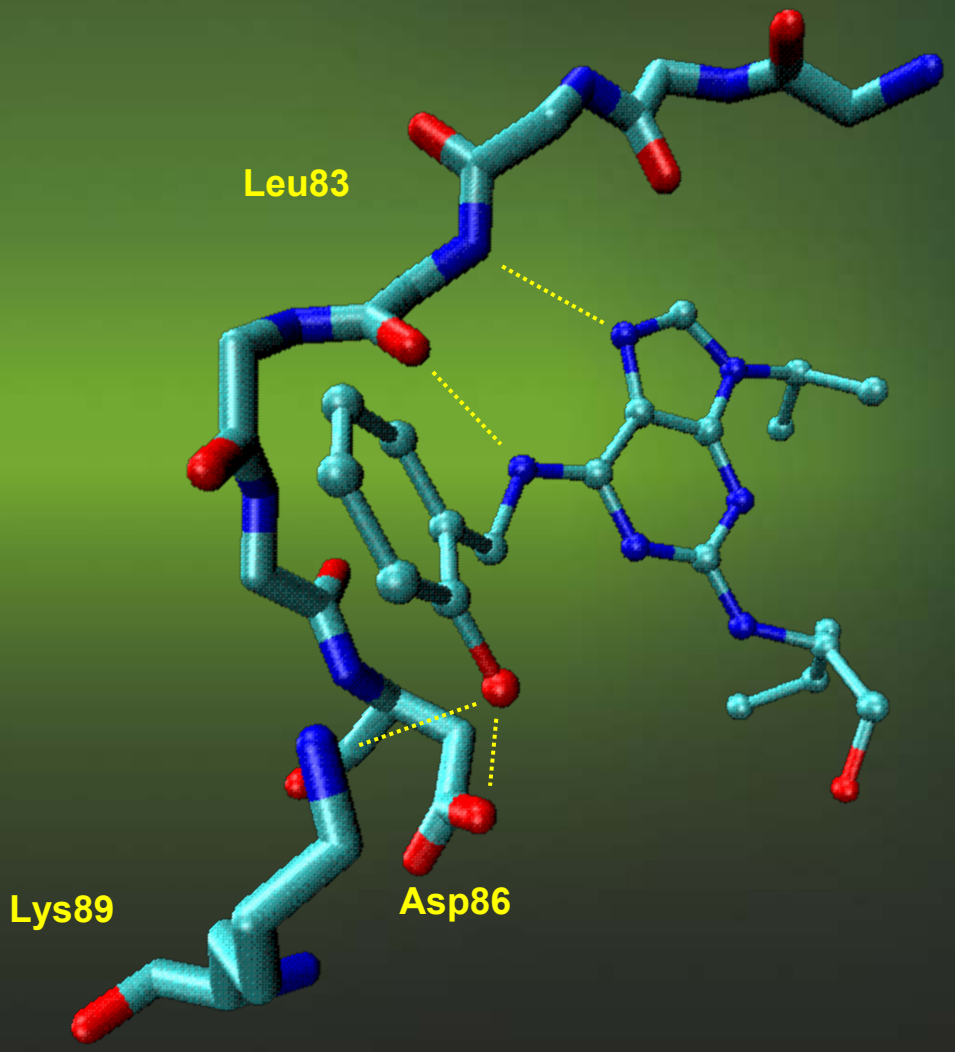
kinase	IC50 / μM
CDK1/B	2.7
CDK2/E	0.84
CDK4/D	14.2
CDK7/H	0.49
CDK9/T	0.72
Erk2	1.17
PKA	>100
PKC	>100
CHK1	>100
c-Abl	>100



