Identificacion de nuevos farmacos antitumorales

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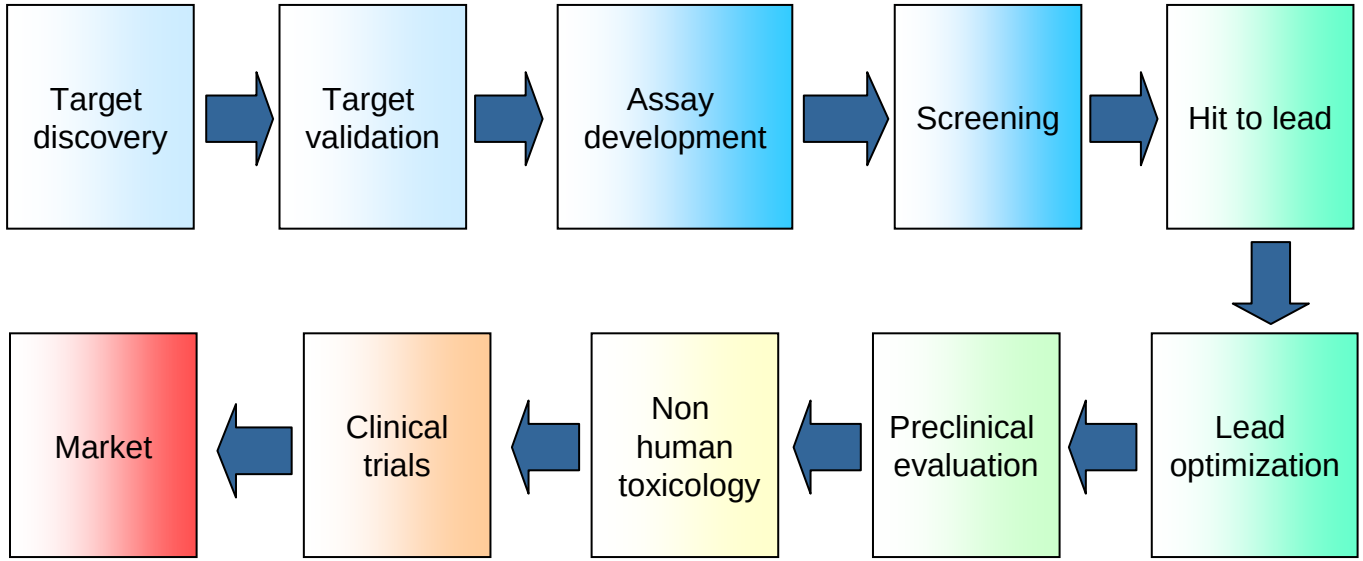
Experimental Therapeutics
CNIO

Drug discovery process



HTS cytotoxicity

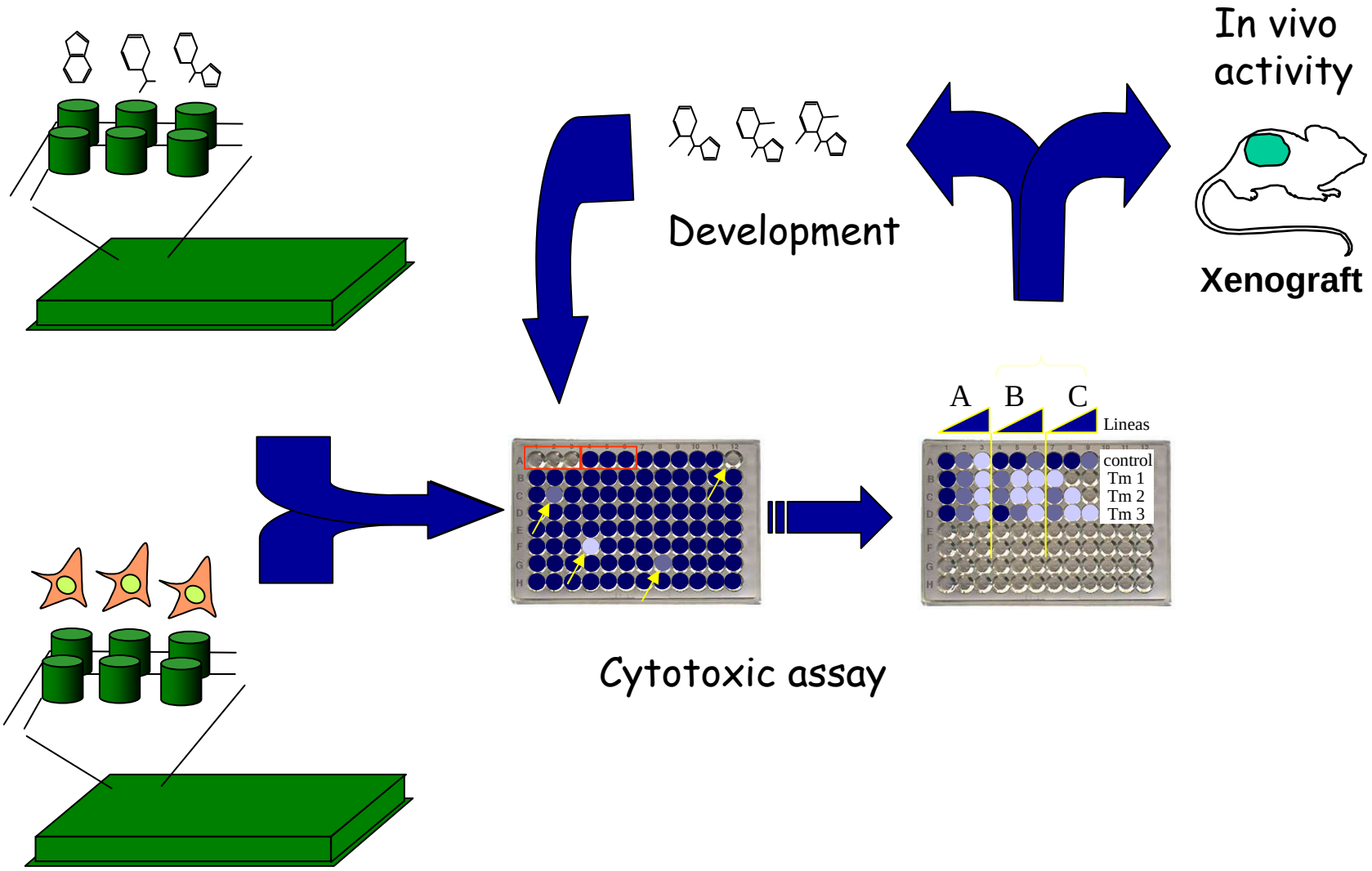
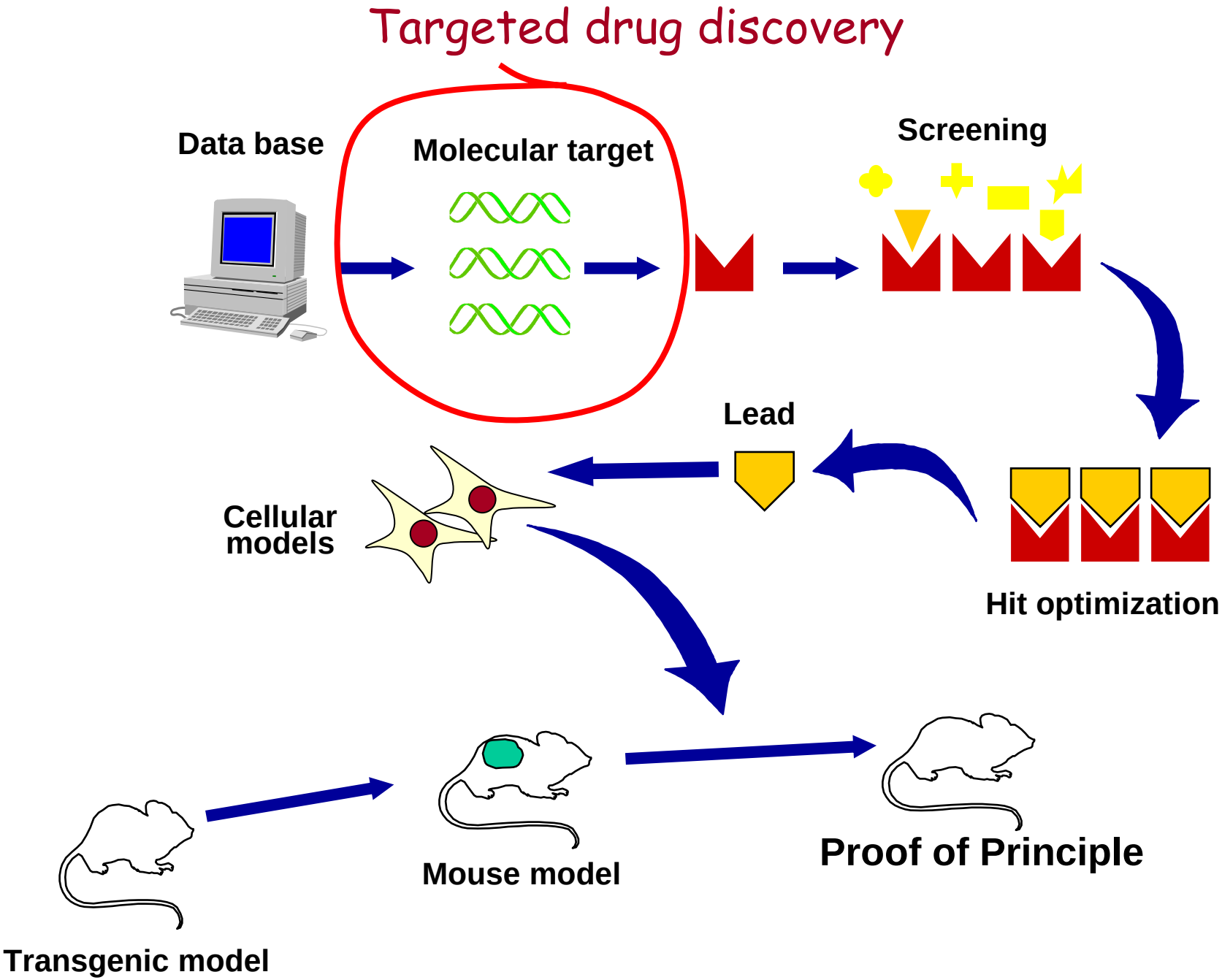
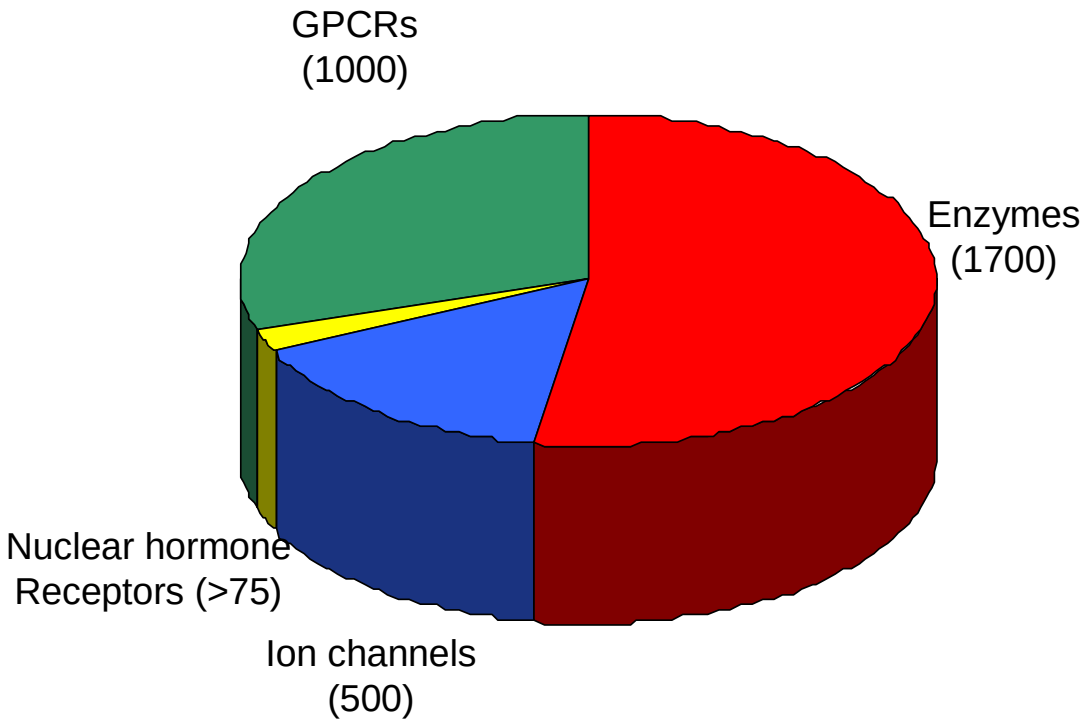


Table 1 **List of Newly Approved Drugs and Their Targets**

Trade/Proprietary Name	Approval	Target	Classification
Alimta/Pemetrexed	2004	DNA synthesis	cytotoxic
Avastin /bevacizumab	2004	VEGF	antiangiogenic
Erbitux (cetuximab)	2004	EGF receptor	semi-selective
Bexxar/Tositumonab	2003	CD20	tissue-selective
Velcade/Bortezomib	2003	proteasome	cytotoxic
Iressa/Gefitinib	2003	EGF receptor	semi-selective
Eloxatin/Oxaliplatin	2002	DNA (alkylating)	cytotoxic
Zevalin/Ibritumomab tiuxetan	2002	CD20	tissue-selective
Gleevec/Imatinib mesylate*	2001	Bcr-Abl*	semi-selective
Campath/Alemtuzumab	2001	CD52	tissue-selective
Trisenox/Arsenic trioxide	2000	multiple	cytotoxic
Mylotarg/Gemtuzumab	2000	CD33	tissue-selective
Temodar/Temozolomide	1999	DNA (alkylating)	cytotoxic
Valstar/Valrubicin	1998	DNA (TOPO-II)	cytotoxic
Herceptin/Transtuzumab	1998	erbB2	semi-selective
Xeloda Capecitabine	1998	DNA (metabolism)	cytotoxic
Rituxan/Rituximab	1997	CD20	tissue-selective
Intron A/Interferon-a	1997	IFN receptor	tissue-selective
Camptosar/Irinotecan	1996	DNA (TOPO-I)	cytotoxic
Hycamtin/Topotecan	1996	DNA (TOPO-I)	cytotoxic
Gemzar/Gemcitabine	1996	DNA (metabolism)	cytotoxic
Taxotere/Docetaxel	1996	microtubules	cytotoxic
Vesanoid/Tretinoin	1995	RAR	cytotoxic**
Vinorelbine/Navelbine	1994	microtubules	cytotoxic
Leustatin/Cladribine	1993	DNA (metabolism)	cytotoxic
Taxol Paclitaxel	1992	microtubules	cytotoxic


Targeted drug discovery
Data base
Molecular target
Screening
Lead
Cellular models
Hit optimization
Transgenic model
Mouse model
Proof of Principle

Predicted numbers of potential drug targets belonging to different biochemical classes.



Molecular target

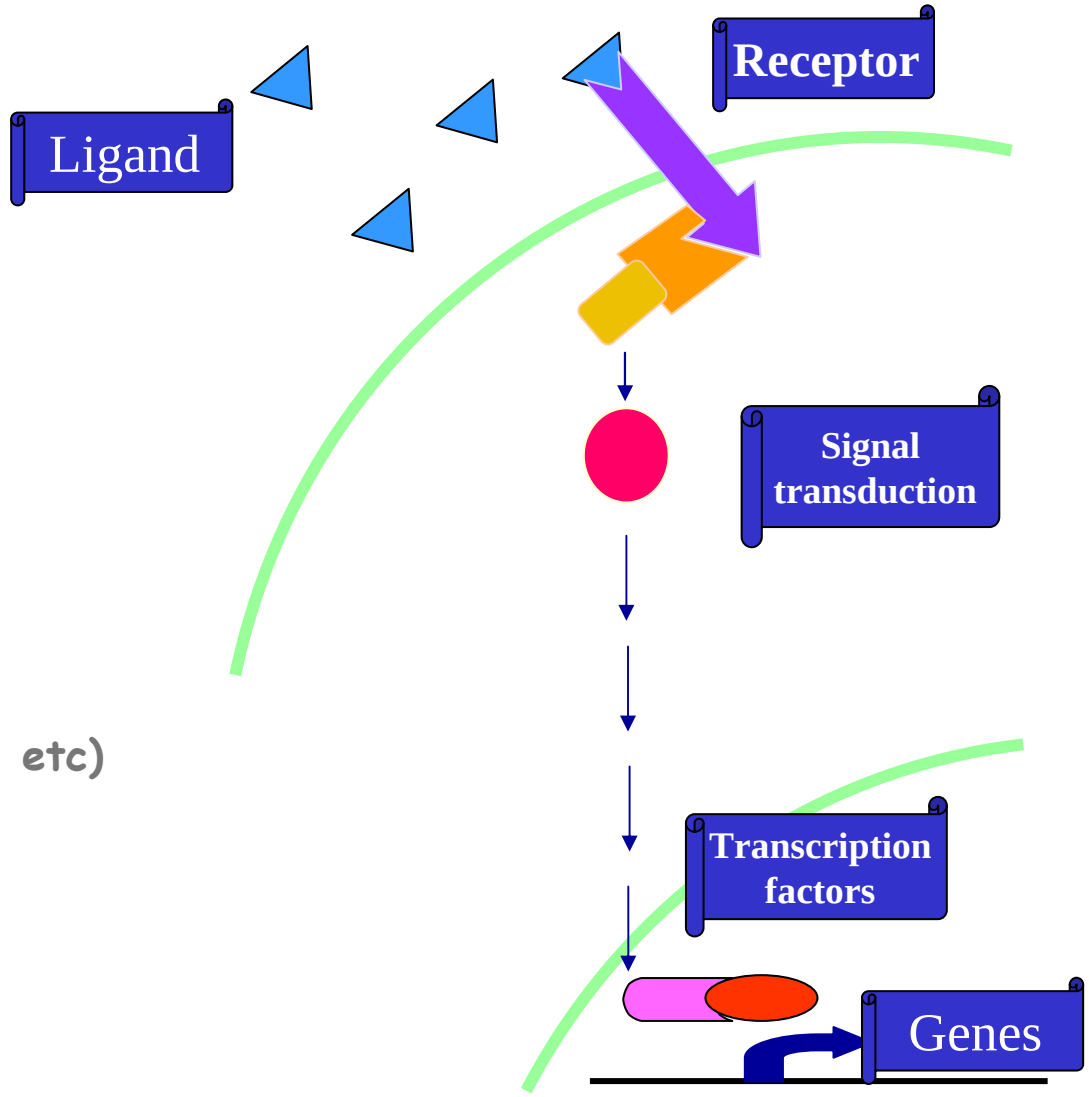
Ligand inhibition
- Ligand production
- Binding to the receptor

Receptor inhibition
- dimerization
- Receptor activity
- Binding adaptor molecules

Signal transduction inhibition
- Binding adaptor molecules
- Binding pathway proteins
- Pathway proteins activity
- Specific localization (membrane, etc)
- Nuclear transport

