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Small molecules solving big problems: present and future of drug discovery

INAUGURAL LECTURE TO THE 775th ACADEMIC YEAR OF THE UNIVERSITY OF SIENA

▶ DIPARTIMENTO DI BIOTECNOLOGIE, CHIMICA E FARMACIA

Maurizio Botta, PhD
Professor in Medicinal Chemistry

SIENA 28/10/2015

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DRUG DISCOVERY

"Drug discovery is the process by which ***new candidate medications*** *are discovered"*

Substances *used to:*

- *diagnose*
- *cure*
- *treat*
- *prevent*

…a ***disease***

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THE EARLY AGES OF MEDICINE

- **2735 BC:** use of *Dichroa febrifuga* reported in China
- **1500 BC:** use of *Drimia maritima* reported in Ebers' papyrus (Egypt)
- **Ancient Greece:** transcripts from Hippocrates and Galen
- **Middle Ages:** many medical plants cultivated in the monasteries
- **1500:** Paracelsus has the idea of moving towards inorganic chemistry
- **1800:** Start to isolate the active ingredient from medical plants

Williams DA, Lemke TL, Foye's Principles of Medicinal Chemistry, 5th Ed

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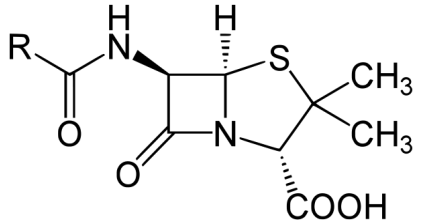
SERENDIPITY: PENICILLIN

1928

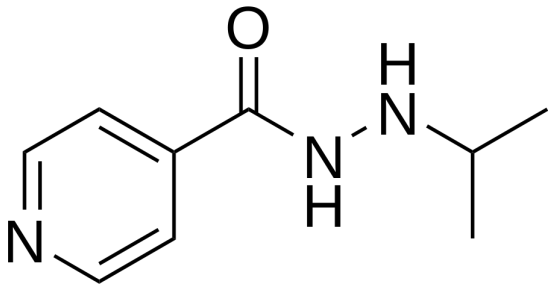
- **Alexander Fleming** is working with bacteria (*Staphilococcus aureus*)
- He goes on holidays for three days, forgetting the incubation plates out
- Plates contaminated with mould (*Penicillium notatum*)



Observation: no bacteria colonies close to the mould

Discovery: PENICILLIN

CLINICAL OBSERVATIONS: IPRONIAZID



- Iproniazid was developed as an **anti-tubercolosis** agent
- **1952: Observation:** Patients given Iproniazid become inappropriately happy

- **1958:** Iproniazid was approved as one of the first **antidepressant** agents
- **1961:** withdrawn due to high hepatitis incidence

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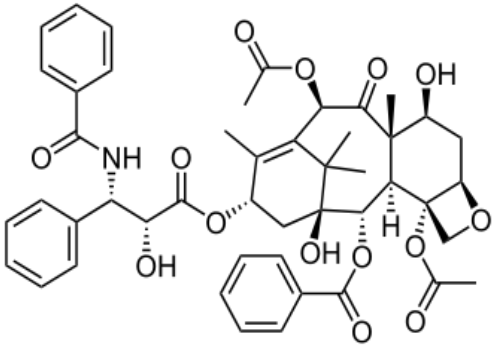
NATURAL PRODUCTS: PACLITAXEL

- **1960: Plant Screening Program** for anticancer activity (NCI, USA): ~1000 plants species screened/year
- **1964:** A sample of *Taxus brevifoliax* cortex was found active in a cytotoxicity assay



- **1992** (USA) and **1993** (Europe): **clinical use** of Paclitaxel
- Forefather of the taxane drug family
- Now used in over 75 Nations
- Cure of **ovarian**, **prostate** and **lung** cancers