CDK2 / roscovitine co-crystal

Olomoucine II Interactions with CDK2

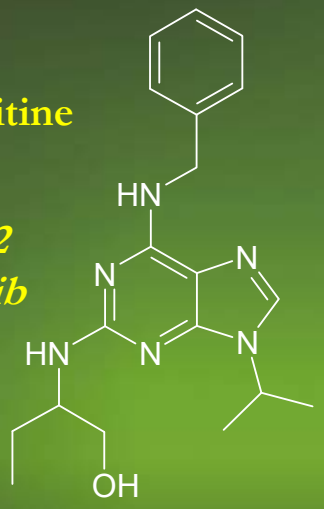
kinase	IC_{50} / µM
CDK1/B	2.7
CDK2/E	0.1
CDK4/D	20
CDK7/H	0.45
CDK9/T	0.06
Erk2	32
PKA	>100
PKC	>100
CHK1	>100
c-Abl	>100



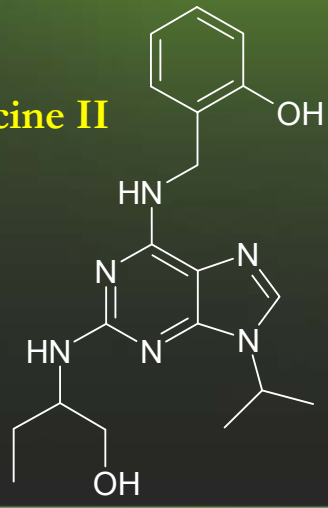
Anticancer effect of olomoucine II

Roscovitine

CYC202
Seliciclib



Olomoucine II

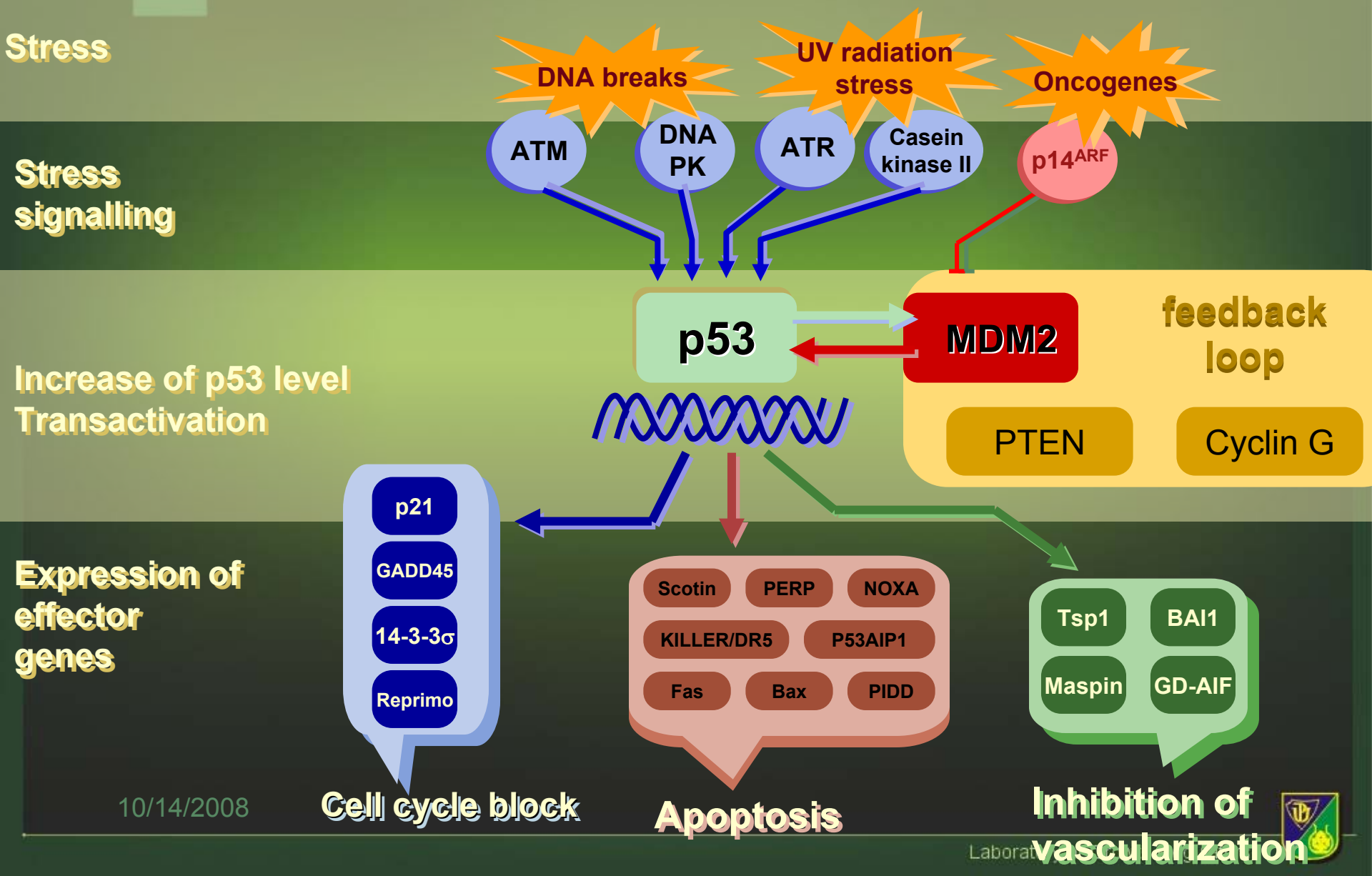


Panel/Cell Line	Log_{10}GI50	GI50
Leukemia		
CCRF-CEM	-5.66	
HL-60(TB)	-5.68	
K-562	-6.20	
MOLT-4	-5.54	
RPMI-8226	-5.36	
SR	-5.87	
Non-Small Cell Lung Cancer		
A549/ATCC	-5.40	
EKVX	-4.82	
HOP-62	-5.25	
HOP-92	-5.30	
NCI-H226	-5.05	
NCI-H23	-5.53	
NCI-H322M	-5.28	
NCI-H460	-5.61	
NCI-H522	-5.75	
Colon Cancer		