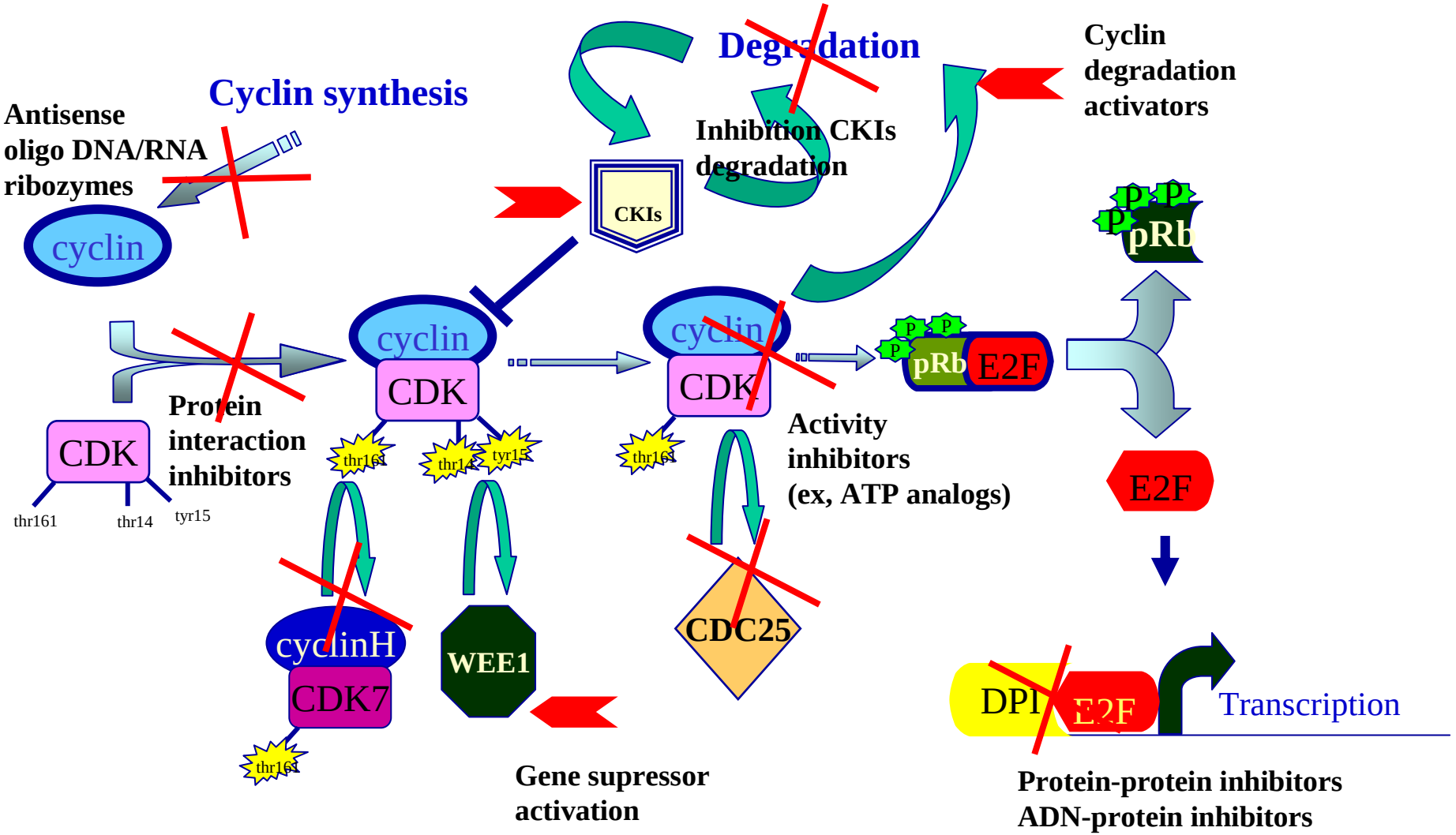
Transcription factor inhibition
- protein-protein interaction
- ADN-protein interaction

Effector genes activity

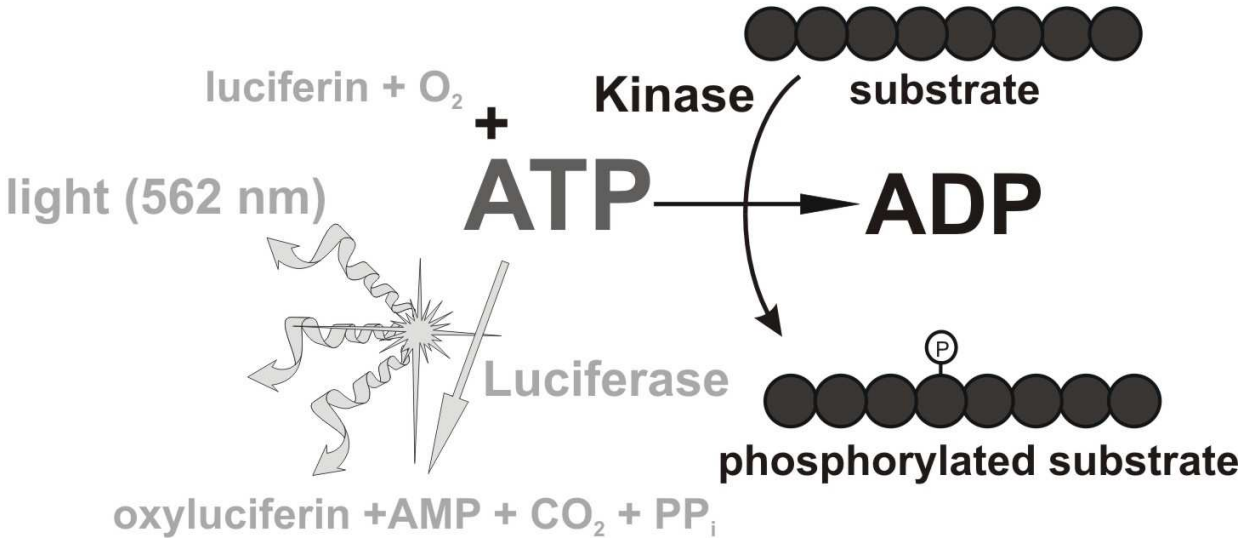


Cell cycle
apoptosis
vascularization
etc

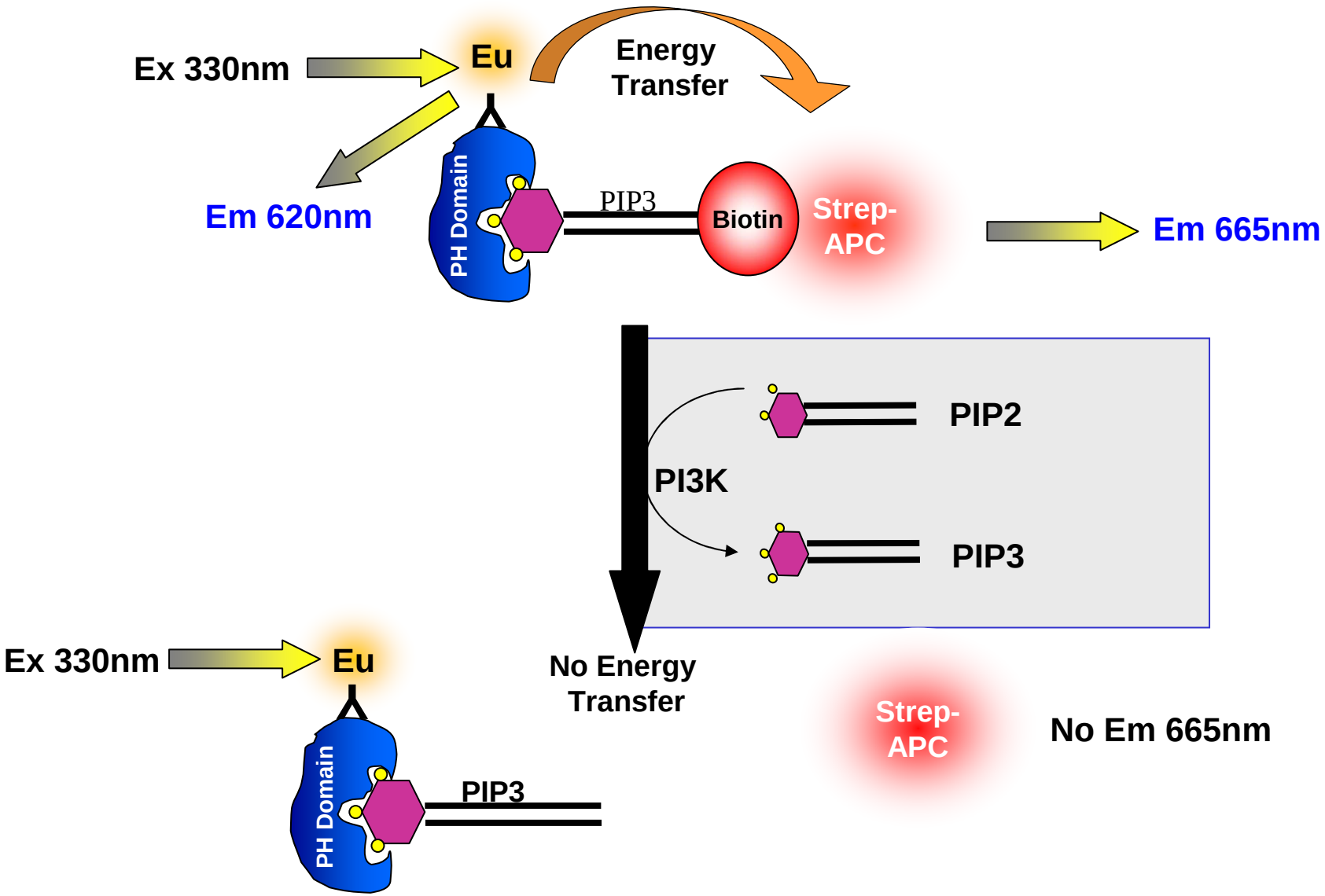
Ex: Cell cycle targets



ATP Consumption Assay:

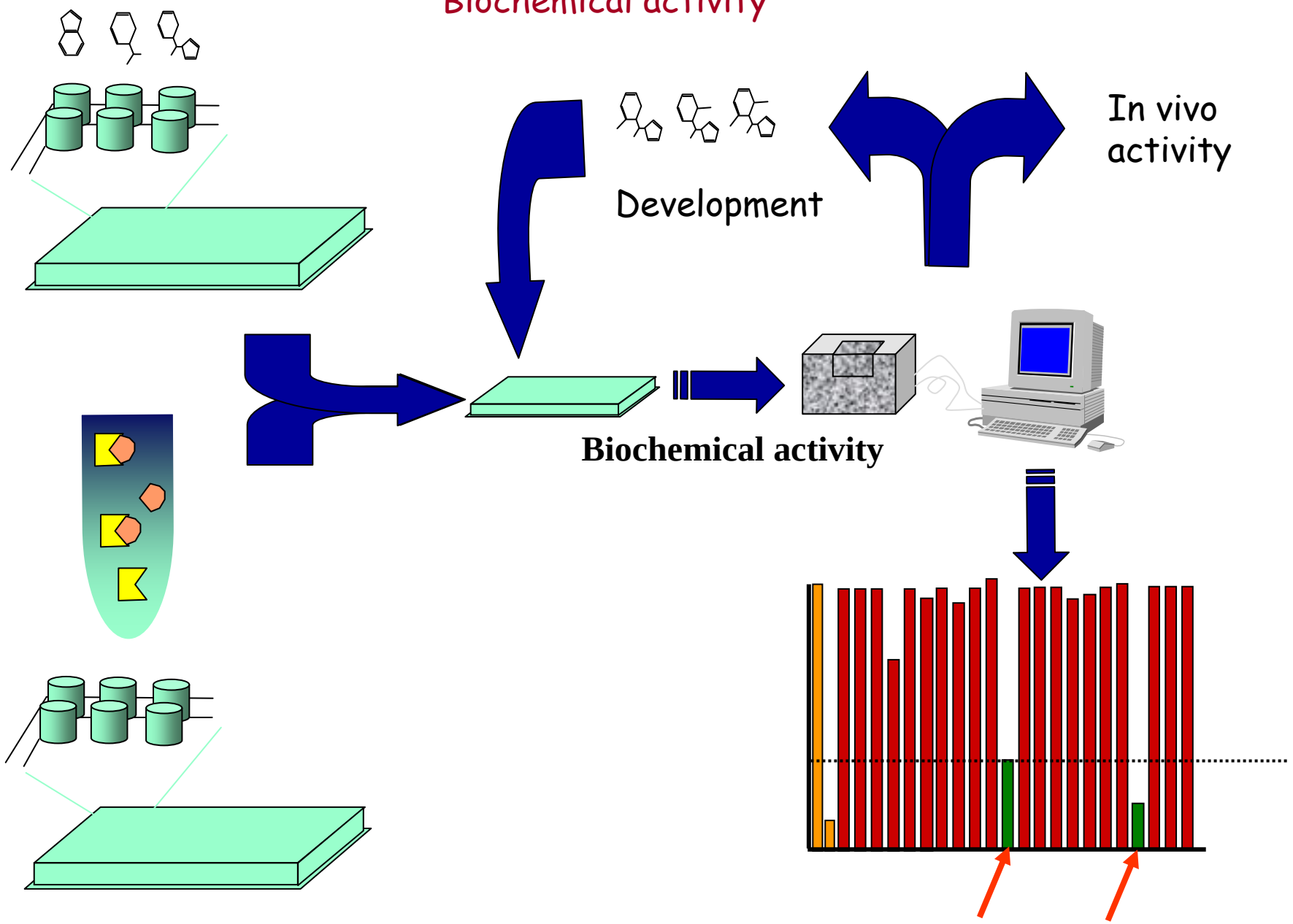


PI 3-Kinase HTRF Assay



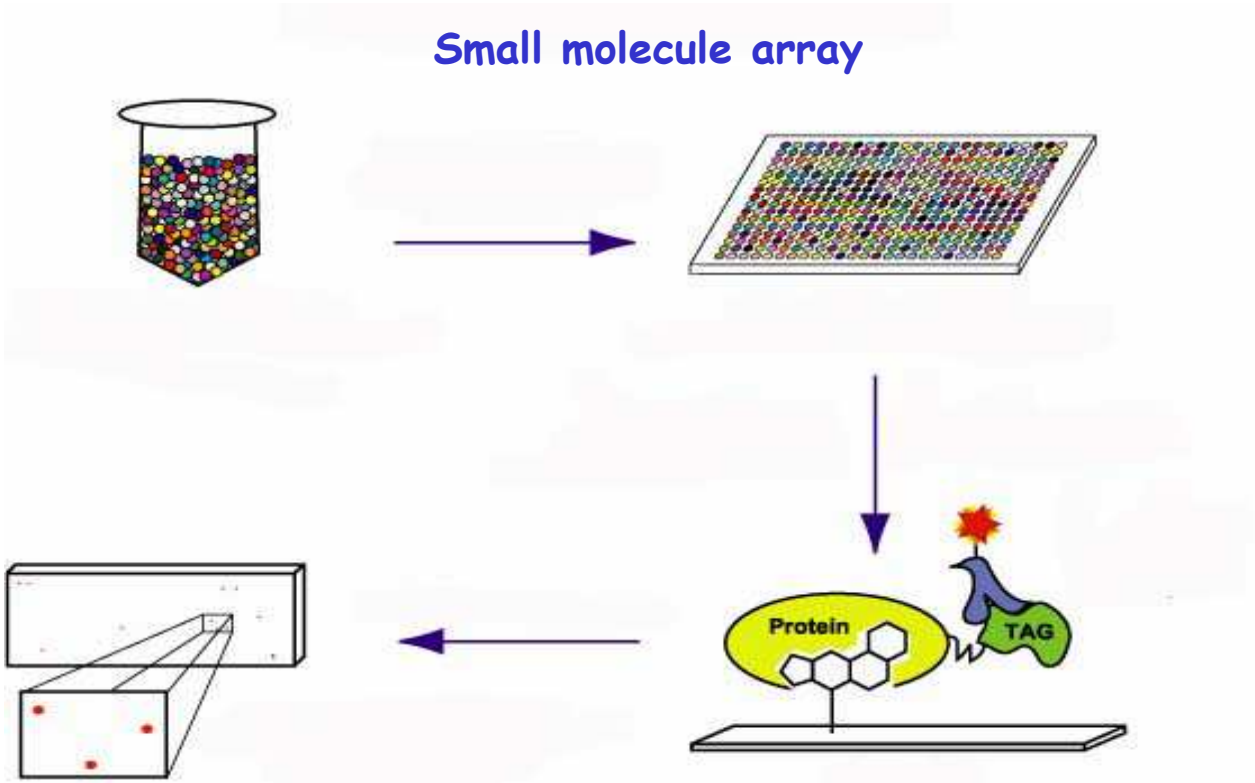
Molecular targeted HTS

Biochemical activity

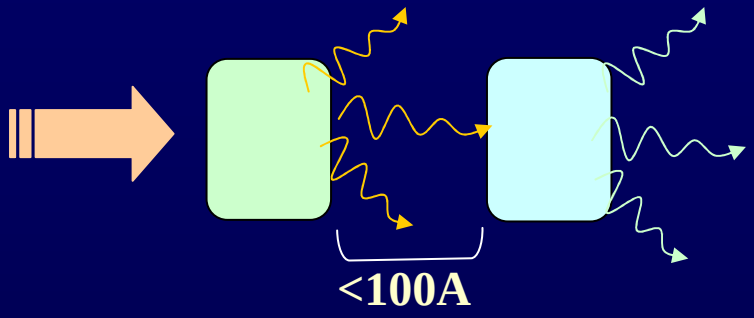


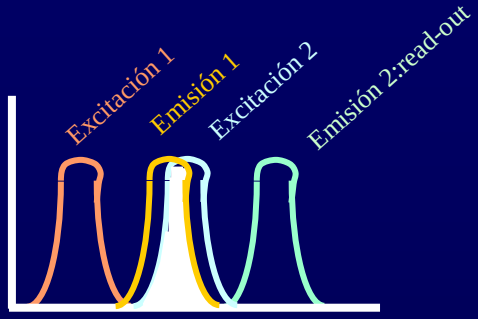
Chemical genomics

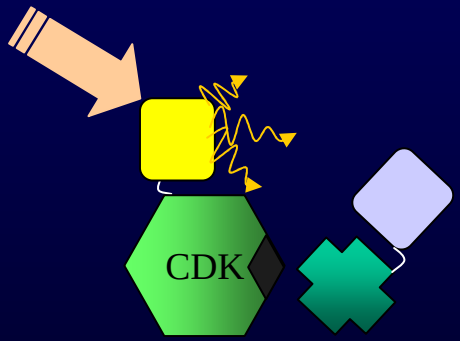
Small molecule partner for every gene product