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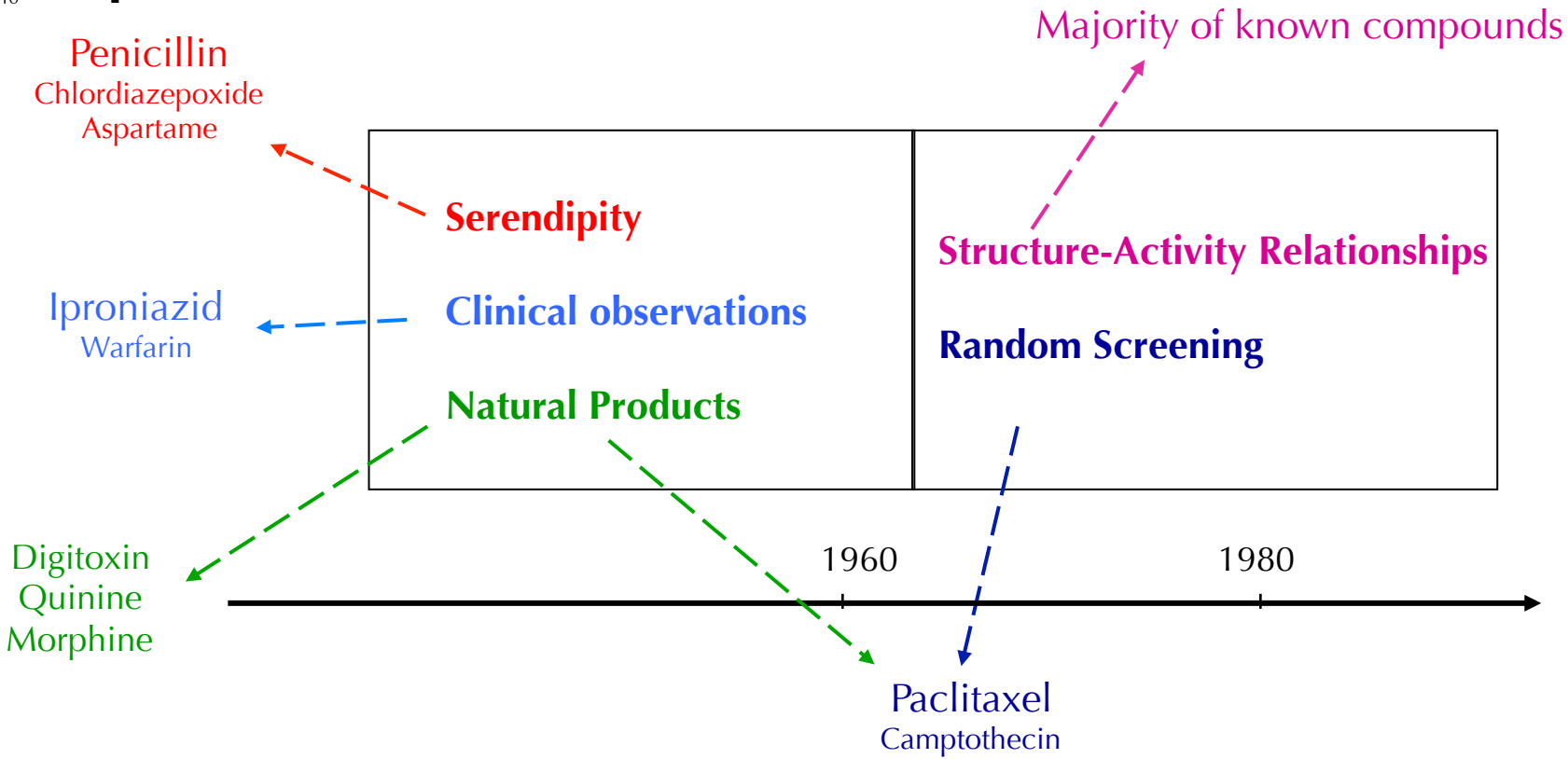
BEGINNING OF DRUG DISCOVERY