COLO 205	-5.71	
HCC-2998	-6.67	
HCT-116	-5.58	
HCT-15	-5.65	
HT29	-5.43	
KM12	-5.41	
SW-620	-5.48	
CNS Cancer		
SF-268	-5.59	
SF-295	-5.53	
SF-539	-5.43	
SNB-19	-5.48	
SNB-75	-5.71	
U251	-5.32	
Melanoma		
LOX IMVI	-5.58	
MALME-3M	-4.40	
M14	-5.32	
SK-MEL-2	-5.75	
SK-MEL-28	-5.63	
SK-MEL-5	-5.60	
UACC-257	-4.49	
UACC-62	-5.64	
Ovarian Cancer		
IGROV1	-5.74	
OVCAR-3	-5.51	
OVCAR-4	-5.27	
OVCAR-5	-5.43	
OVCAR-8	-5.38	
SK-OV-3	-5.23	
Renal Cancer		
786-0	-5.35	
A498	-5.44	
ACHN	-5.71	
CAKI-1	-4.81	
RXF 393	-5.48	
SN12C	-5.56	
TK-10	-4.99	
UO-31	-7.11	
Prostate Cancer		
PC-3	-5.25	
DU-145	-5.35	
Breast Cancer		
MCF7	-5.54	
NCI/ADR-RES	-5.18	
MDA-MB-231/ATCC	-5.37	
HS 578T	-5.51	
MDA-MB-435	-5.58	
BT-549	-5.56	
T-47D	-5.30	