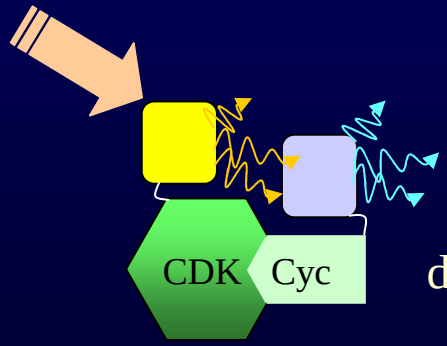


FRET: Fluorescence resonance energy transfer

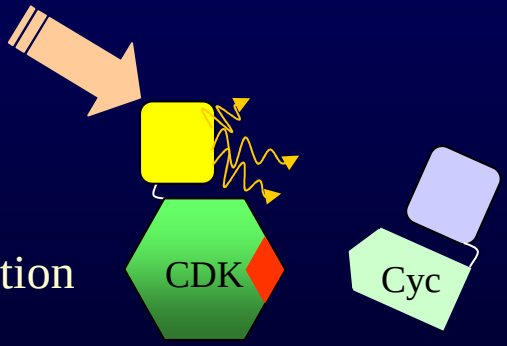


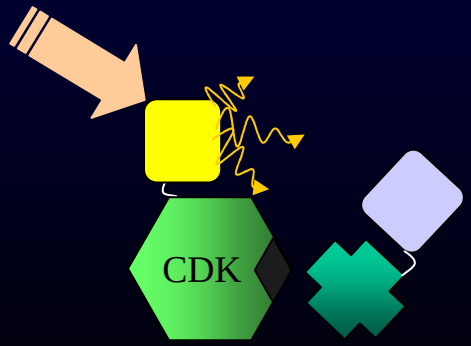


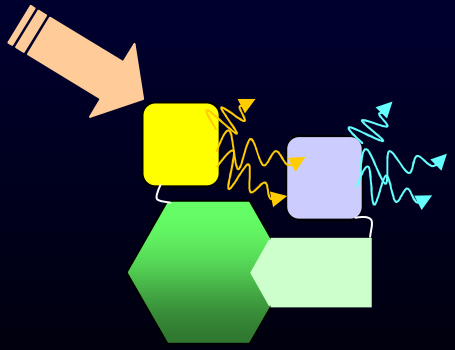




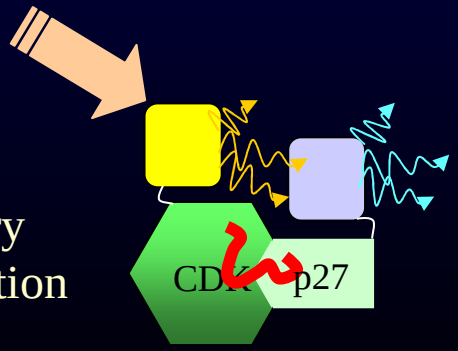
dissociation





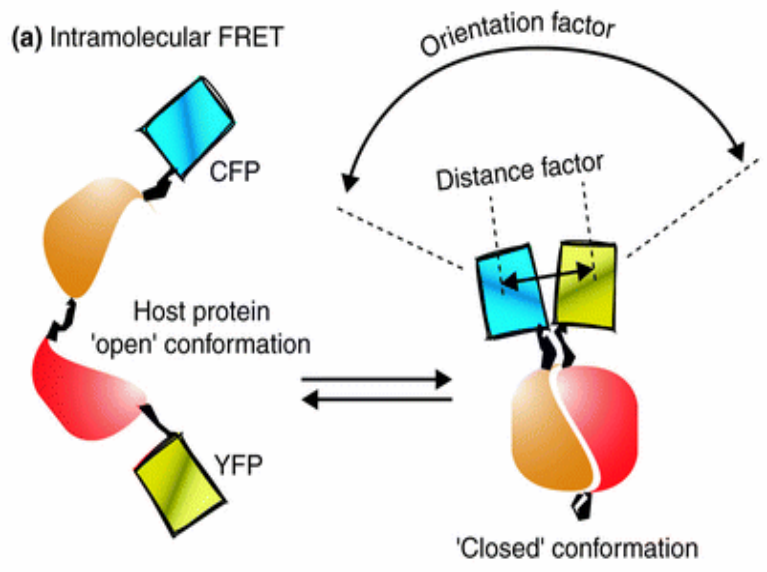


Inhibitory stabilization

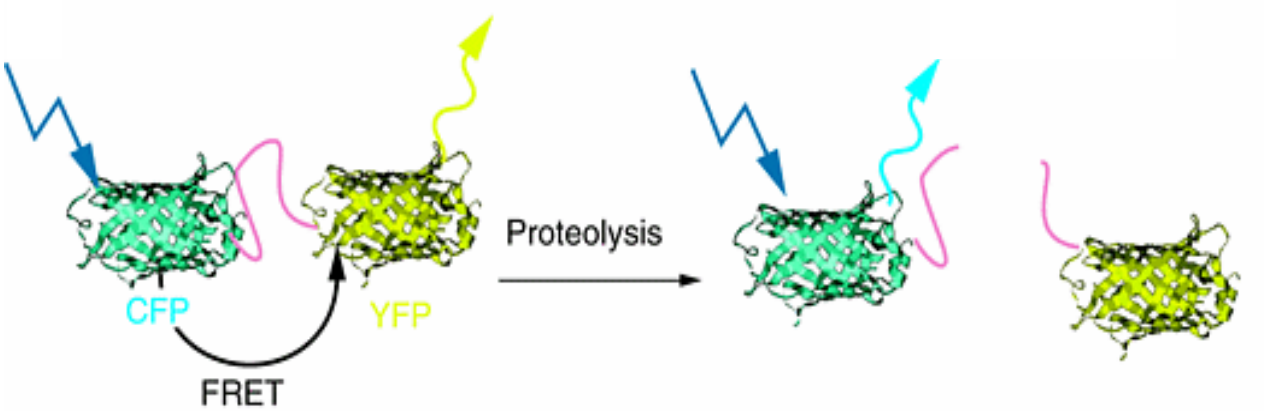


Intramolecular FRET

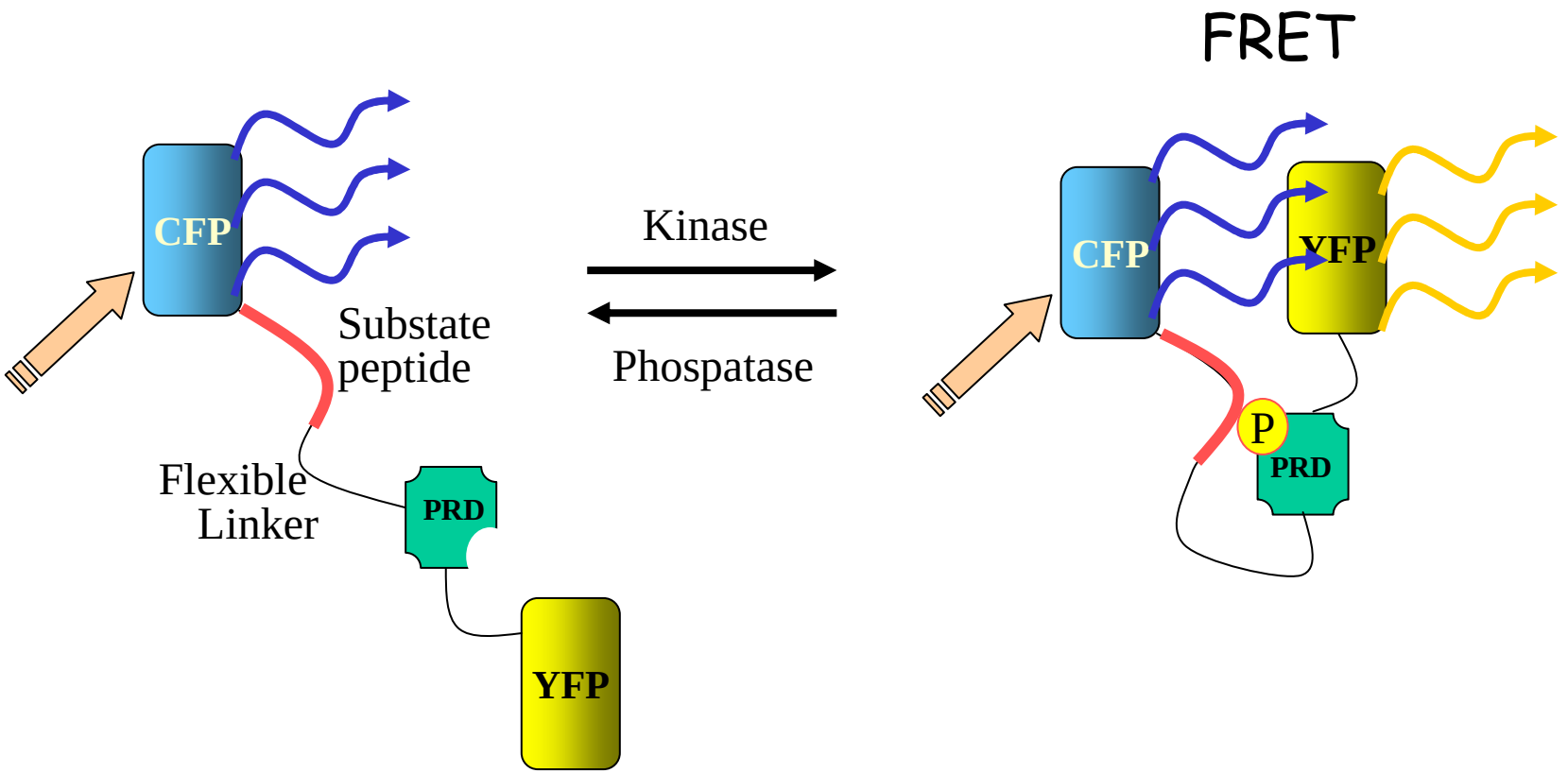
Conformational change



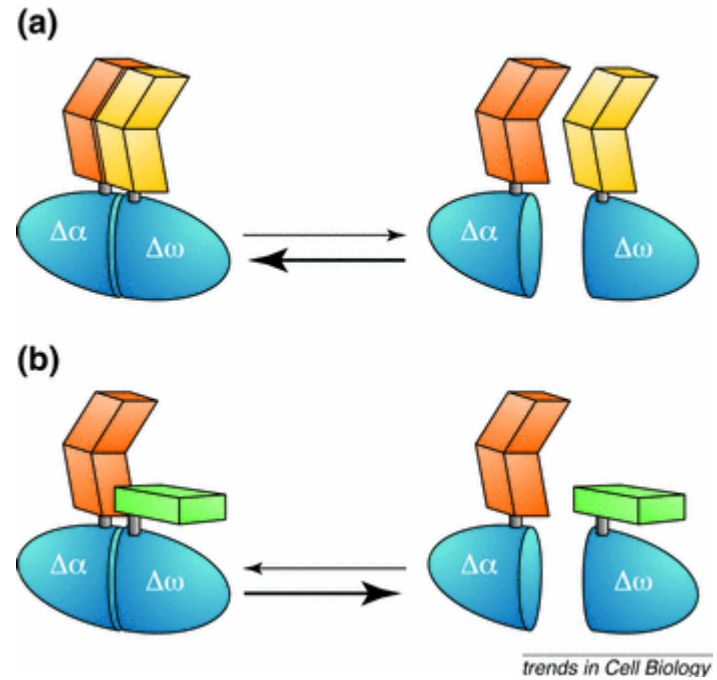
proteolysis



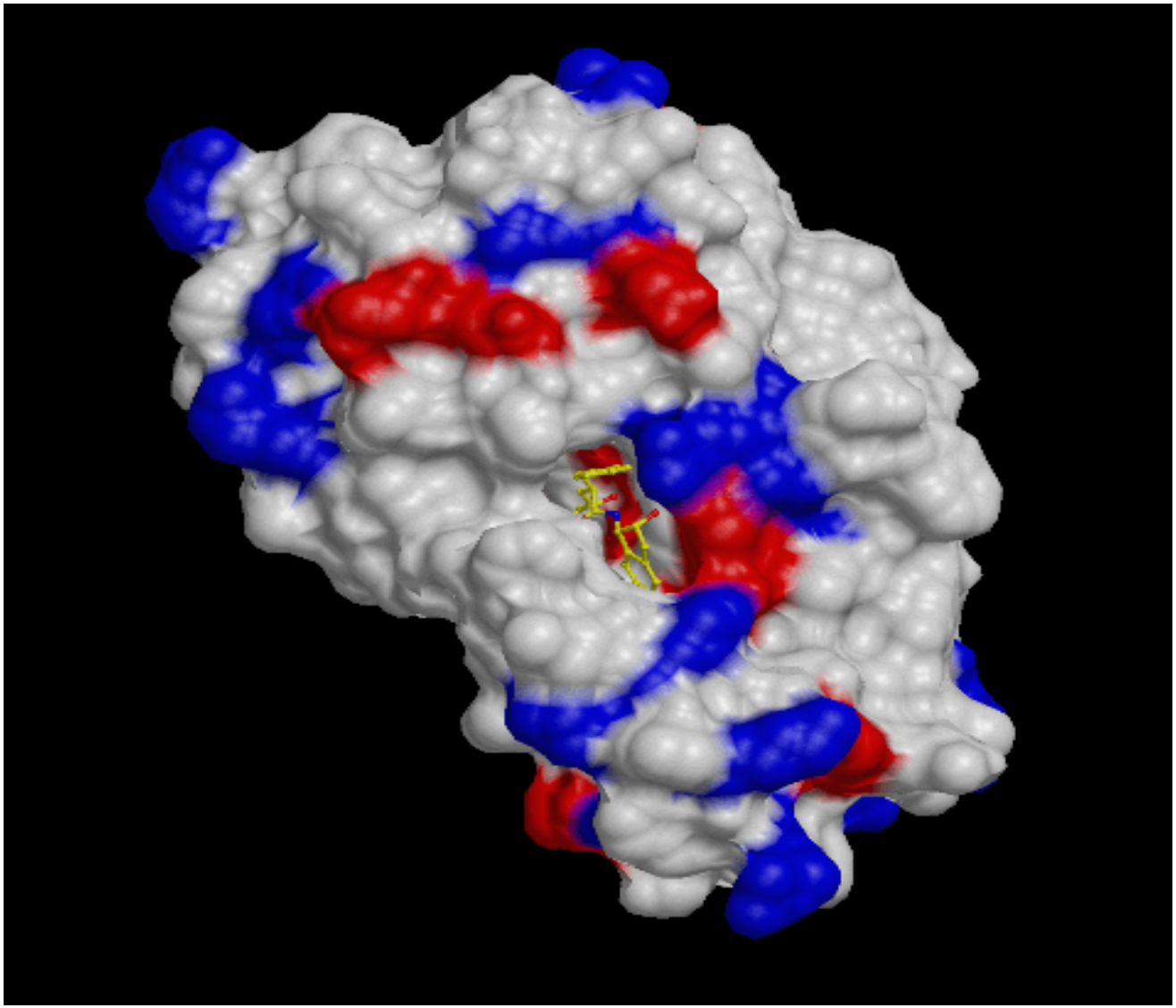
FRET as read-out for phosphorylation



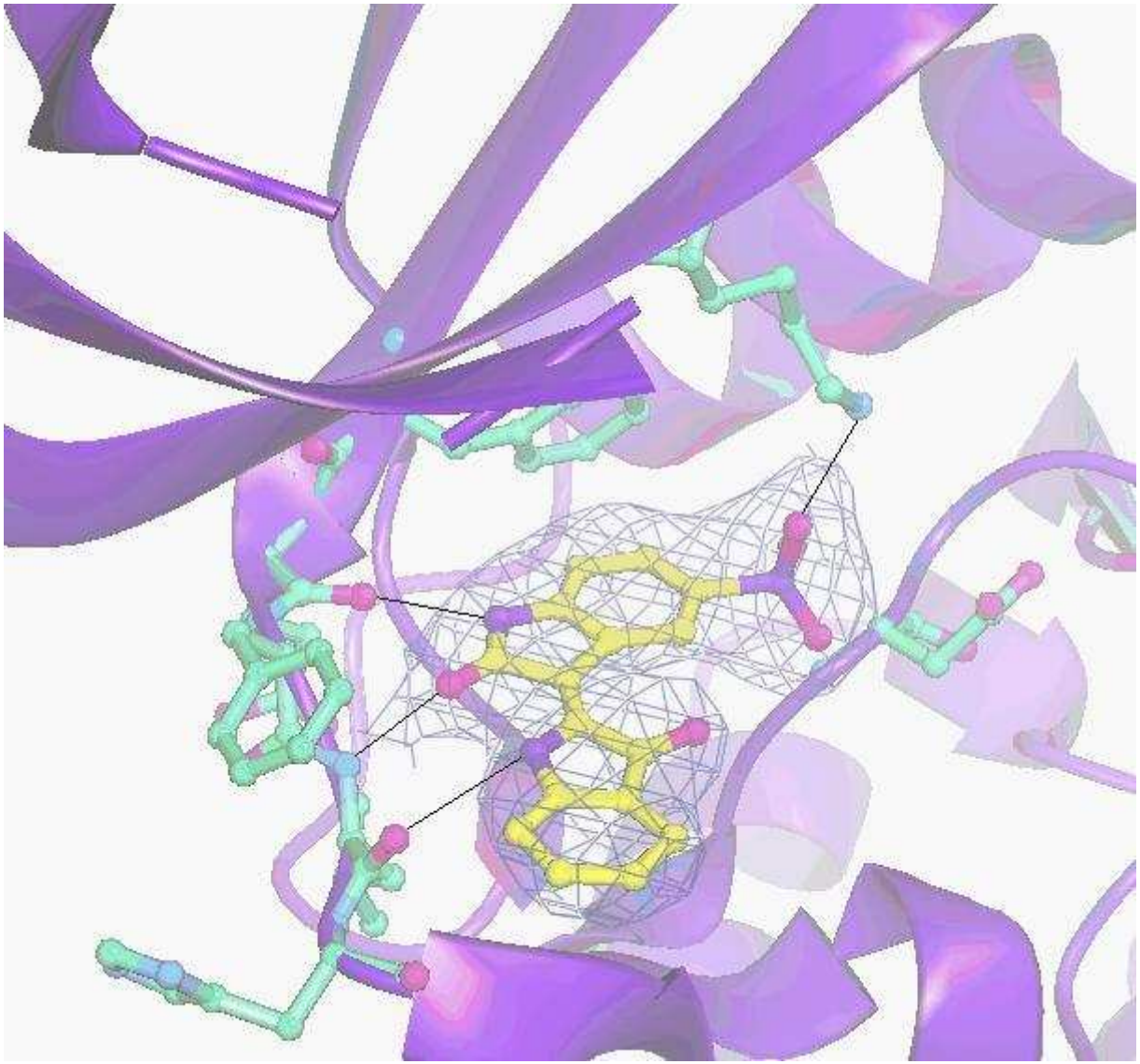
B-Galactosidase Complementation system
Effect of partners domain interaction