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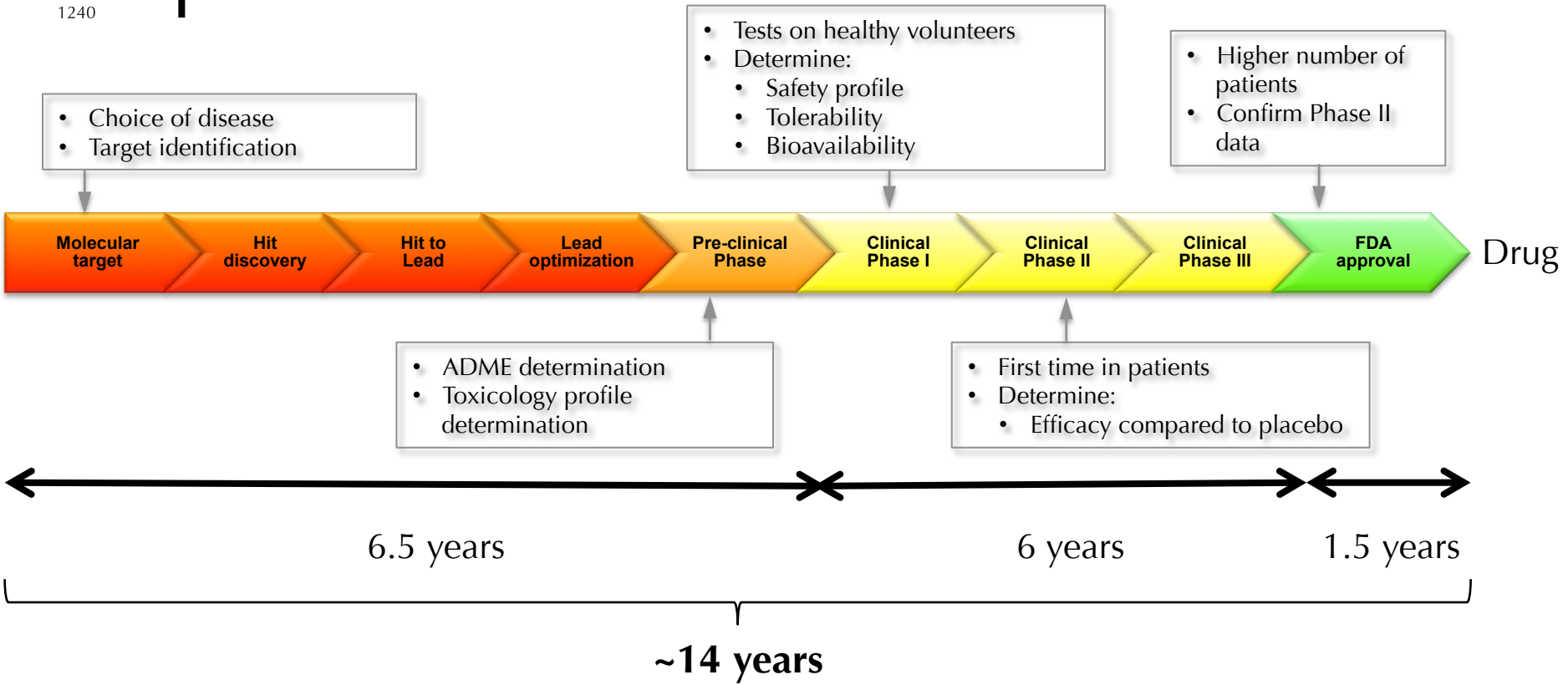
THE LONG AND WINDING ROAD



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THE DRUG DISCOVERY PROCESS TODAY

Drug Discovery is a more and more expensive process

0.8 Bln $

2003

2.6 Bln $

2014

Trufts Center for the Study of Drug Development

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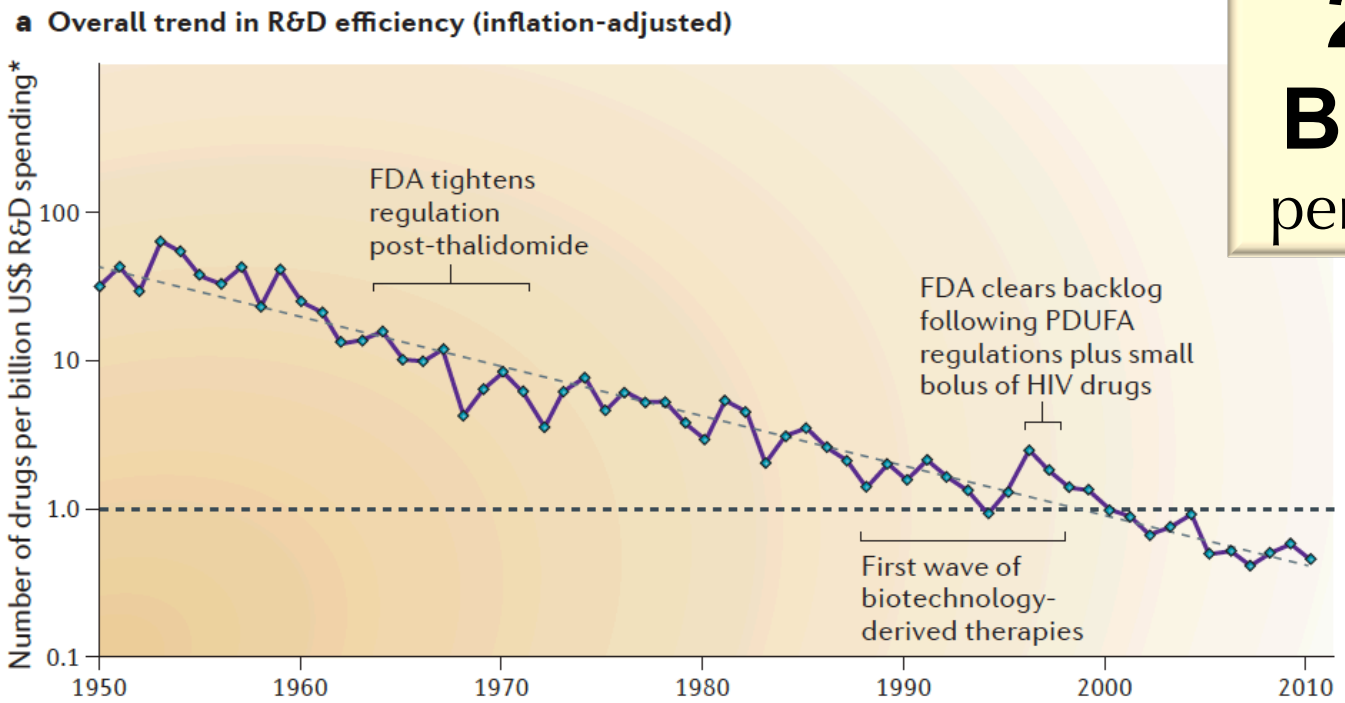
THE DRUG DISCOVERY PROCESS TODAY