roscovitine Mean GI_{50}= 19 μM

olomoucine II Mean GI_{50}= 3.3 μM

p53 signalling



Stabilization of p53/Induction of p21

CDK2
Cyklin E

CDK1
Cyklin B

MDM2
p14 ARF

p14 ARF

p21 CIP1

apoptoses

p53

p53 P

MDM2

Chk2

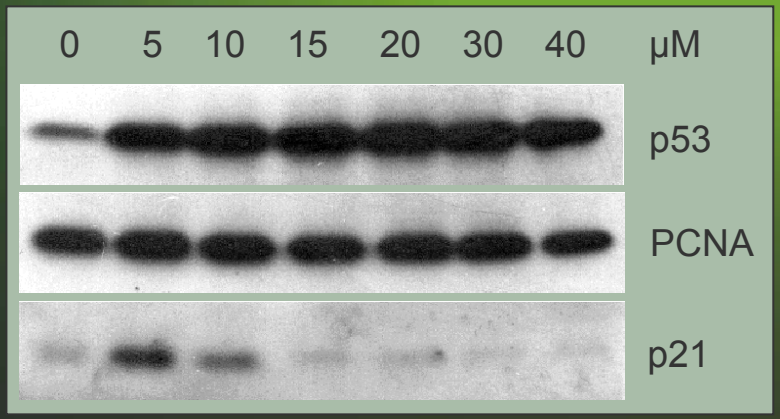
MDM2 P

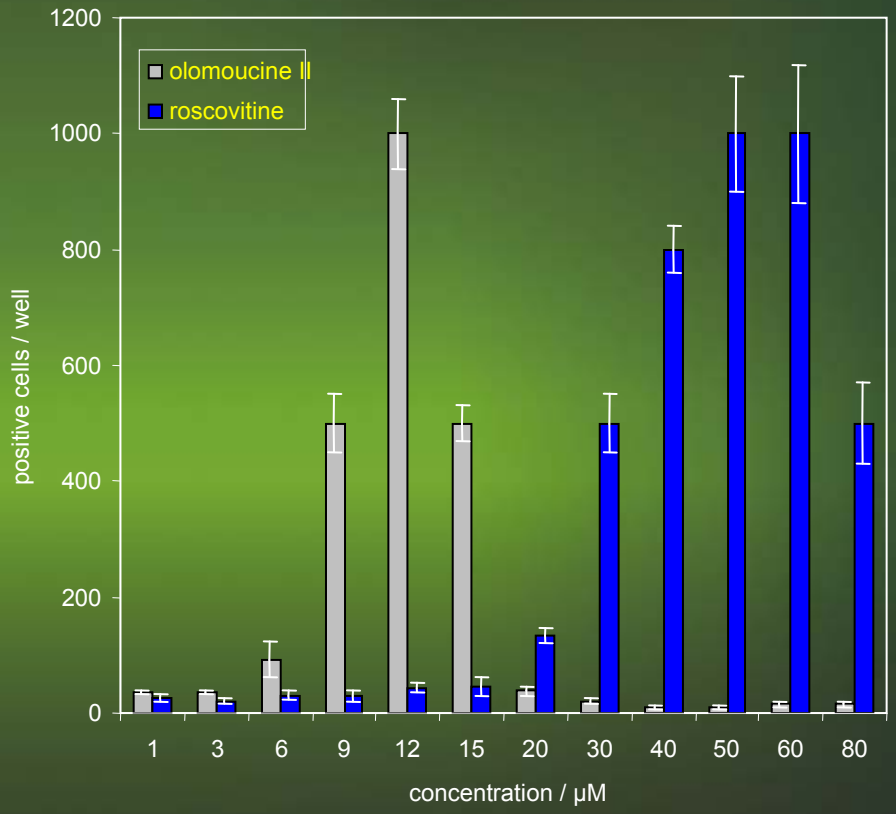
ATM

DNA damage, hypoxia and temperature shock ...