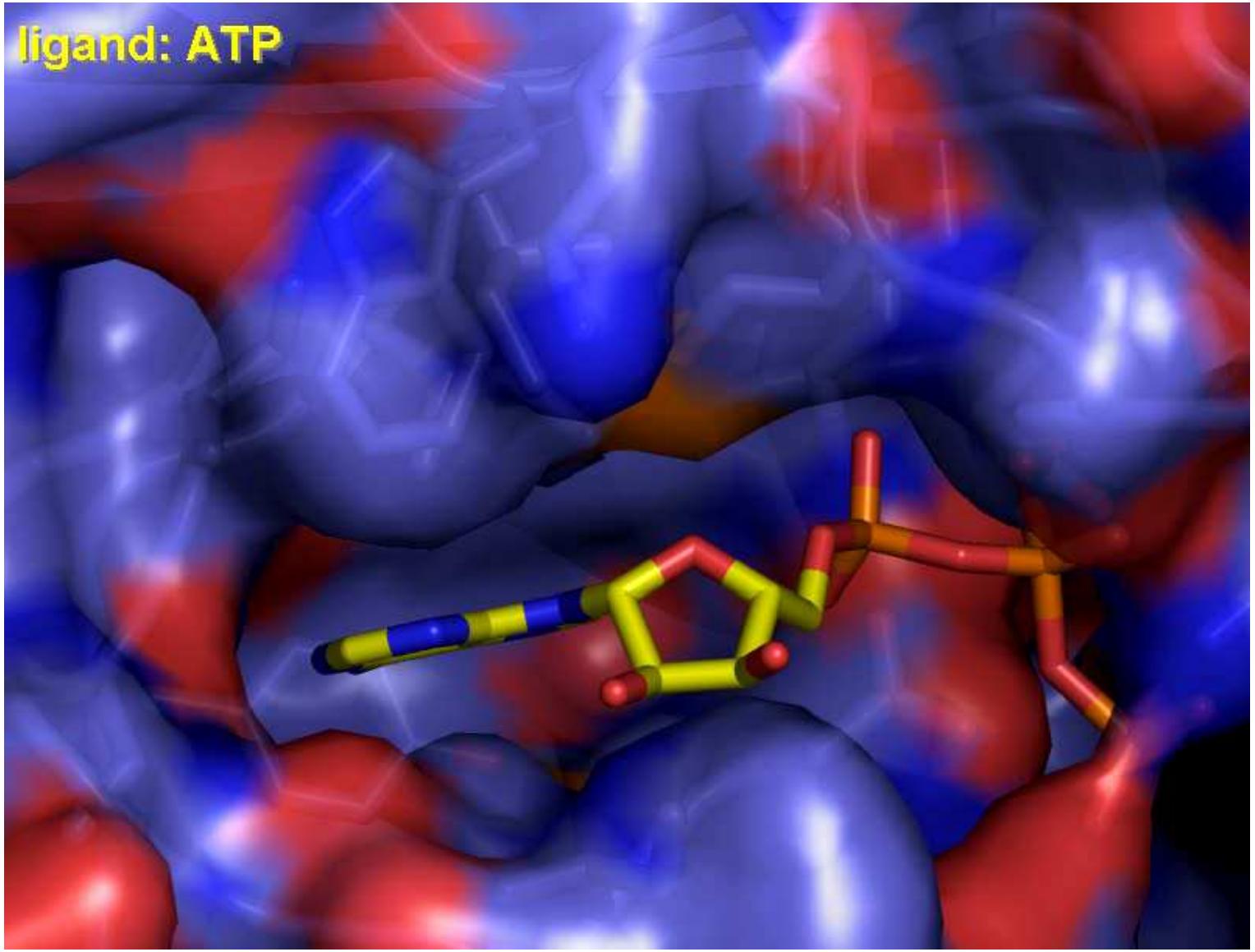


Going in silico

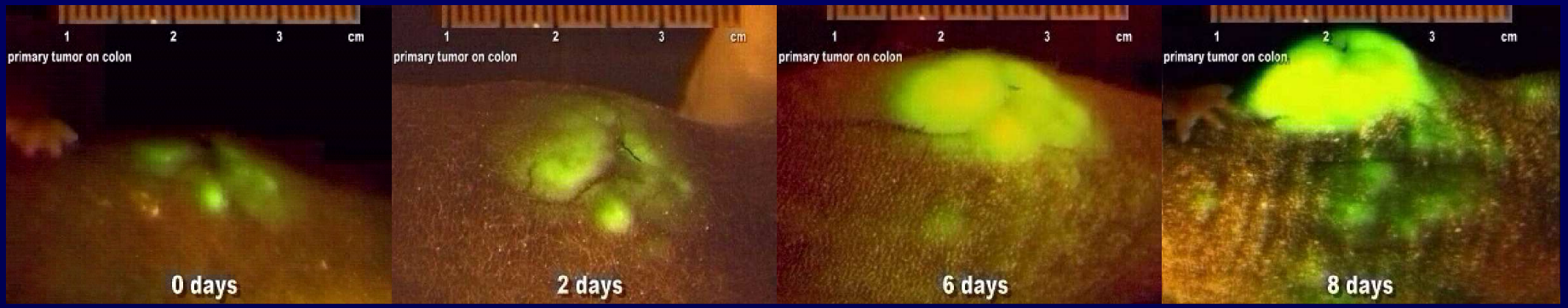


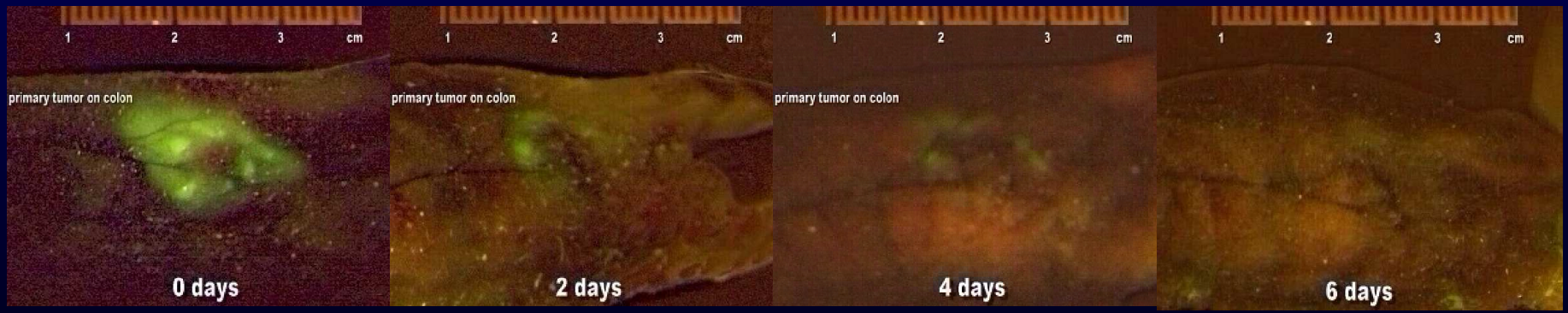
Mejora de actividad in silico



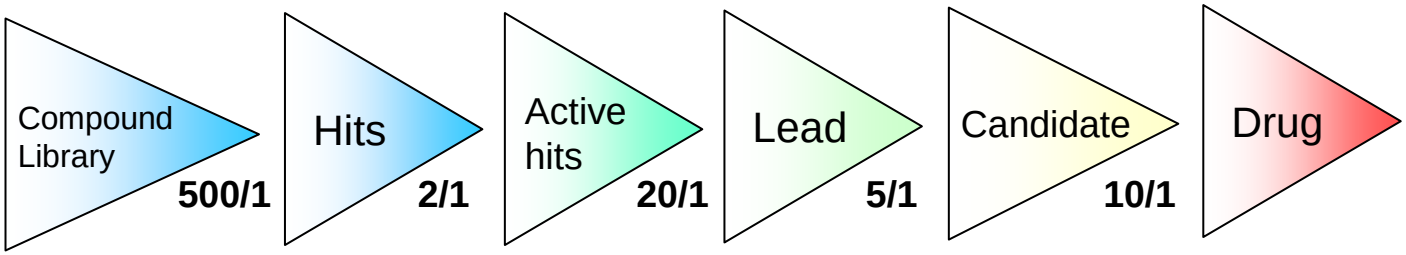

ligand: ATP

Going *in vivo*, xenografts



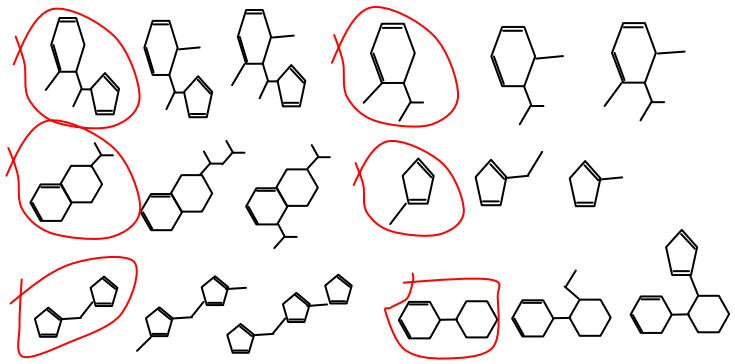


High attrition rate in drug development

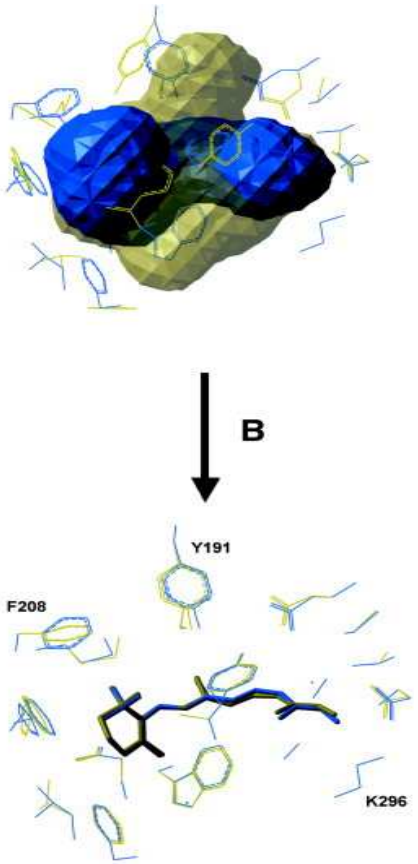


In silico library selection

Diversity design selects a small sub-library from a larger compound library in such a way that the full range of chemical diversity is best represented. When no structural information about the target or ligands is available, the library should be chosen by diversity design. The results of such in silico diversity selections (in silico screening) are smaller sub-libraries of manageable size with a high degree of chemical diversity that are then subjected to HTS in vitro.



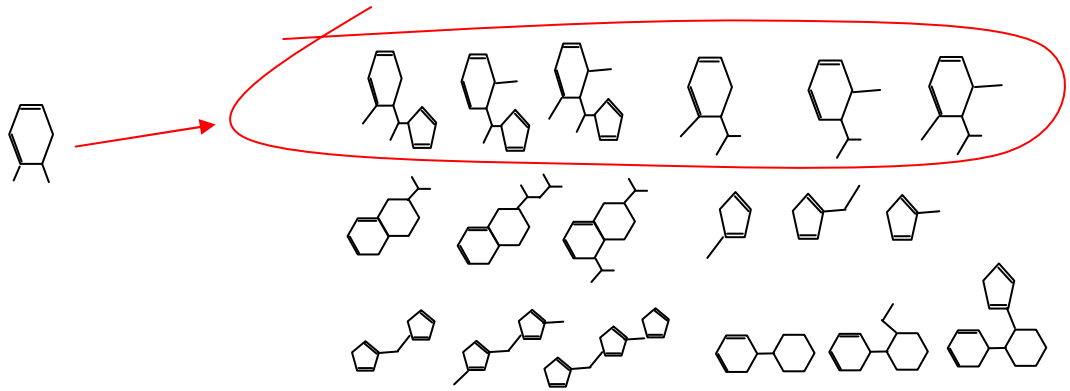
Virtual screening: pharmacophores



In silico library selection

Structure-based library design requires information of the target structure (X-ray or nuclear magnetic resonance). The goal is to select from existing compound libraries or to design compounds with three-dimensional complementarity (i.e. shape, size and physicochemical properties) to the target-binding site. In this case, new approaches can directly guide the design of virtual combinatorial libraries, which are first screened in silico for target complementarity, thus reducing the number of compounds that will have to be synthesized and tested in vitro. It can be expected that the hit-rate of such focused libraries will be higher than that of diversity screening.

Alternatively, compounds can be selected on the basis of previously known active compounds. The basic assumption for all molecule based hypothesis is that similar molecules have similar activities. This principle is also called Structure-Activity Relationship (SAR).



Prediction of drug-likeness

Once lead molecules have been identified, they have to be optimized in terms of potency, selectivity, pharmacokinetics (absorption, distribution, metabolism and excretion, ADME) and toxicology before they can become candidates for drug development

Lipinski's rule of five can be used indicate whether a molecule is likely to be orally bioavailable (bioactive). In general, an orally active drug has:

- not more than 5 hydrogen bond donors (OH and NH groups)
- not more than 10 hydrogen bond acceptors (notably N and O)
- a molecular weight under 500
- a LogP under 5

Molecules that possess this rule are not automatically drug-like. For classifying a molecule as drug-like or non-drug-like a proper cheminformatic approach should be used, e.g. QSAR.

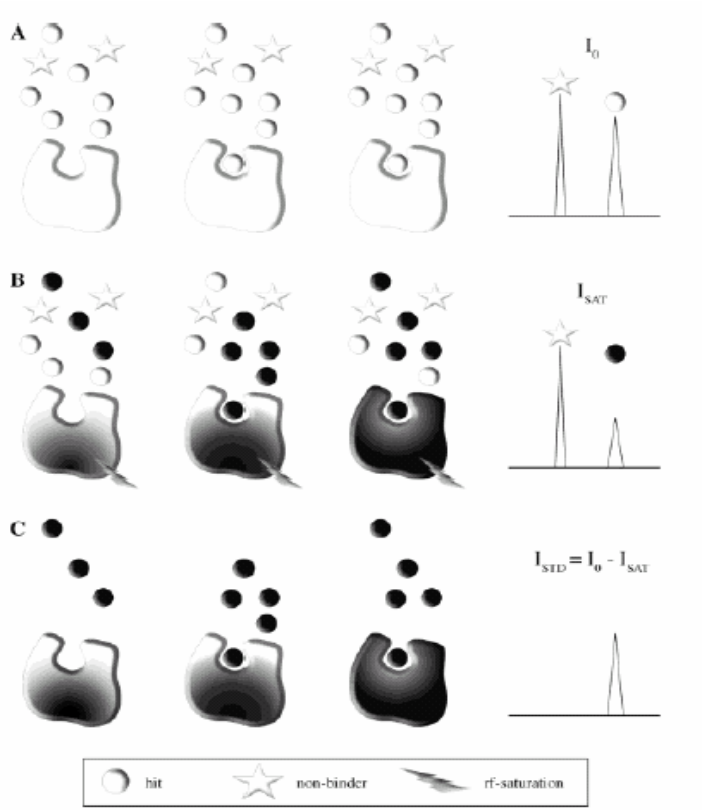
This rule was derived for drugs and not for lead structures, which usually have a lower molecular weight, fewer rings, fewer rotatable bonds, and a lower lipophilicity.

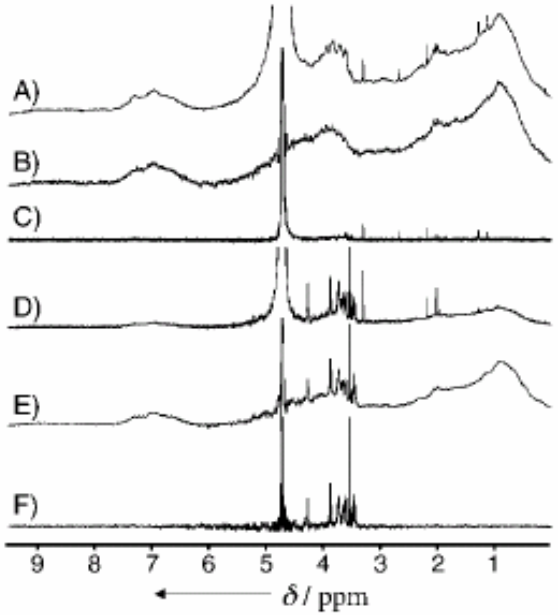
NMR screening

Detection of binders through changes on the ligand signals (saturation transfer difference)

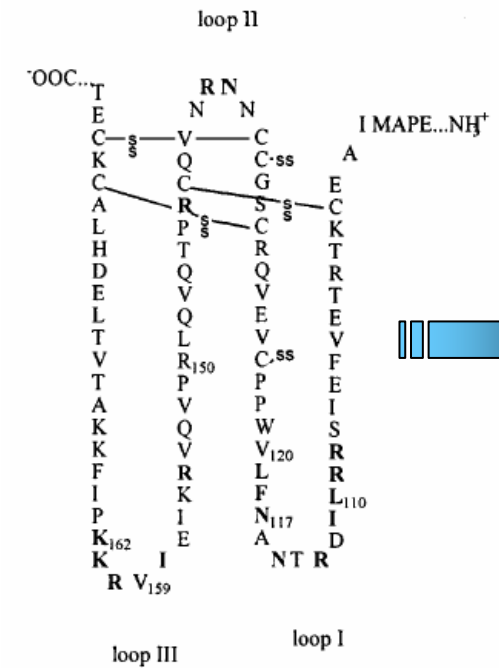
- Protein MW > 30 kDa
- No isotope enrichment
- 50 µM (0.5 mgr) protein sample, 5 compounds per sample
- 0.5 hour experiment, 50 compounds/day
- K_D < 10 mM

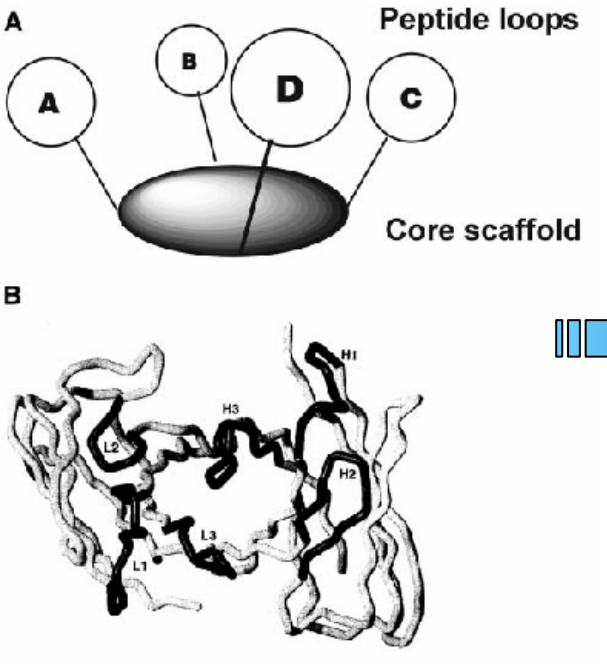
- Fragment library
- Limit KD<mM (cut-off at 0.5 mM)
- Not HTS, maximun 25 compounds/day

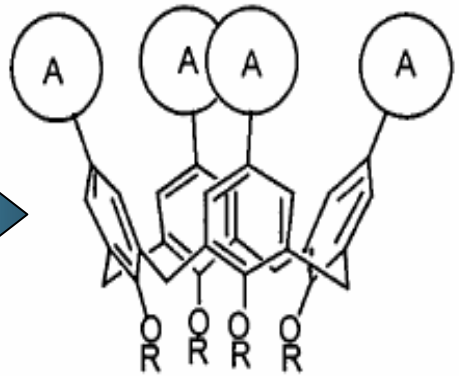


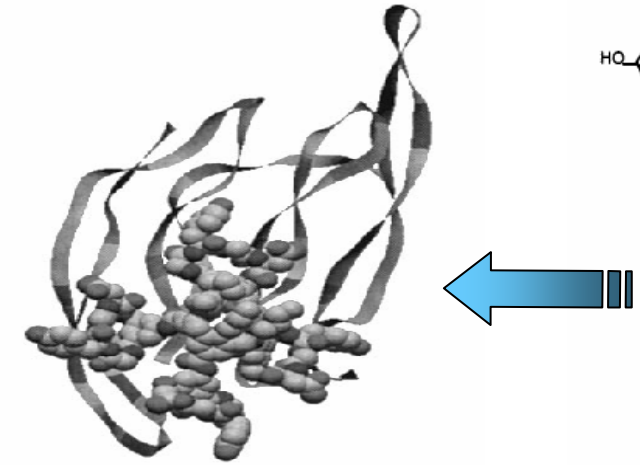


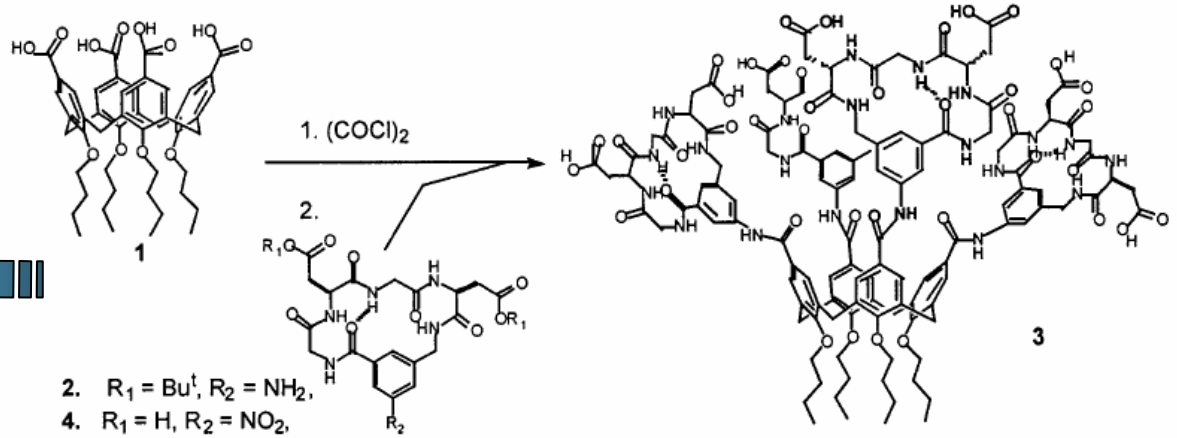
Rational Design: PDGF Inhibitor











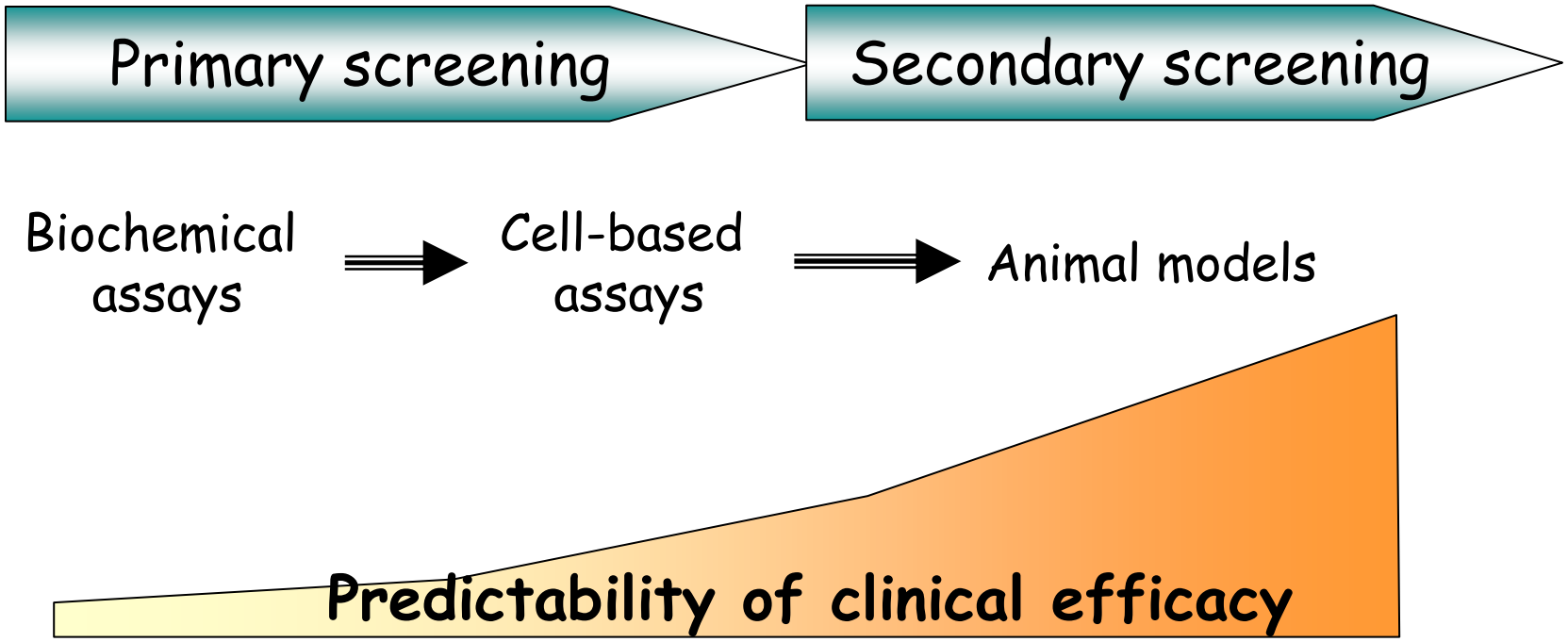
Clinically approved kinase-targeted agents

Small molecules		
Imatinib (Gleevec, STI-571)	Abl, PDGFR, cKit	CML, ALL,GIST
Gefitinib (Iressa, ZD-1839)	EGFR	NSCLC
Erlotinib (Tarceva, OSI-774)	EGFR	NSCLC
Sorafenib (Nexavar, BAY43-9006)	VEGFR, PDGFR, FLT3 cKit, B-Raf, RAF11	Renal Ca.
Sunitinib (Sutent, Su11248)	VEGFR, PDGFR, FLT3 cKit	Renal Ca.
Biologicals		
Trastuzumab (Herceptin)	Erb2	Breast Ca.
Cetuximab (Erbitux)	EGFR	
Bevacizumab (Avastin)	VEGF	Colon, Pancreatic Ca