2.6 Bln $ per drug



Scannell et al., Nature Reviews Drug Discovery 2012, 11:191-200

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THE PHARMA CRISIS

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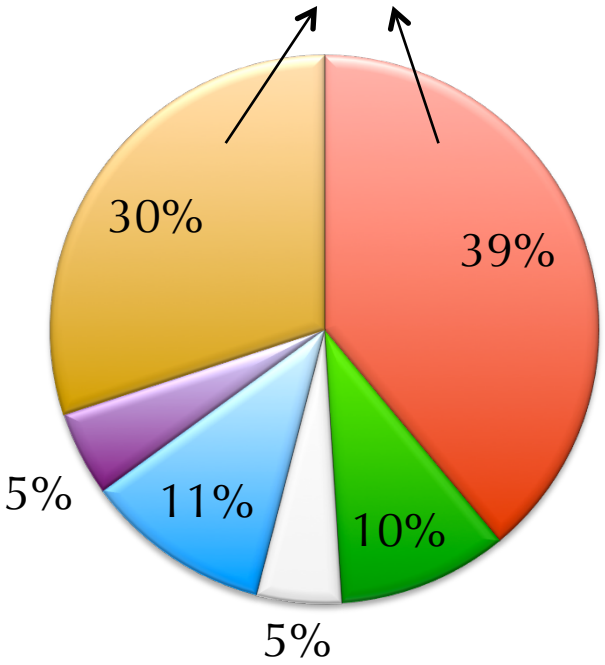
THE CAUSES OF CANDIDATES DROP

Discovered **late** in the drug development process



Big **loss** of **investments**

- ADMET
- Undesired effects
- Commercial reasons
- Preclinical toxicity
- Oher
- Limited efficacy