Stabilization of p53/Induction p21 expression

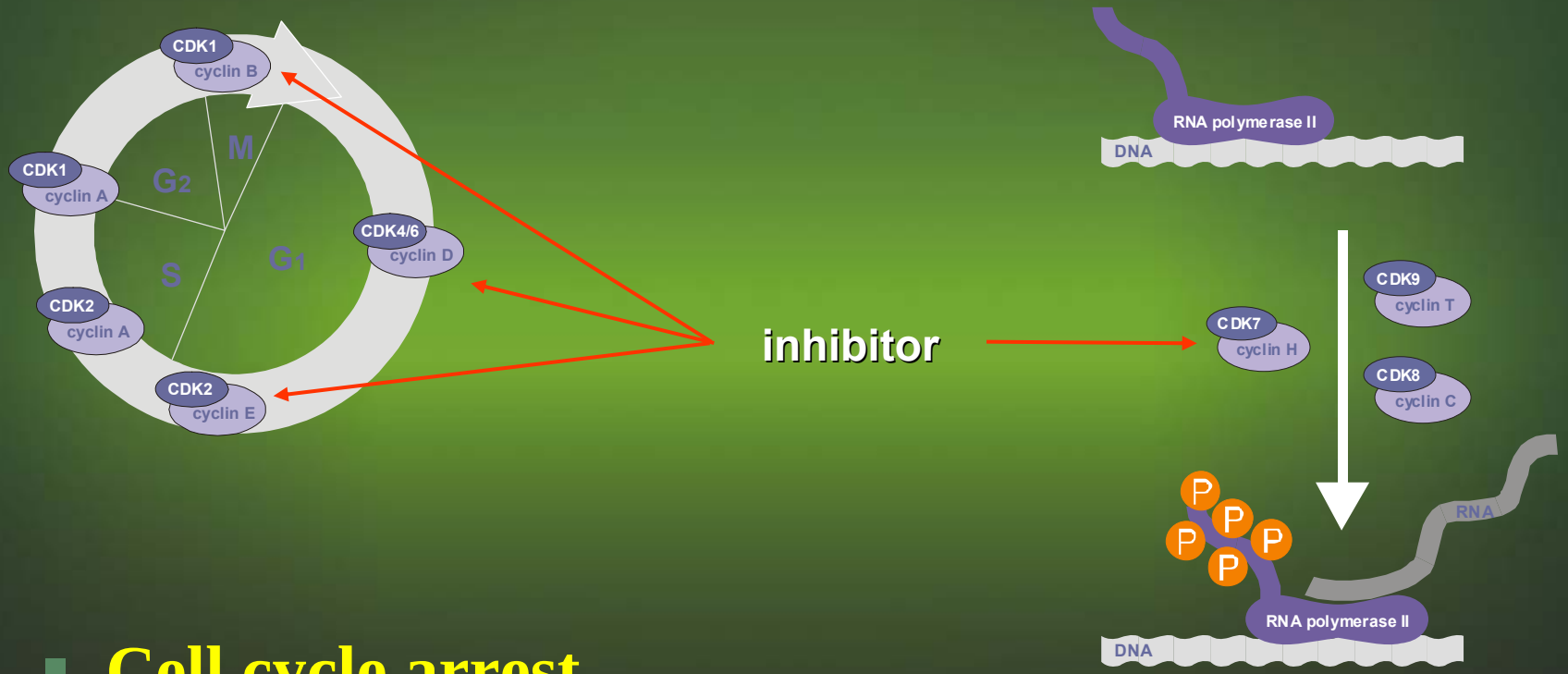
Accumulation of p53 and p21^{WAF} proteins in olomoucine II treated MCF7 cells





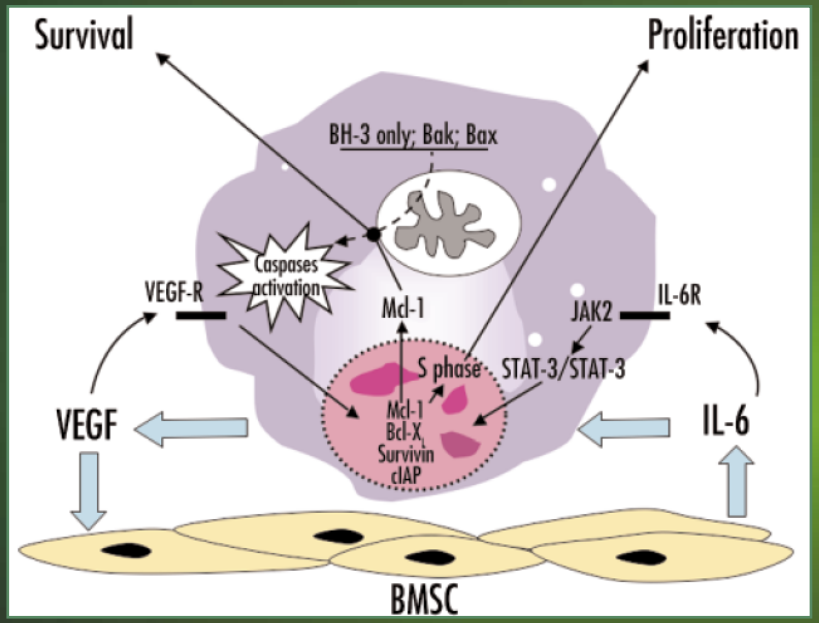
Induction of p53-transcriptional activity by olomoucine II in Arn8 cells with reporter system