In the pipeline:
Representative kinase-directed oncology drug targets

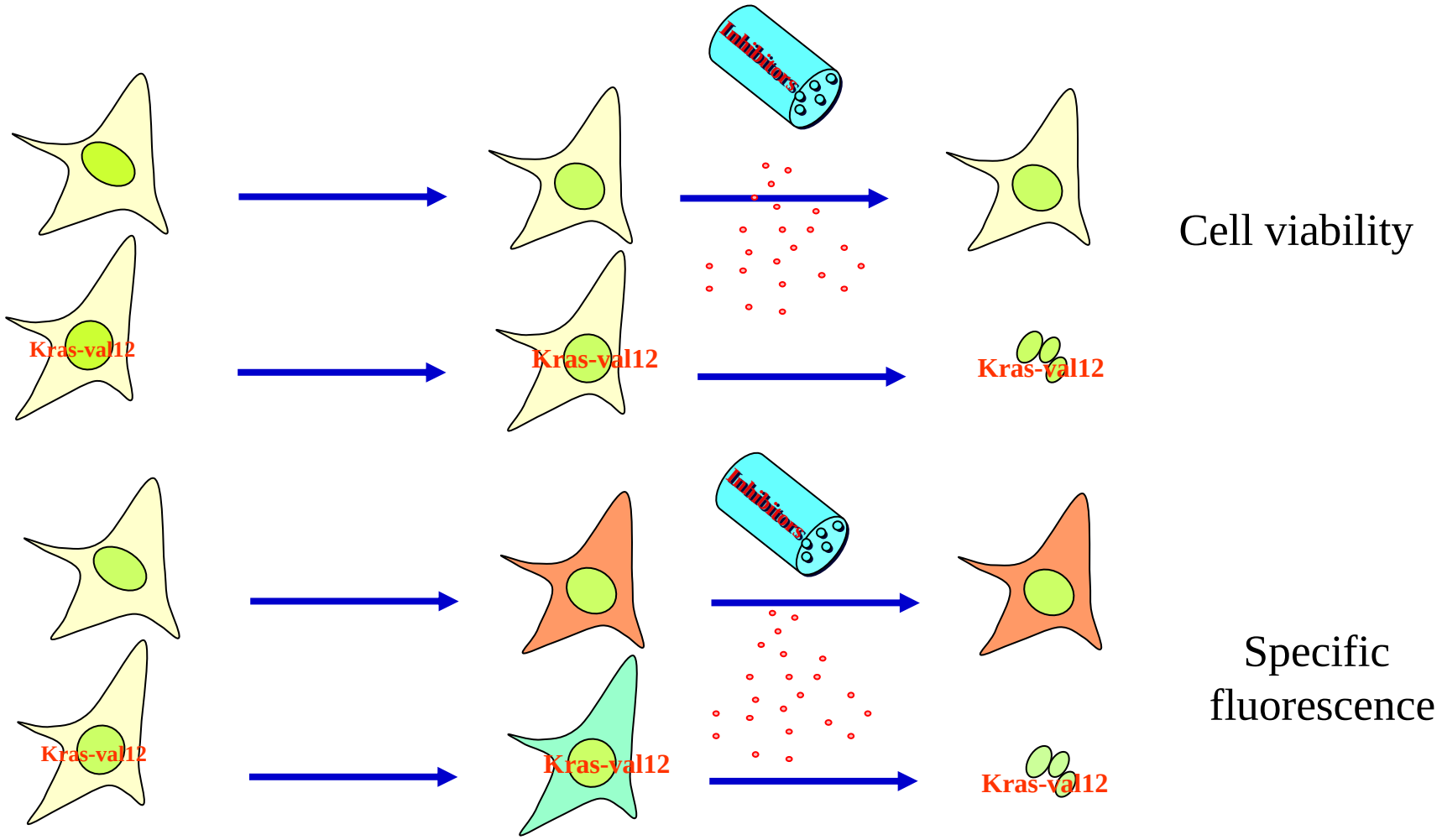
Kinome branch	known target	compound
TK	VEGFR	AZD-2171
	VEGFR, EGFR	Vandetanib, ZD6474
	VEGFR, PDGFR, cKit	Vatalanib, PTK787
	EGFR,Erb2	Lapatinib, GW572016
	Src, Abl	BMS-354825
	Anti-IGFR (antibody)	CP-751871
Cell cycle	CDKs	Flavopiridol
	CDK4	PD332991
Signal transduction	MEK	PD0325901
		ARRY-142886

Assay development



Molecular targeted HTS

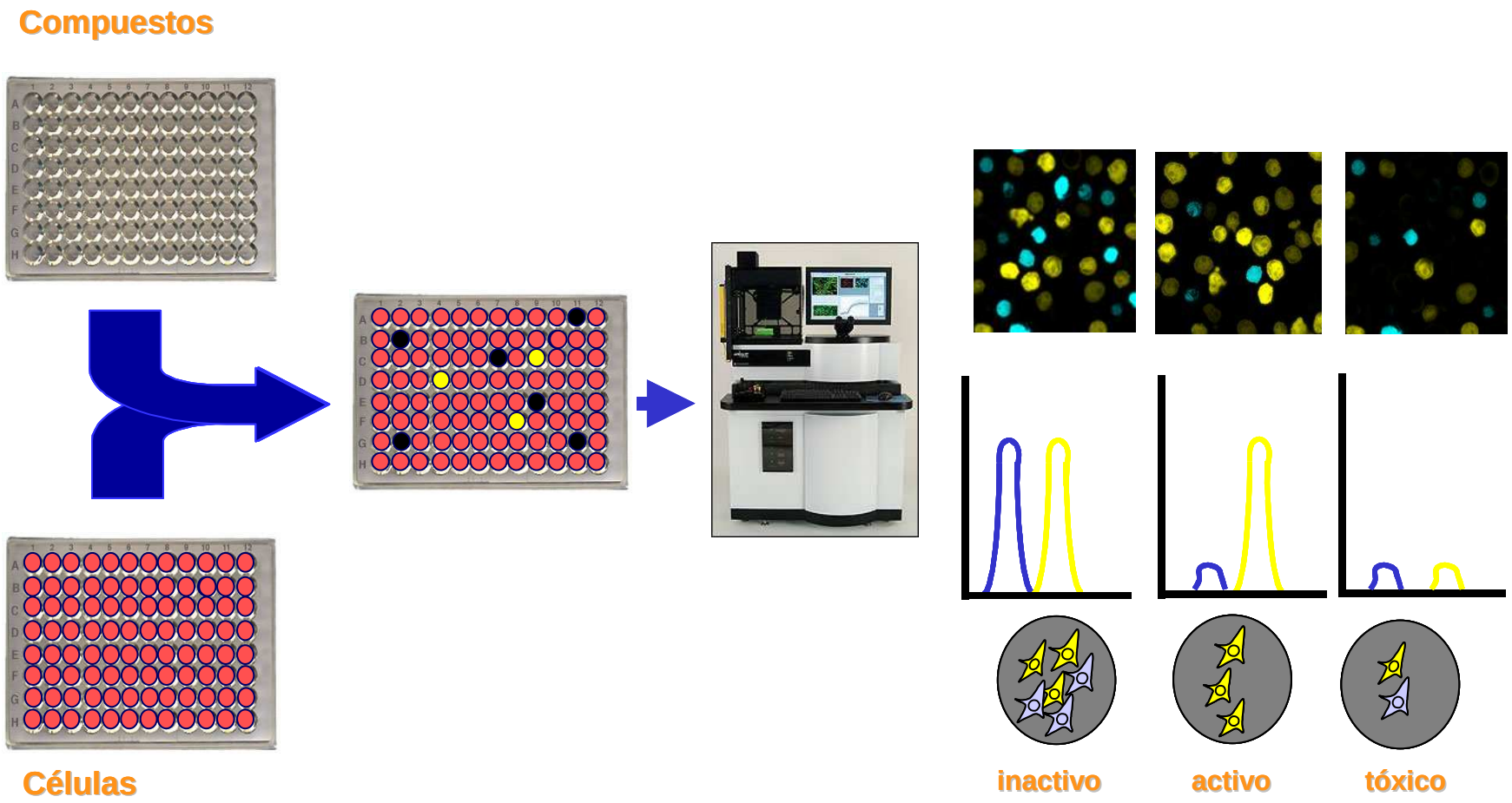
Mutated cell line specific assays



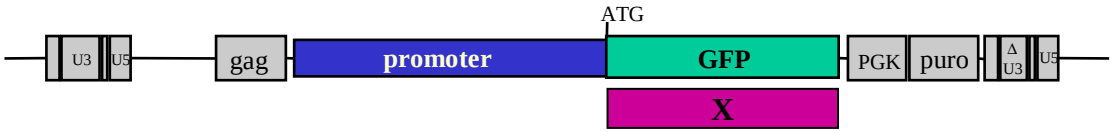
Cell viability

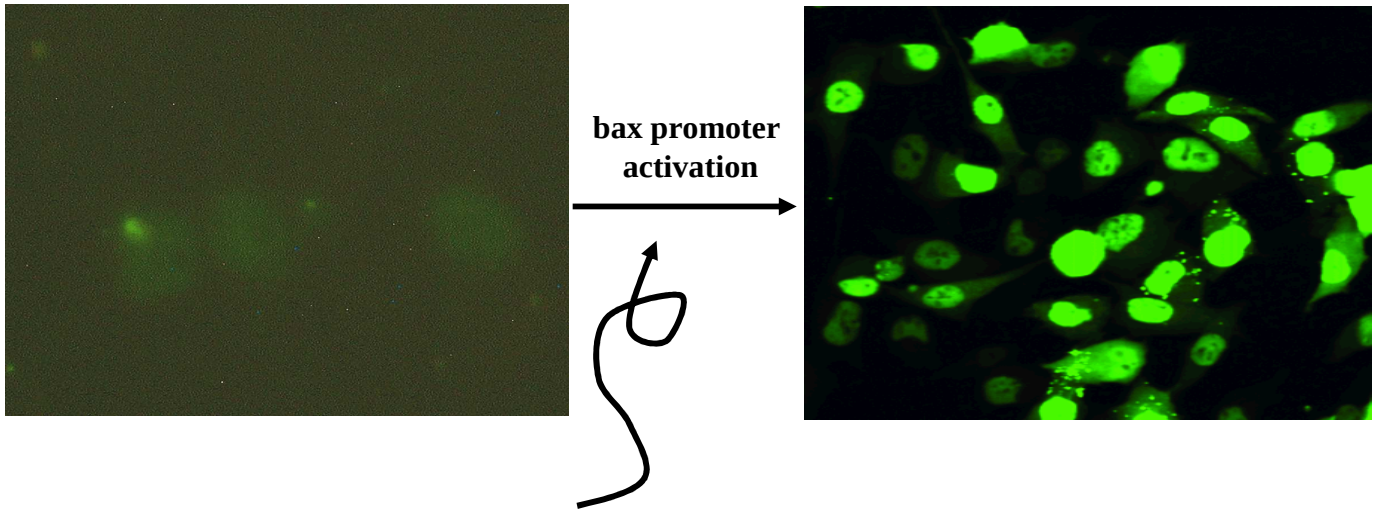
Specific fluorescence

Pathway bassed screening

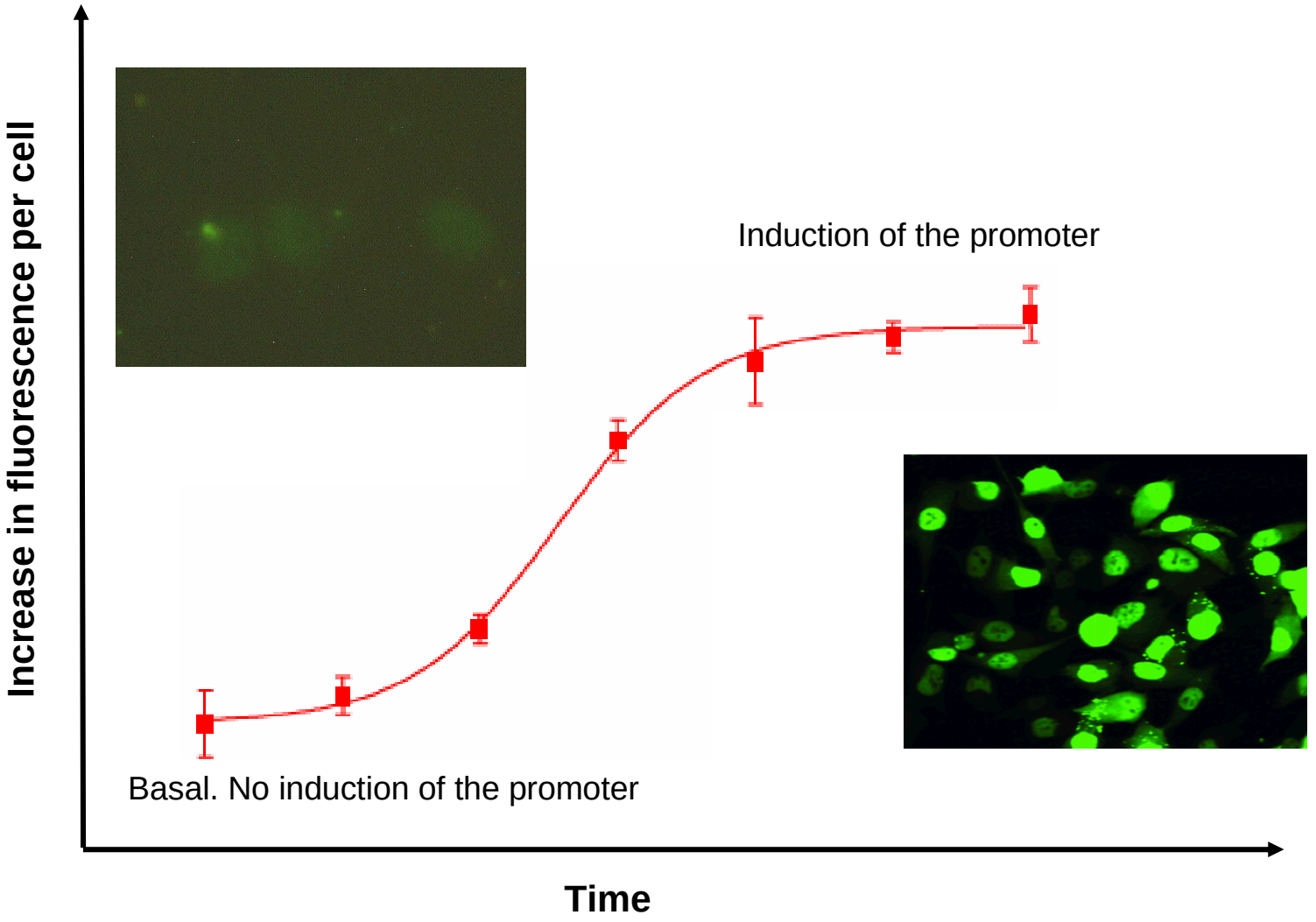


cell lines with specific-response promoters.



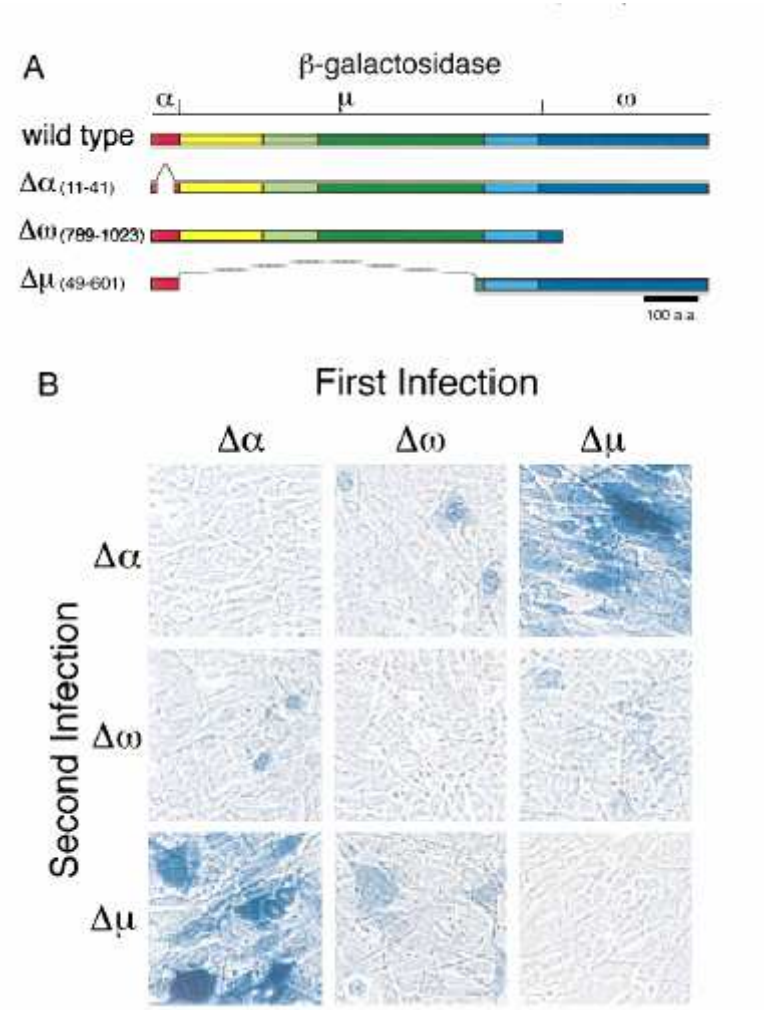


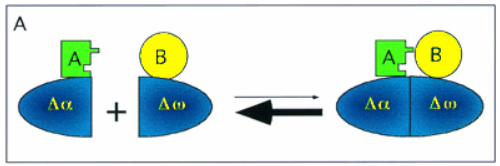
Quantification of promoter response using GFP as read-out

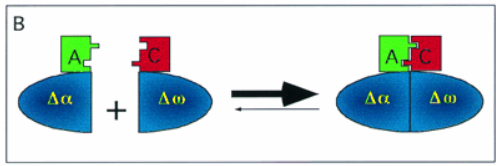


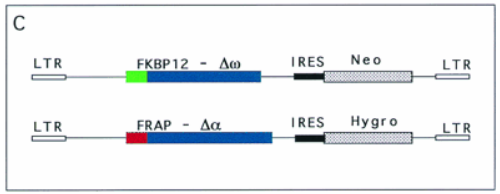
The construct carry a chimaera of a nuclearprotein tagged to GFP under a inducible promoter

B-Galactosidase Complementation system



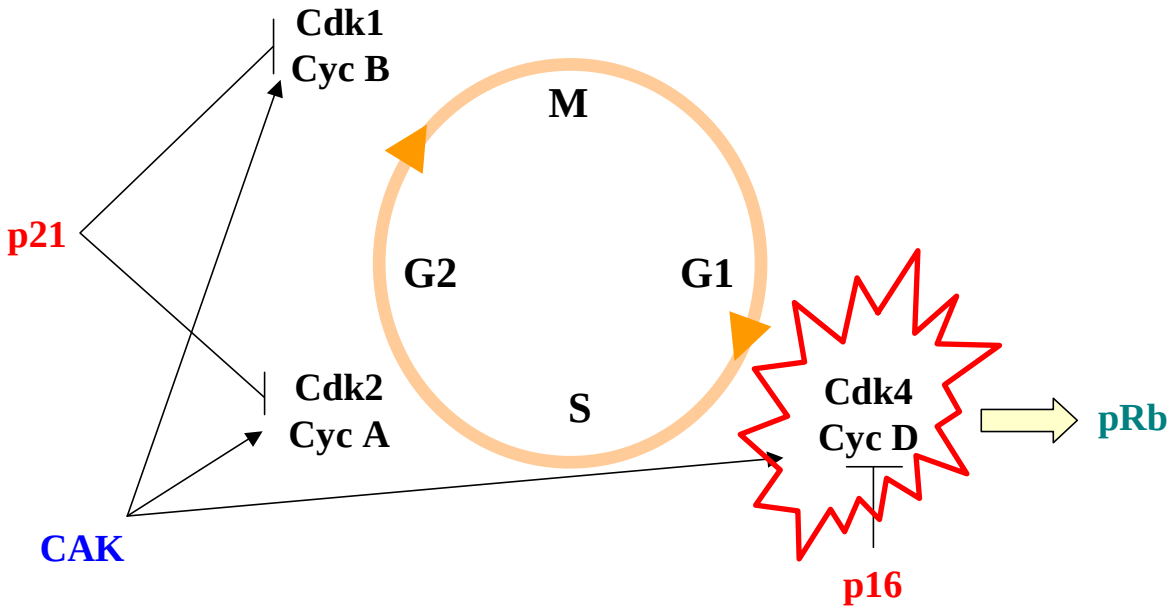






Cell-based models

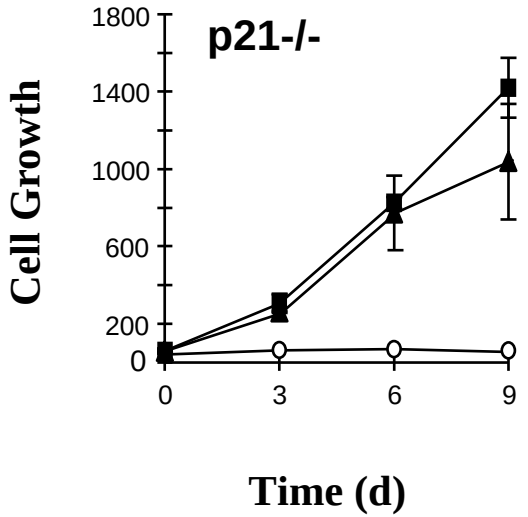
Cell Cycle components selected for our studies