H. van de Waterbeemd, E. Gifford, Nature Rev. D,D 2003, 2:192-204

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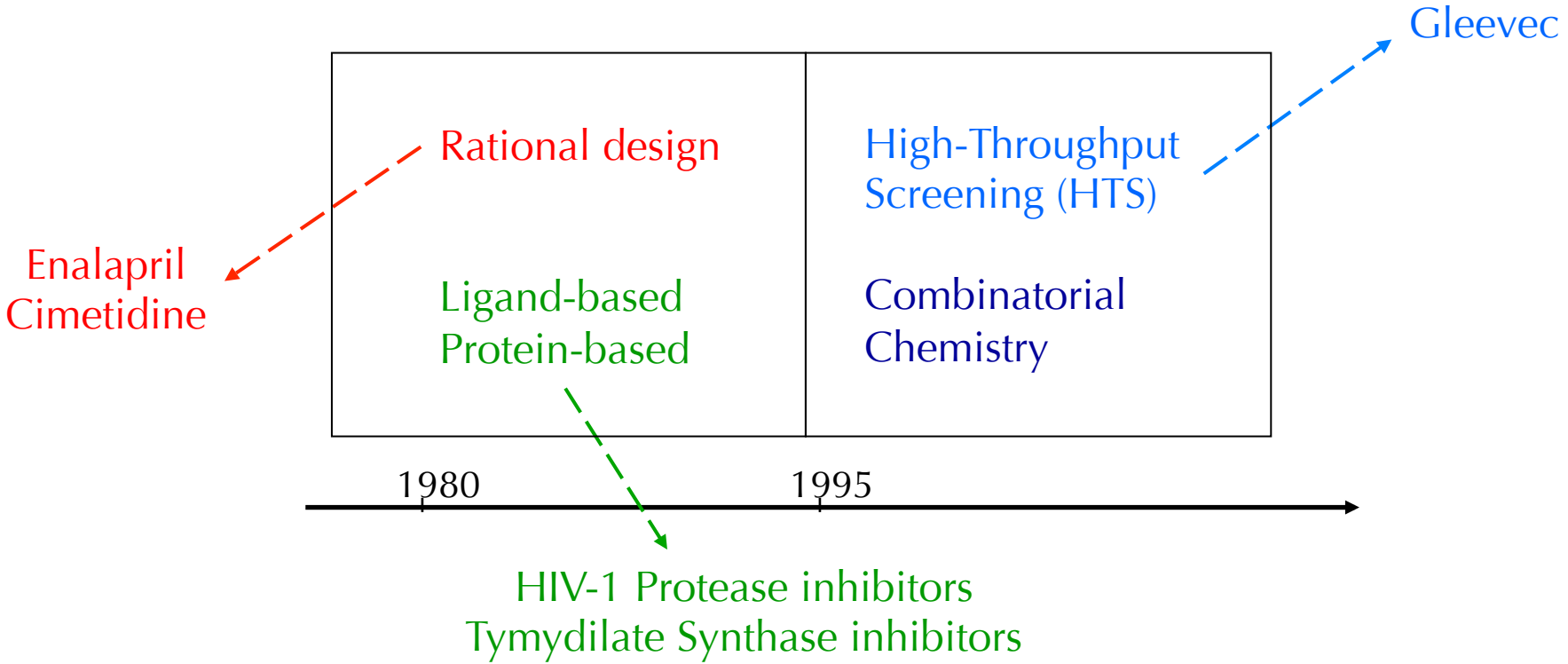
PRESENT OF DRUG DISCOVERY



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COMBINATORIAL CHEMISTRY

Chemical synthesis method that allows the **preparation** of a **large number** of small molecules or peptides in a single process.

Up to **millions of compounds!**

Lead discovery	Lead optimization
• > 10,000 compounds • < 1mg/compound • Synthesis in solid phase • Split and mix	• < 1,000 compounds • > 1mg/compound • Synthesis in solid phase or in solution • Parallel synthesis

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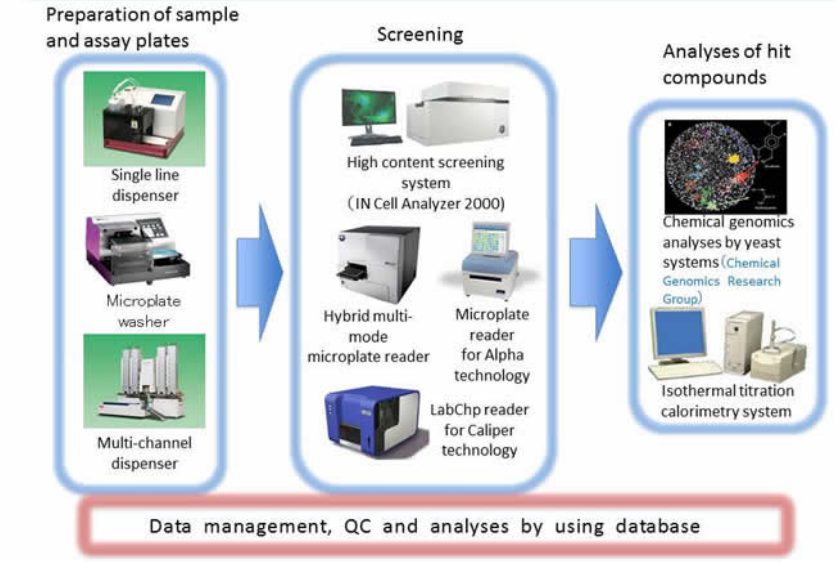
HIGH THROUGHPUT SCREENING (HTS)

Scientific method for the **rapid performance** of **millions of tests** for the identification of active compounds that modulate a specific biomolecular pathway

Needed Resources:

- Robotics
- Sensitive detectors
- Control software
- Data processing programs



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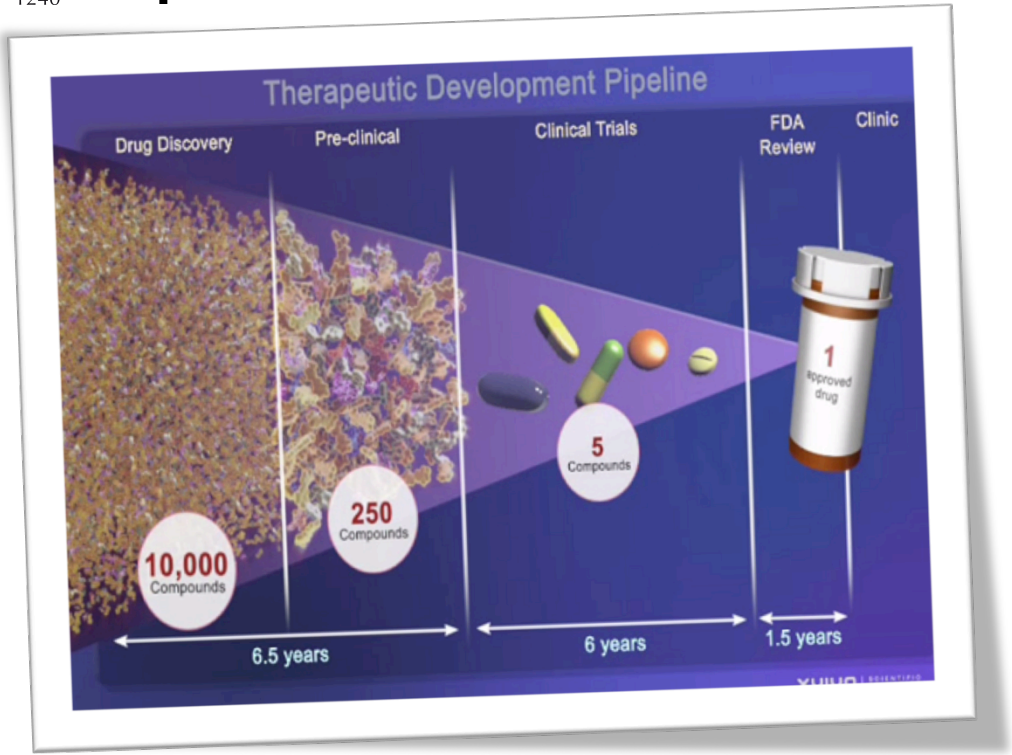
MORE MOLECULES MORE EXPENSES



- Statistics: 10,000 compounds evaluated for each drug
- HTS and combinatorial chemistry increase the number of compounds synthesized and tested
- **The cost of drug discovery increases**

Image: http://www.ncats.nih.gov/research/reengineering/process.html

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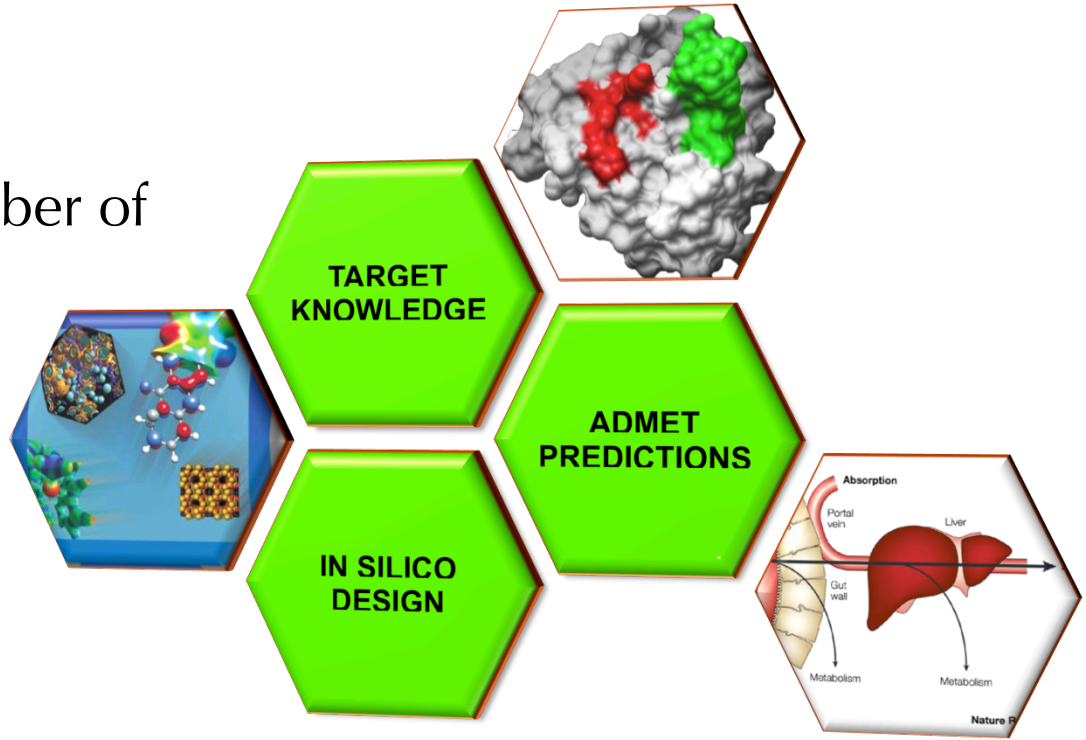
THE FUTURE OF DRUG DISCOVERY