Anticancer activity of CDK inhibitors



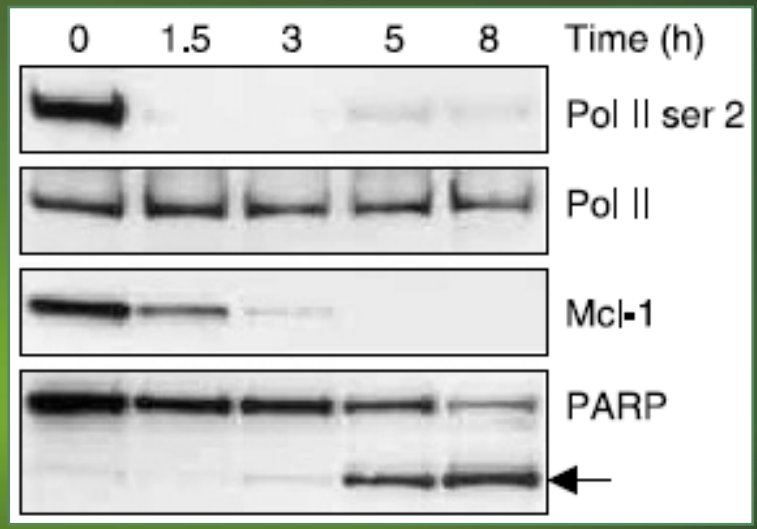
- **Cell cycle arrest**
- **Induction of apoptosis**
- **Activation of p53**