Engineered mammalian cell lines

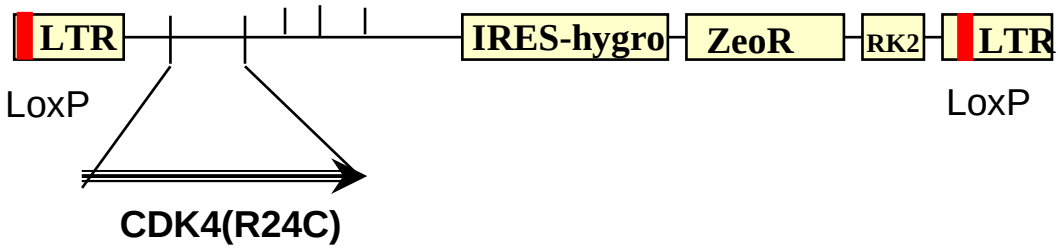
CDK4(R24C) mutant immortalizes p21 null MEFs

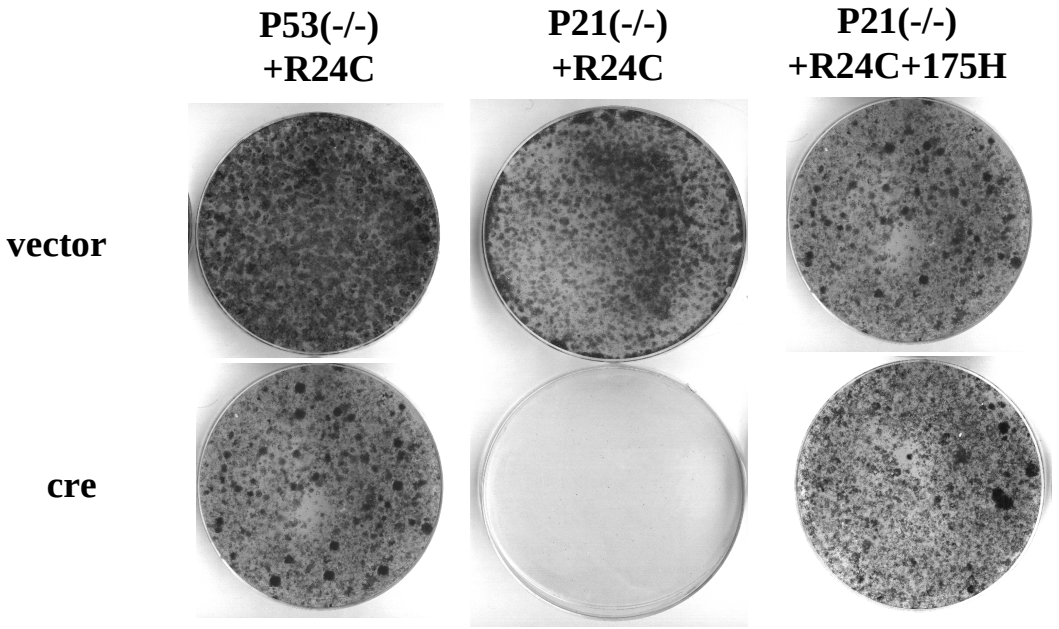
Gene	p21-/-
Vector	22±14
p53(175H)	+ 500
CDK4(R24C)	+ 500



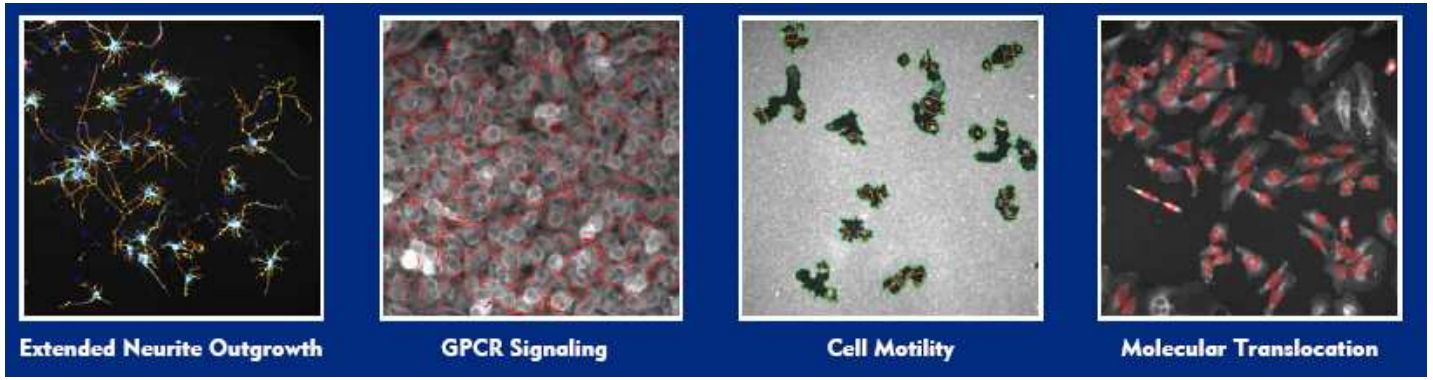
Engineered mammalian cell lines

Dependence of CDK4(R24C) expression for constitutive immortalisation

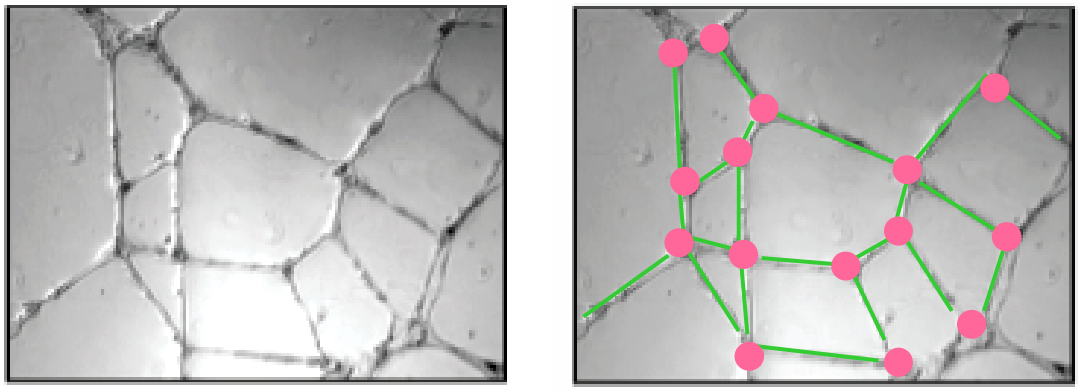




High Content Screening

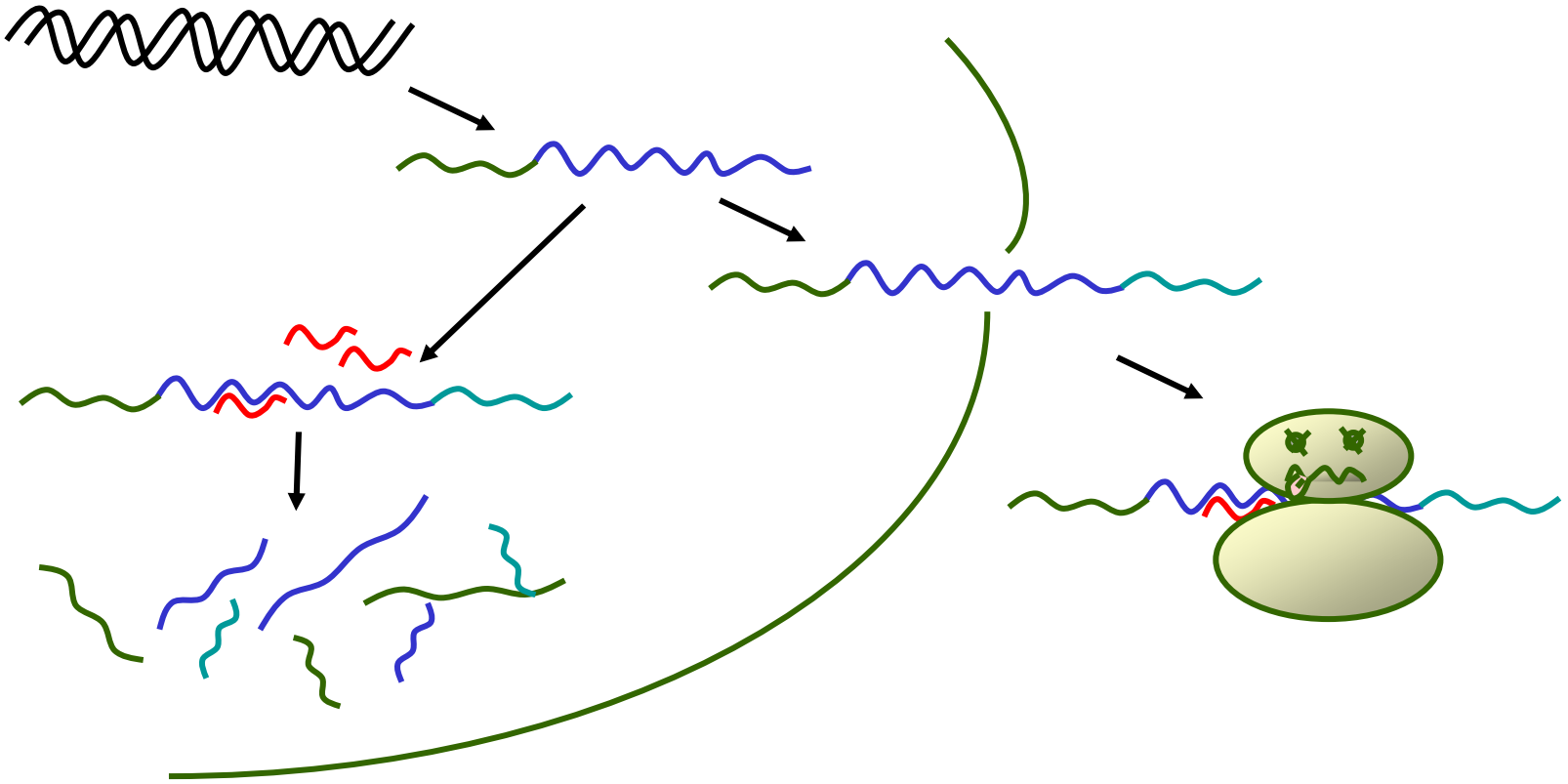


Inhibitors of neovasculature formation



$$\frac{\text{Total length}}{\text{Total nº nodes}} = K$$

Nucleic acids



Small oligoDNAs

Small RNAi

RNA antisense

In clinical trials

Isis 2503
αs- Hras

Fase II: terapia combinada con quimioterapia en
Cancer de mama metastatico, cancer de pancreas
NSCLC

Isis 5132
αs- cRaf

Fase I:
colon, carcinoma renal, pancreas: Estabilizacion 7-10 meses
Carcinoma ovarico: 97% de casos disminucion del tumor

Isis 3521
αs- PKCα

Fase II: Terapia combinada carboplatino/paclitaxel
NSCLC 83% casos mejora oestabilizacion del tumor,
50% de casos se observa reduccion del 50% del tamaño
Aumento supervivencia 8 a 16 meses.
Primera linea tratamiento para NSCLC en combinacion
gemcitabine, cisplatino, docetaxel
agente unico para linfoma non-Hodgskin´s
Fase III: Tratamiento primera linea en combinacion Carboplatino
y paclitaxel para NSCLC

In clinical trials

G3139
αs- Bcl2

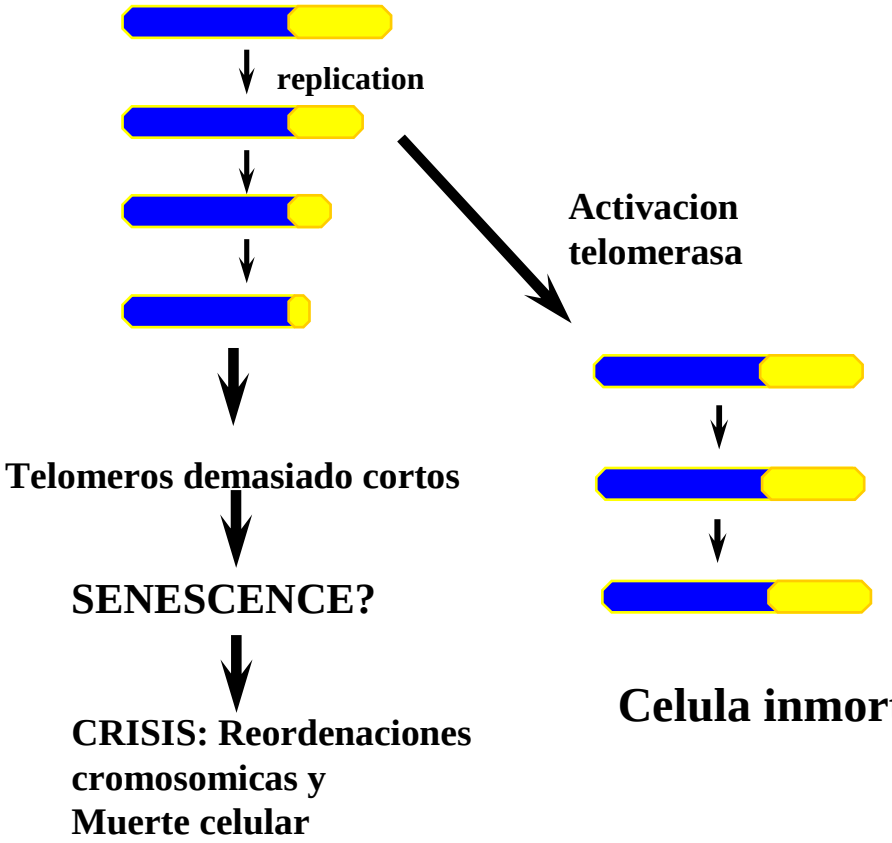
Fase I: 21 pacientes, ninguno remision completa
2 respuestas parciales, 8 estabilizacion, resto progresion + lenta

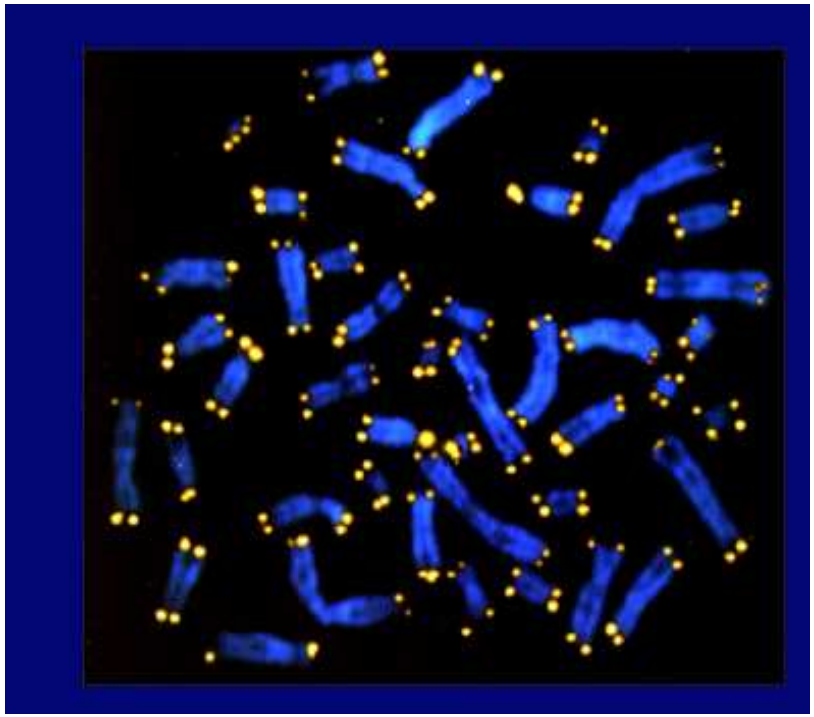
OGX-011
αs- clusterin

Proteina secretora que se sobreexpresa en respuesta a quimiot.
Responsable de resistencia a drogas
Fase I/II: cancer de prostata refractario a hormonas
como agente unico y en combinacion con docetaxel

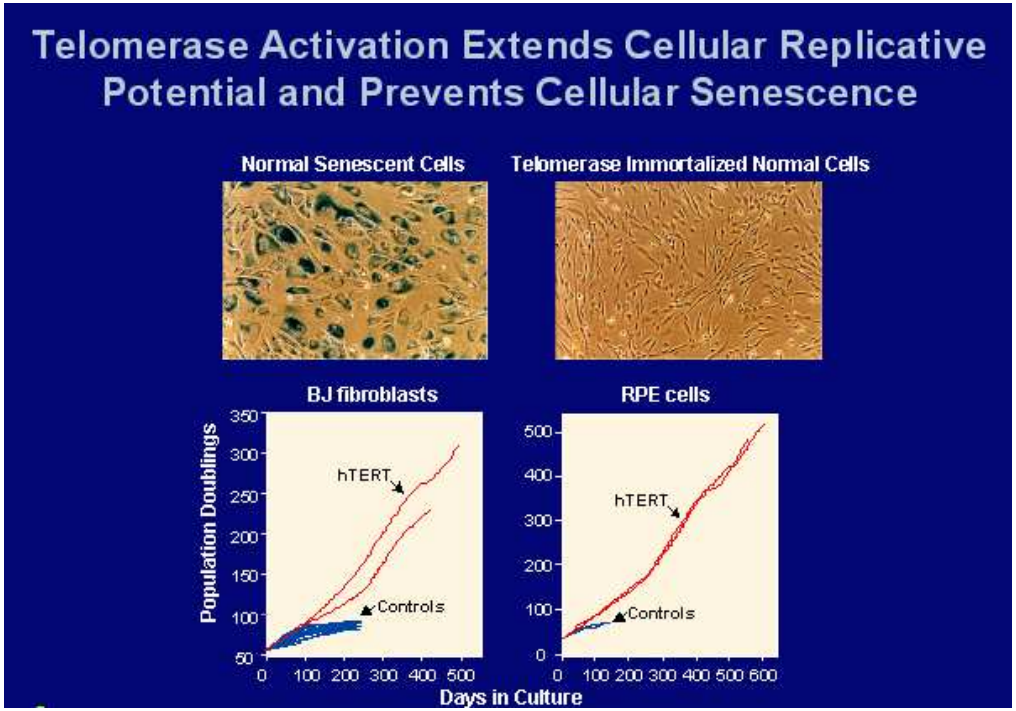
αs-cmyb: Fase I leucemias
αs-DNMT1: Fase I tumores solidos;
Fase II carcinoma renal y de cabeza y cuello
αs-PKA: Fase I Prostata en combinacion con paclitaxel
αs-cmyc: Fase I tumores solidos