- ✓ Smarter design
- ✓ Reduce the number of compounds



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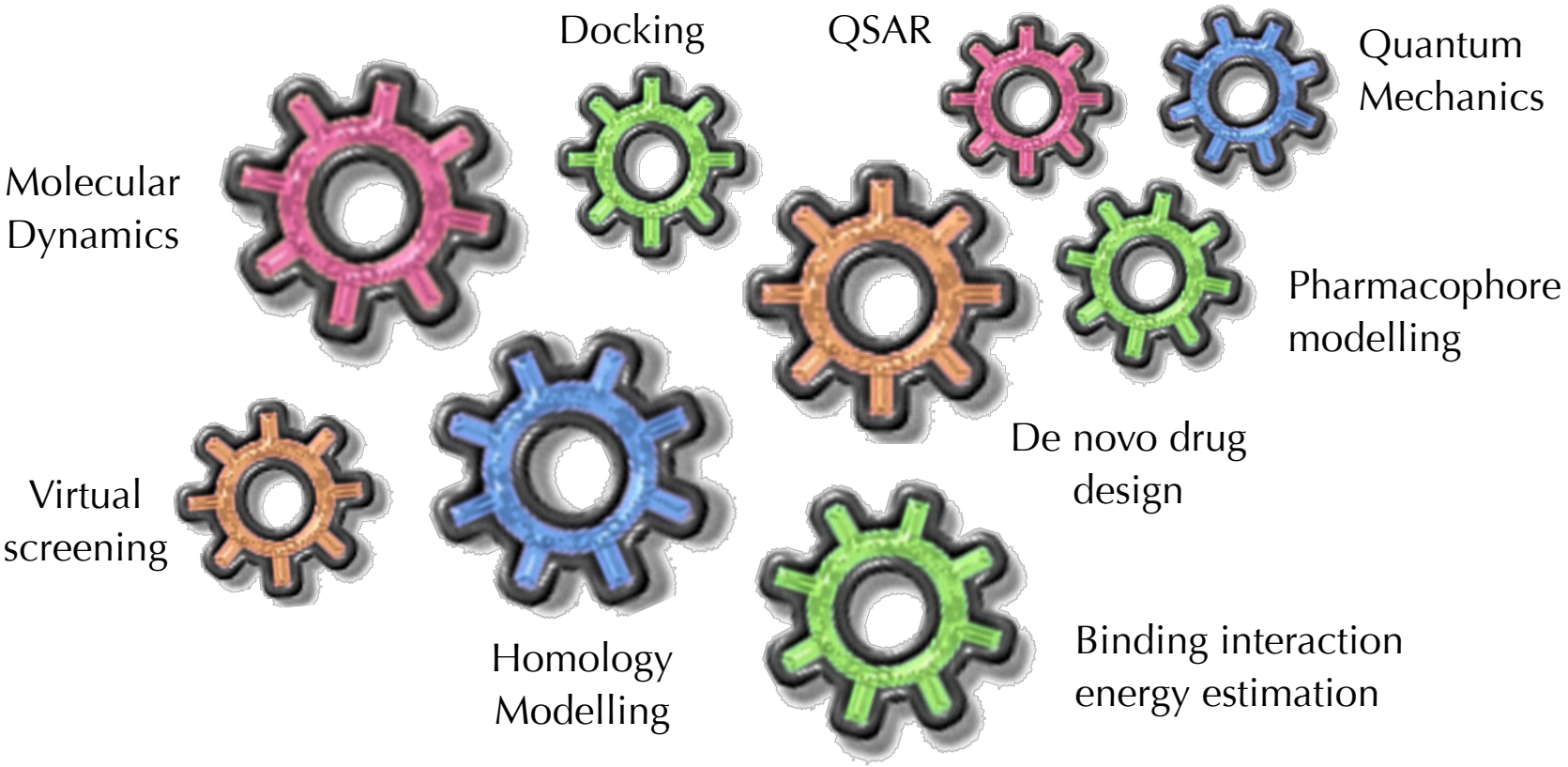
IN SILICO DRUG DESIGN