Anticancer activity of CDK inhibitors



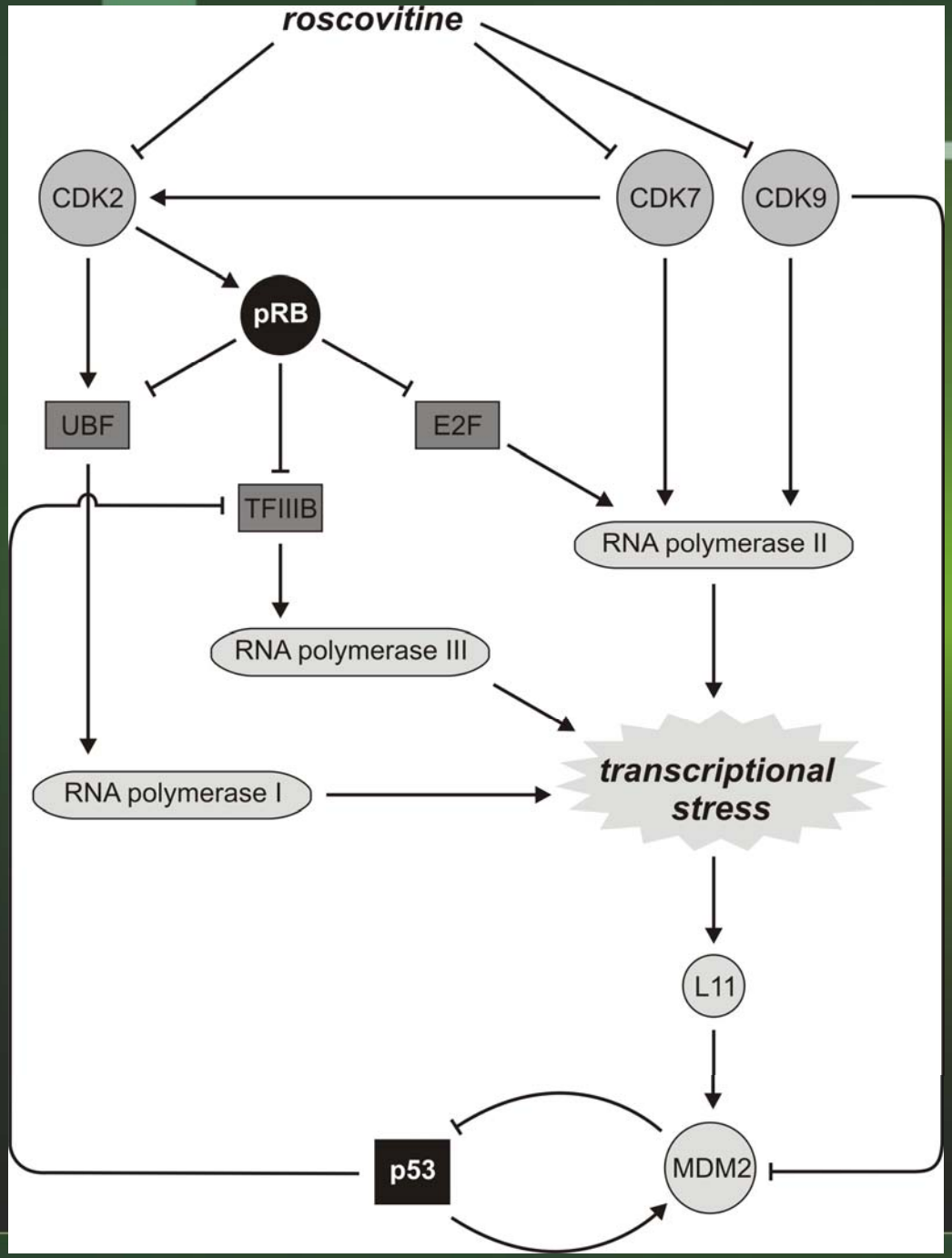
Cell Cycle. 2004 Oct;3(10):1259-62

Pivotal role for Mcl-1 in multiple myeloma cells. Mcl-1 (a member of the Bcl-2 family) antagonizes apoptosis upon mitogenic and survival stimuli. It is rapidly degraded in response to cell death signals.



Roscovitine inhibits phosphorylation of RNA polymerase II by CDK9.
Inhibition of transcription exerts its greatest effect on gene products where both mRNA and protein have short half-lives, resulting in rapid decline of the protein levels. Mcl-1, crucial for the survival of a range of cell types including multiple myeloma, is rapidly down-regulated, which precededs the induction of apoptosis.

Cancer Res. 2005 Jun 15;65(12):5399-407.



Roscovitine blocks transcription through combined inhibition of CDK2, CDK7 and CDK9. Being activated by roscovitine, tumor suppressor proteins pRB and p53 potently repress transcription factors necessary for RNA polymerase I (UBF), RNA polymerase II (e.g. E2F) and RNA polymerase III (TFIIIB).

Structure of Our Research

- Cell Cycle and CDKs
- Cytokinin-Like Inhibitors of Cyclin -Dependent Kinases 1/2
- Cytokinin-Like Inhibitors of CDK7 a CDK9 – p53 activation
- Selective Inhibitors of CDK9
- Anticytokinins as New Database for Development of CDK Inhibitors
- Anticancer Drugs Combining CDK Inhibition with Antiinflammatory properties
- Anticancer Drugs Exhibiting Antiviral Activities

CDKI Therapeutic Applications

- oncology (new generation of drugs affecting cell cycle)
- neurology (Alzheimer's disease, stroke)
- virology (human cytomegalovirus, herpes virus ...)
- parasitology (*Plasmodium*,*Trypanosoma*, *Toxoplasma* ...)

... and other diseases resulting from uncontrolled proliferation

atherosclerosis
post-angioplastic restenosis
tumor angiogenesis
glomerulonephritis
psoriasis

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