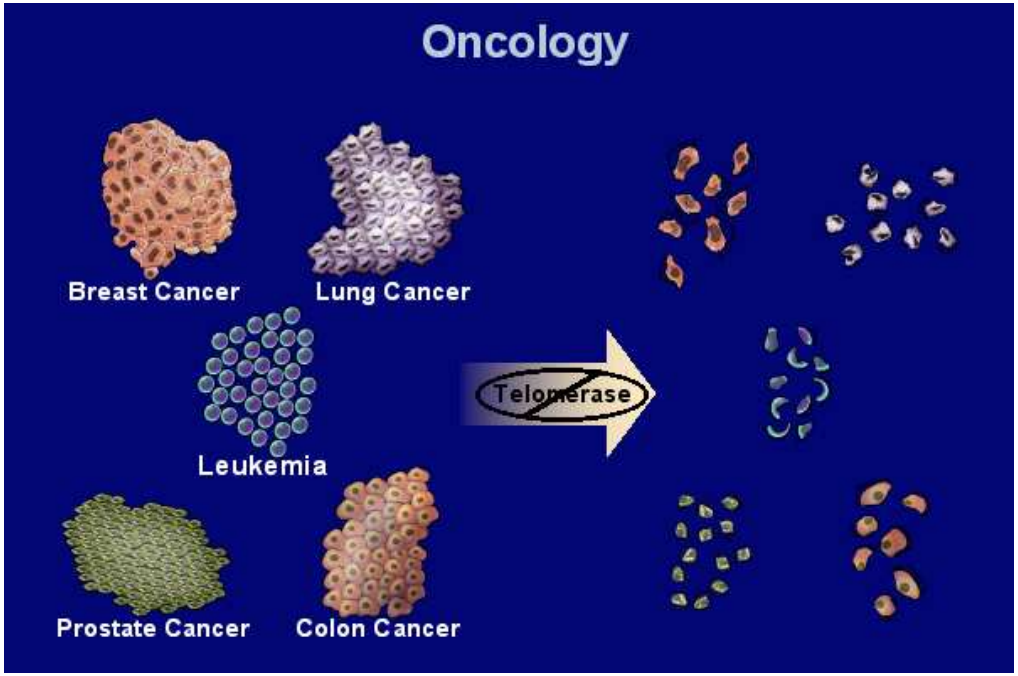
Telomerase as an example





90% tumores presentan telomerasa activada
10% restante presentan un mecanismo alternativo elongación de telómeros


Telomerase Activation Extends Cellular Replicative Potential and Prevents Cellular Senescence
Normal Senescent Cells
Telomerase Immortalized Normal Cells
BJ fibroblasts
RPE cells
Population Doublings
hTERT
Controls
Days in Culture


Oncology
Breast Cancer
Lung Cancer
Leukemia
Telomerase
Prostate Cancer
Colon Cancer

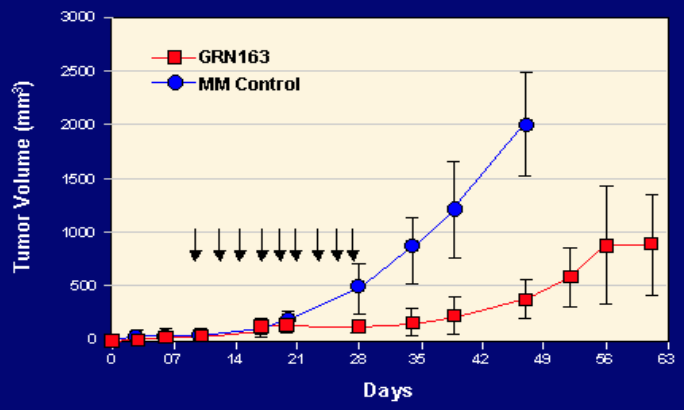
Drugs to Inhibit Telomerase

Template Antagonists

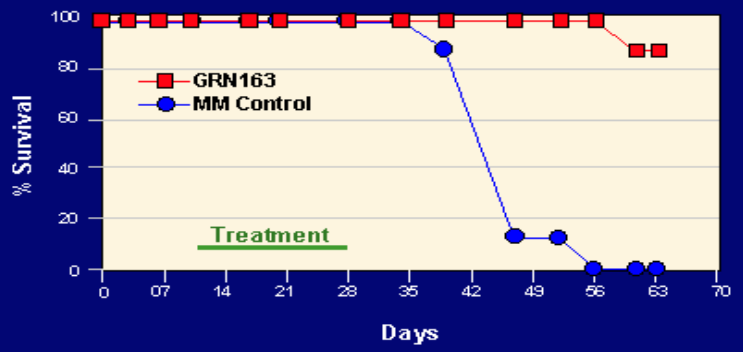
GRN163 - Development Candidate

- A 13-mer oligonucleotide
- IC_{50}: high pM to low μM range
- Time to apoptosis dependent on telomere length
- Potent activity against glioblastoma, prostate cancer, myeloma, lymphoma, breast and renal cancers

Effect of GRN163 on Growth of U-251 Malignant Glioma Tumors *In Vivo*
(UCSF Study #3)



Survival of Mice With U-251 Malignant Glioma Tumors Treated With GRN163



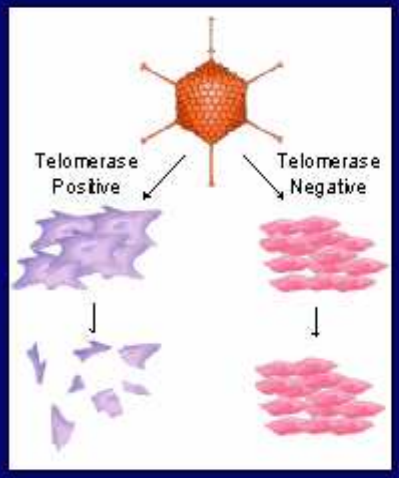
Drugs to Inhibit Telomerase

Small Molecule Inhibitors

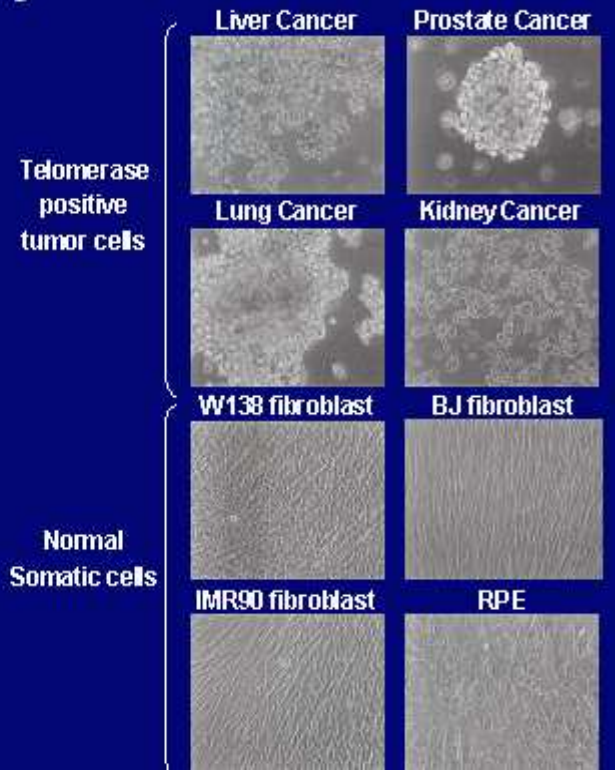
GRN138098 - Potential Development Candidate

- Equipotent in cell culture to GRN163 template antagonist
- In vivo toxicology and xenograft efficacy studies in progress

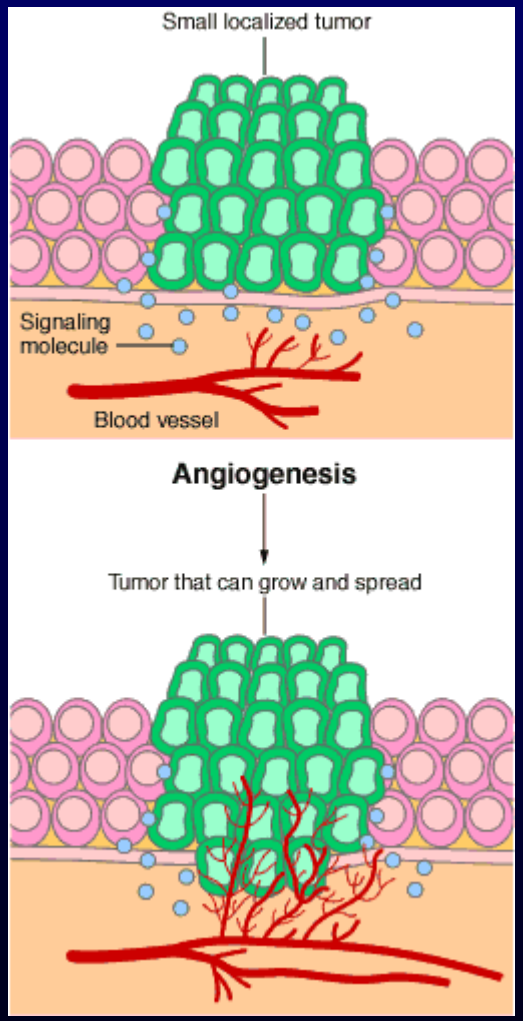
Telomerase Promoter Oncolytic Adenovirus Selectively Lyses Tumor Cells

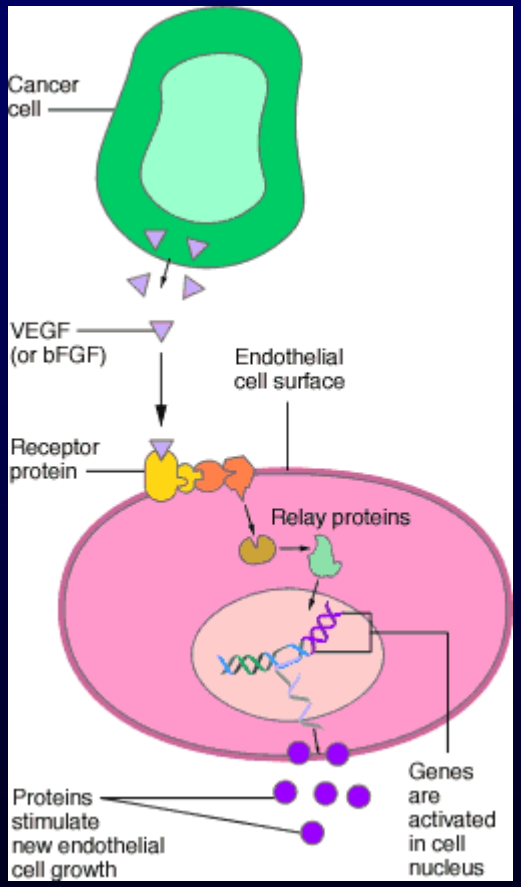


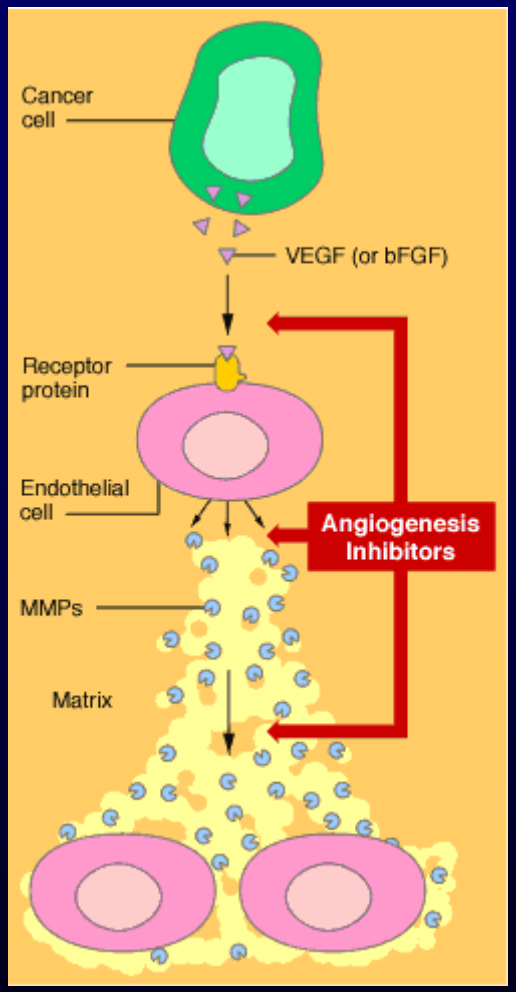
Currently in Animal Xenograft Models



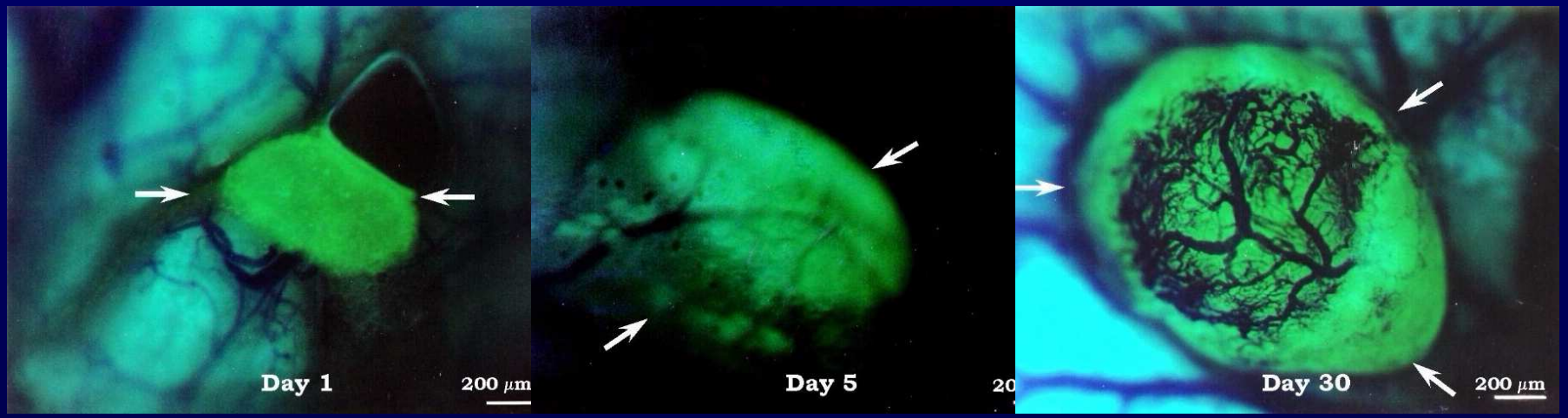
ANGIOGENESIS

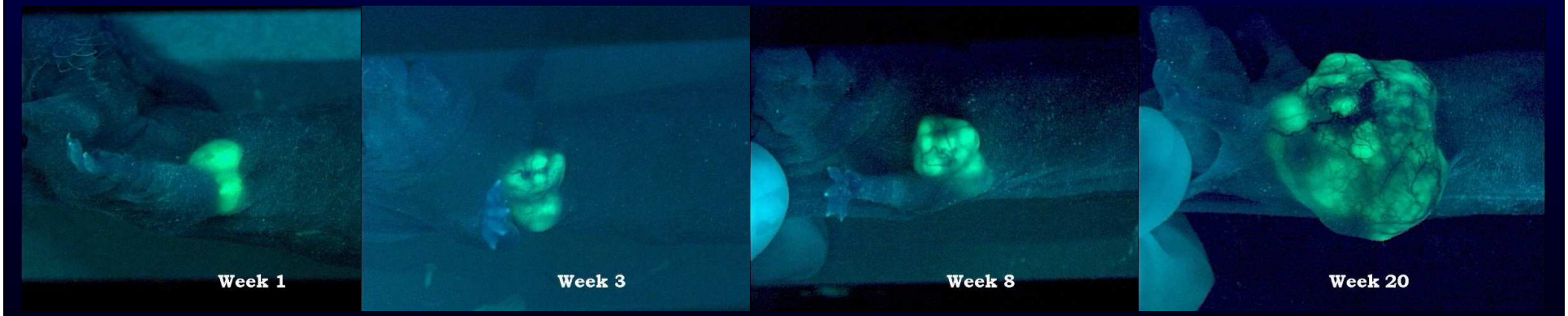


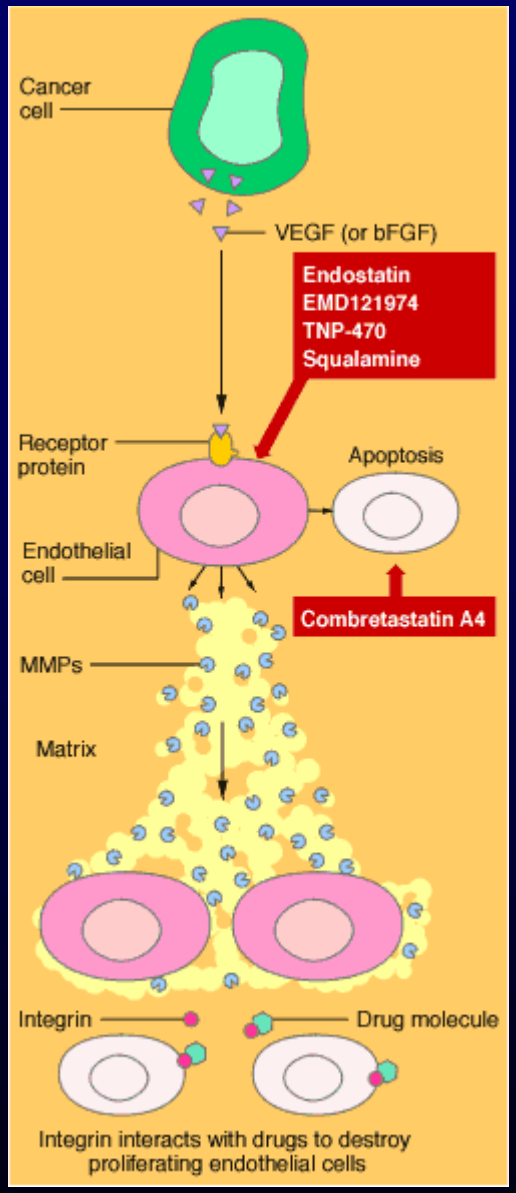


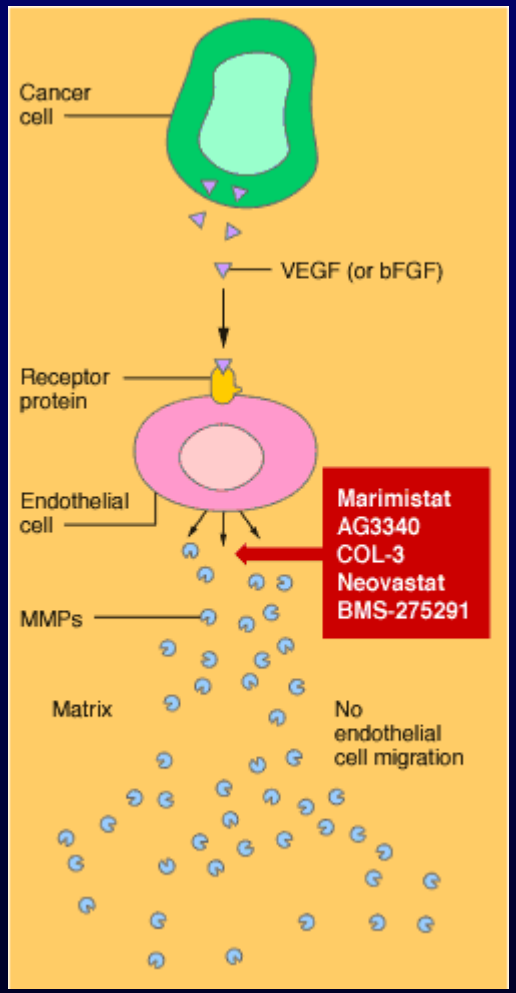


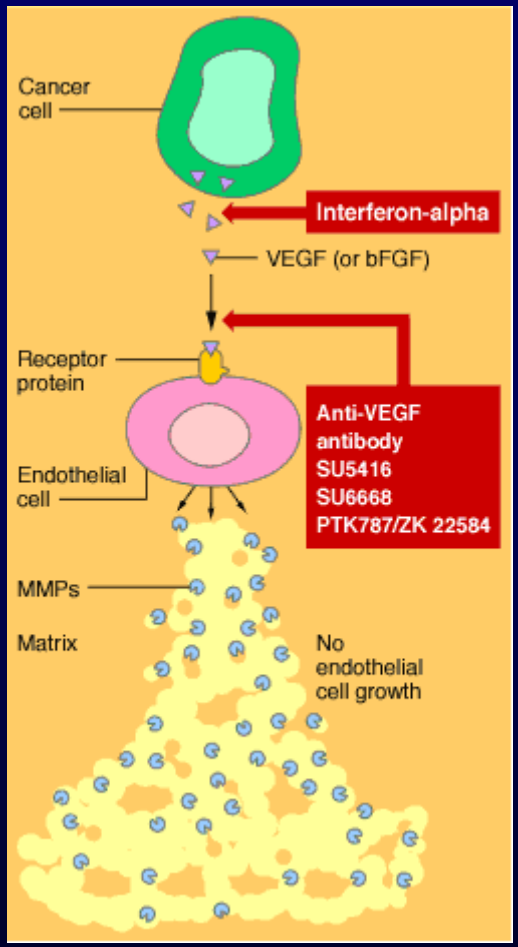
Tumor angiogenesis



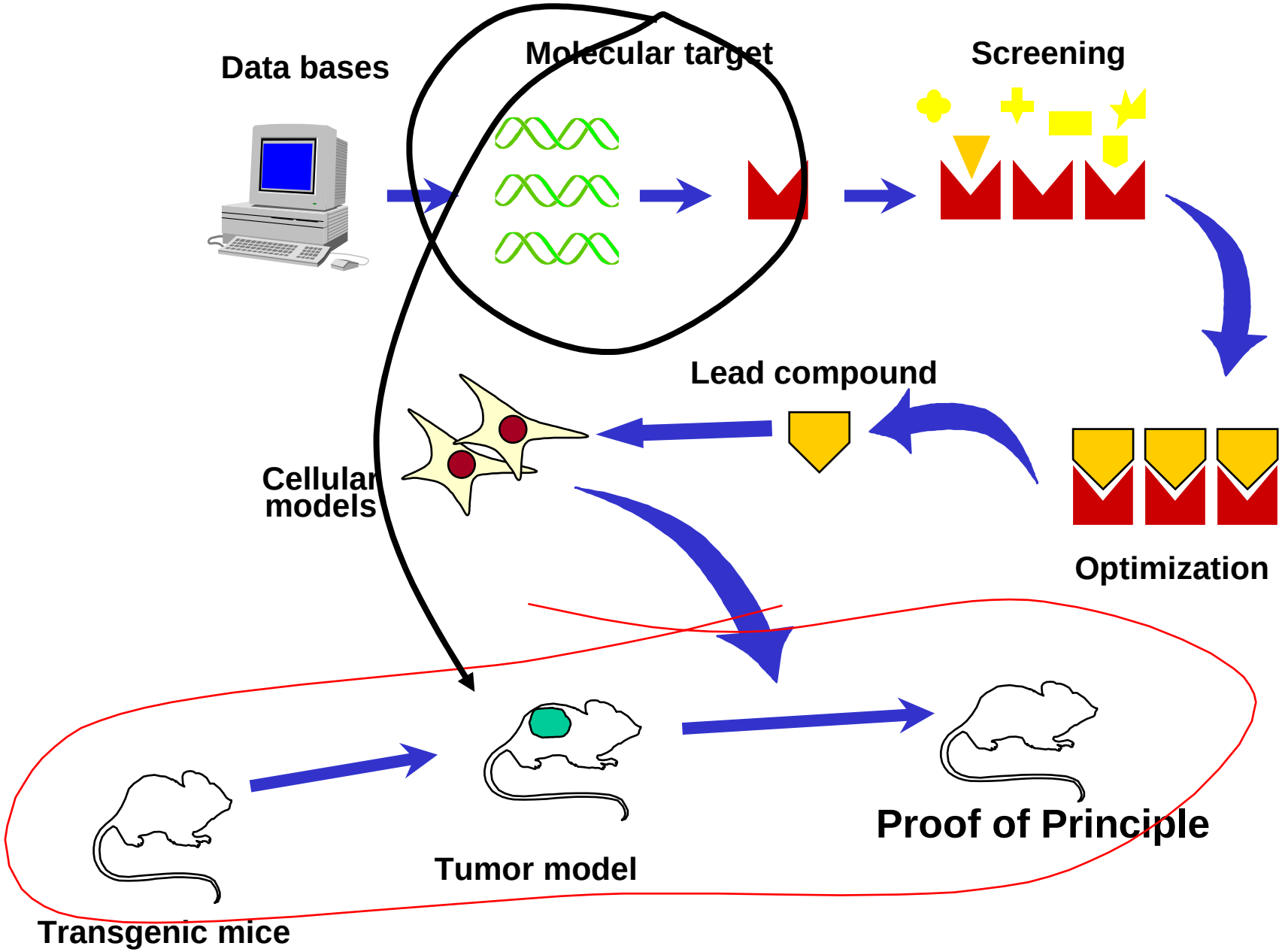






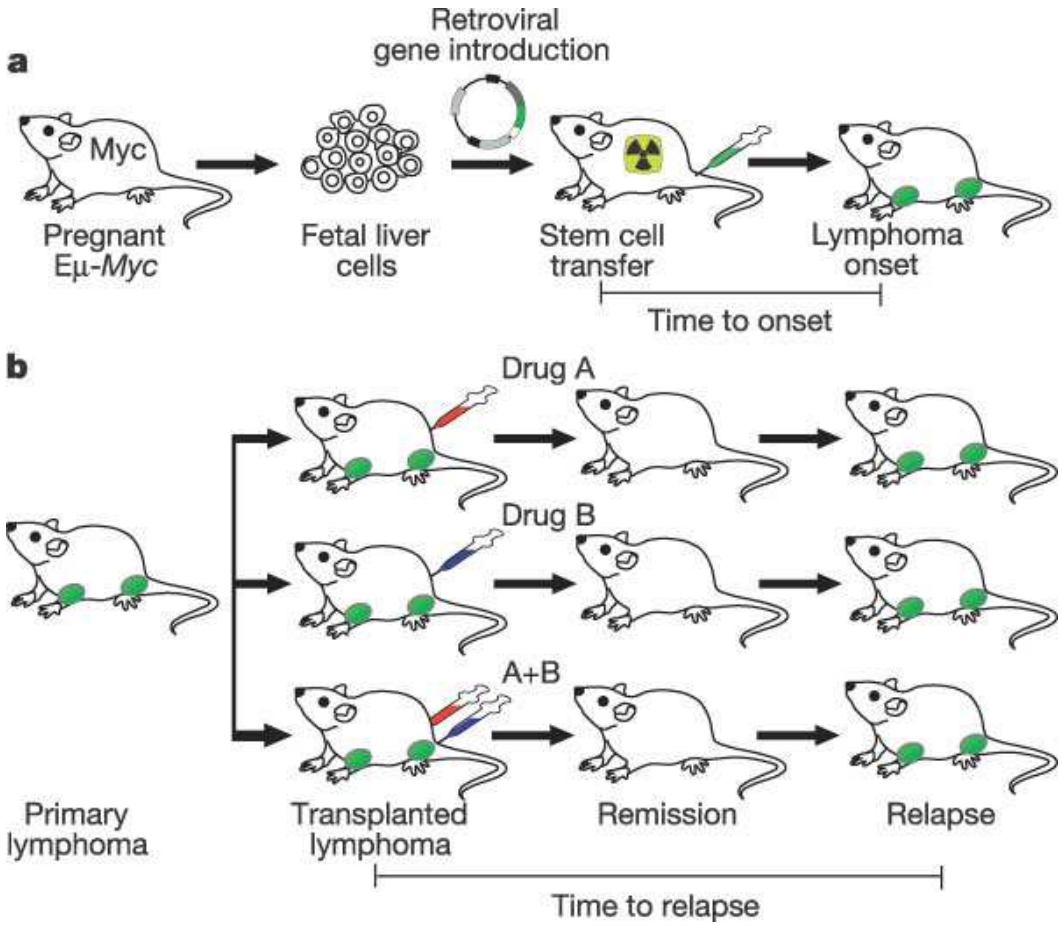


Drug	Mechanism
CAI	Inhibitor of calcium uptake
Interleukin-12	Up-regulation of interferon-gamma and IP-10
IM862	Unknown
Thalidomide	Unknown


Data bases
Molecular target
Screening
Lead compound
Cellular models
Optimization
Transgenic mice
Tumor model
Proof of Principle

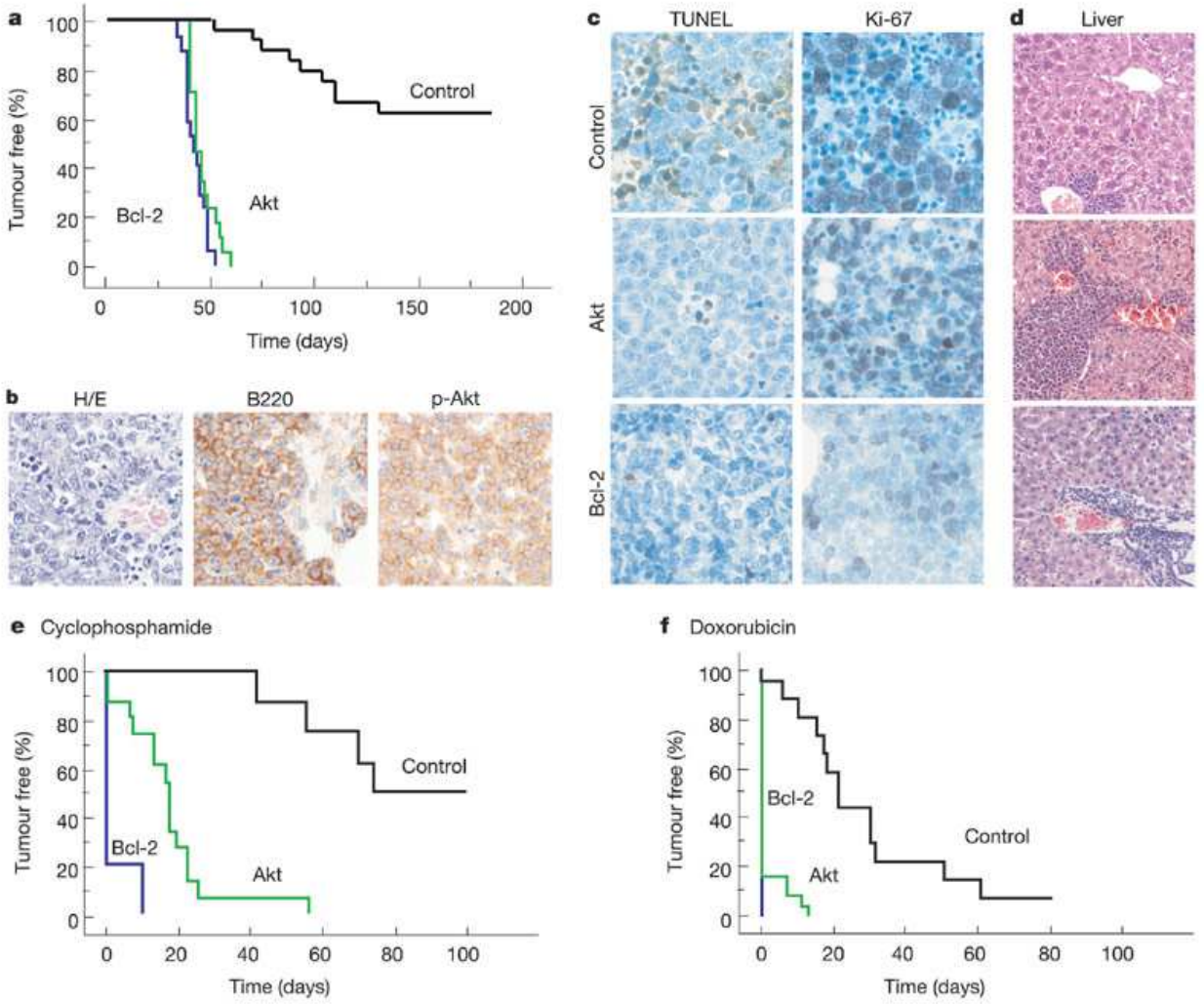
Mouse tumor models: Em-Myc

- Lymphomagenesis dependent on the molecular target
- Study of the different molecular targets
- Proof of principle of targeted compounds



Em-Myc model to study molecular targets and proof of principle

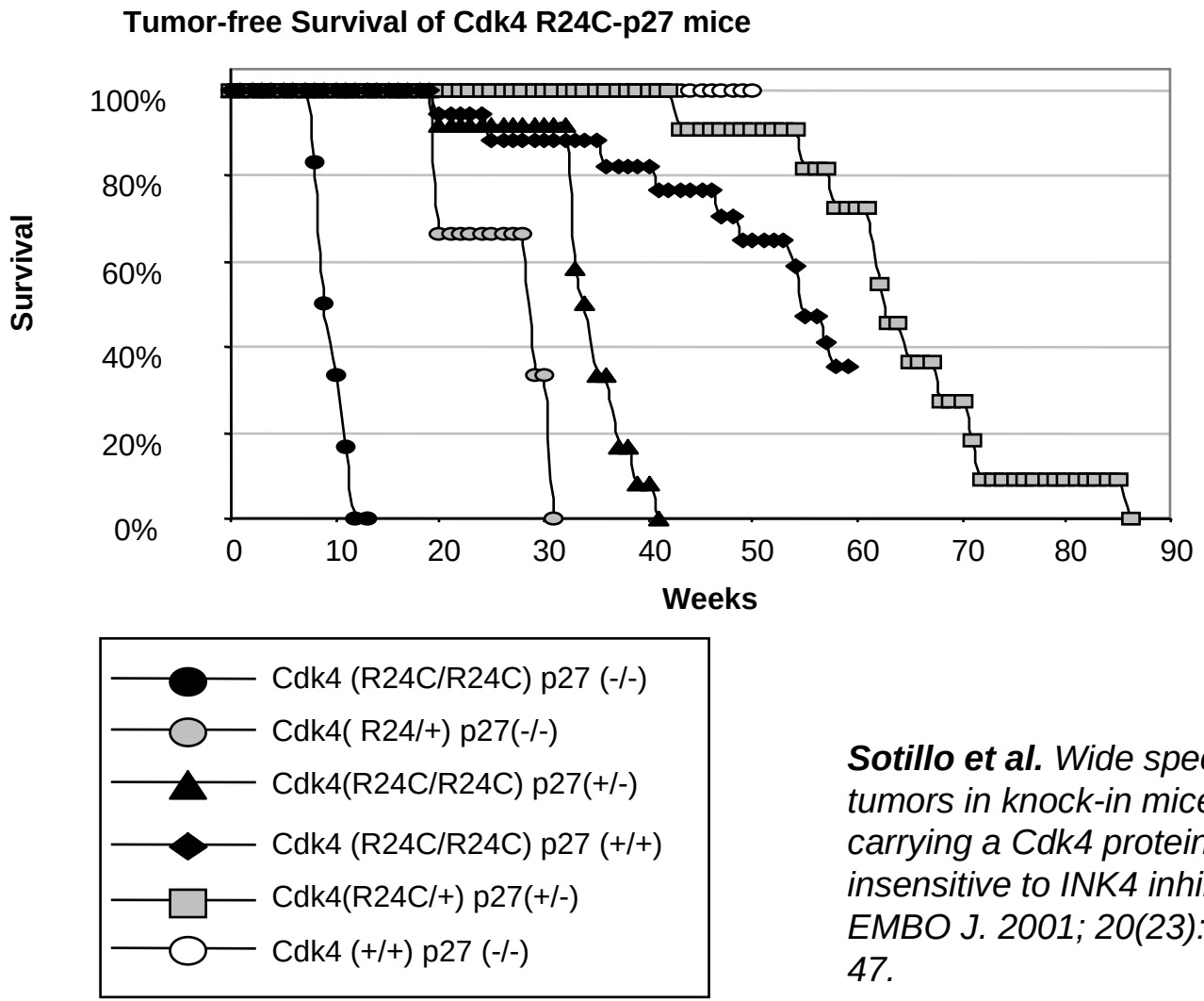
Akt accelerates lymphomagenesis and promotes drug resistance *in vivo*



Wendell et al, *Nature* **428**, 332 - 337

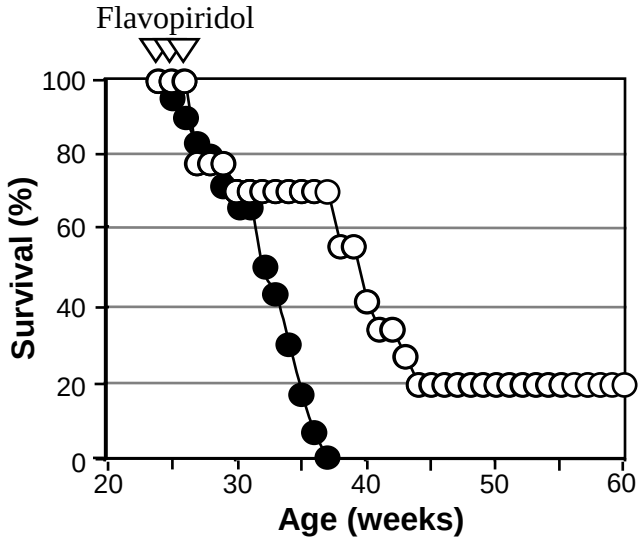
Mouse model: Cdk4 R24C / p27 mice

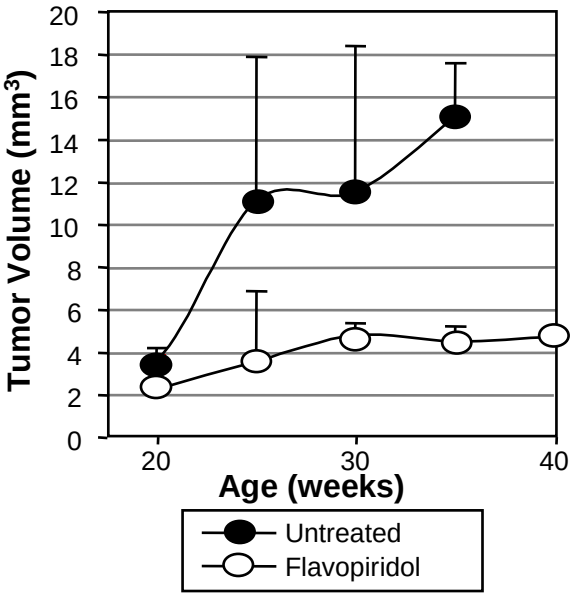
Survival

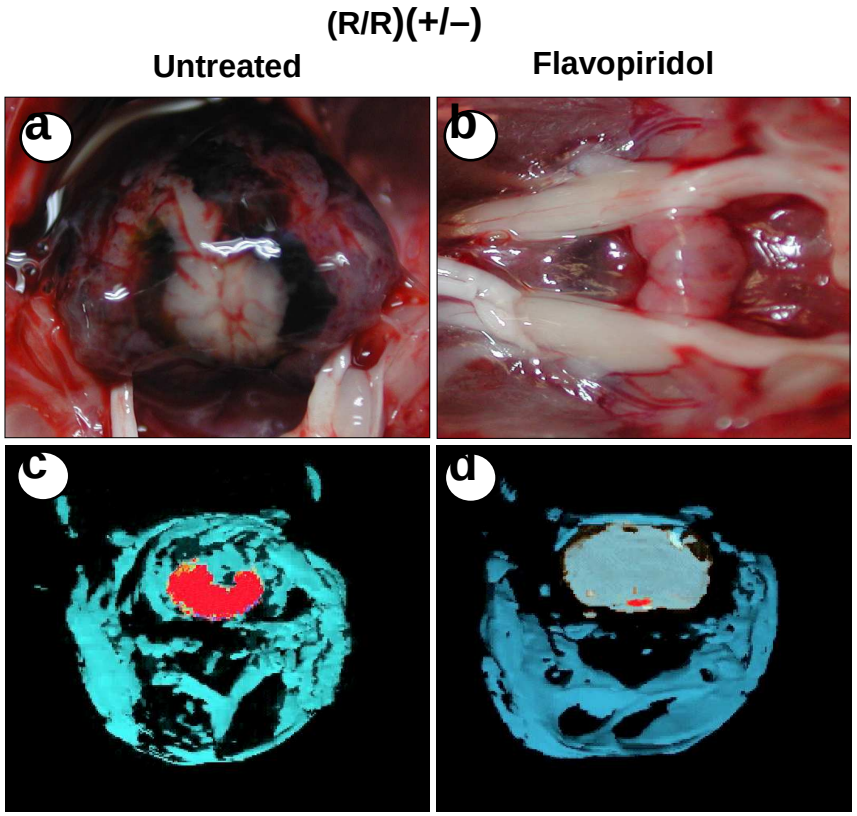


Sotillo et al. *Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors. EMBO J. 2001; 20(23):6637-47.*

Effect of Flavopiridol
In the pituitary tumors of Cdk4 (R24C);p27(+/-) mice







Sotillo et al, Cancer Research, 2005

Tipos de agentes antitumorales en desarrollo

- Pequeñas moléculas
- Péptidos/Peptidomiméticos
- RNAs antisentido/Ribozimas/siRNAs
- DNAs (oligonucleótidos, vacunas génicas)
- Anticuerpos
- Virus (Terapia Génica)
- Células (Células Dendríticas, Inmunoterapia)

Procesos desregulados en células tumorales susceptibles de ser usados en desarrollo de drogas

- Factores de crecimiento/Transmisión de señales
- Ciclo Celular
- Daño en el DNA/Supresores de tumores
- Localización celular de moléculas o proteínas
- Apoptosis
- Proteolísis dirigida por el sistema Ub-Proteasoma
- Senescencia/Elongación de telómeros
- Angiogénesis

Conclusiones

•Aunque existen muchos mecanismos de aproximación a una terapia antitumoral y muchos más de identificación de compuesto activo, todos están basados en un conocimiento de la molécula diana.

•Un mayor conocimiento bioquímico y molecular de la diana molecular permitirá identificar el/los tipos de antitumorales posibles para dicha diana.

•La identificación de compuestos activos con potencial antitumoral depende del tipo de principio buscado y la estrategia elegida para obtenerlo.