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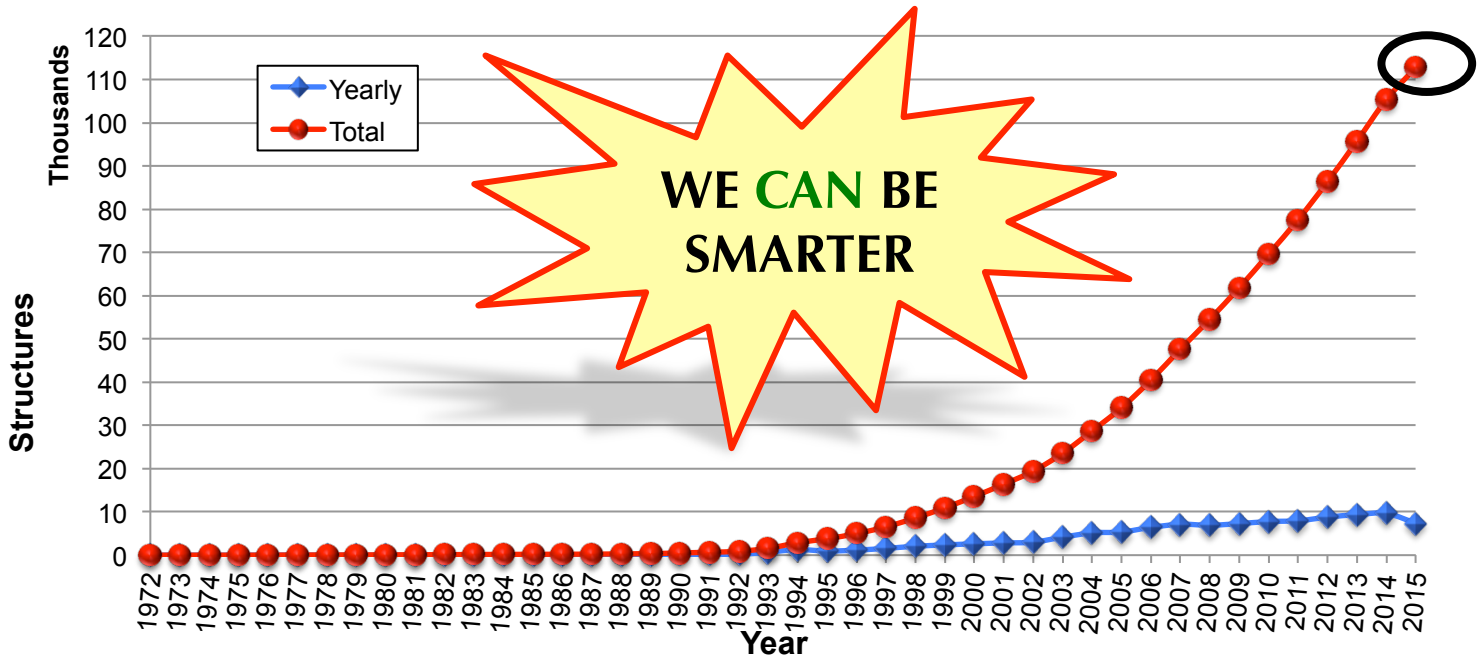
AVAILABLE TARGET STRUCTURES

RCSB PDB
PROTEIN DATA BANK

On 21st Oct 2015:
113,130
structures deposited



www.pdb.org

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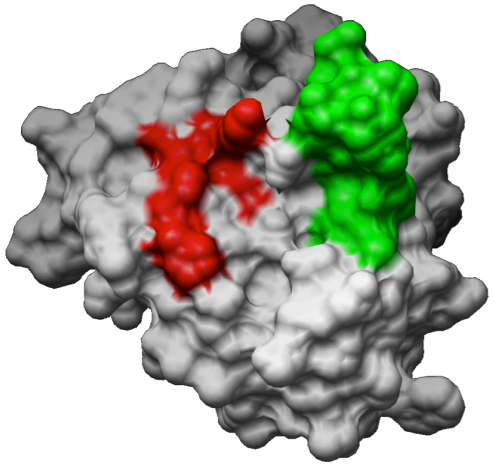
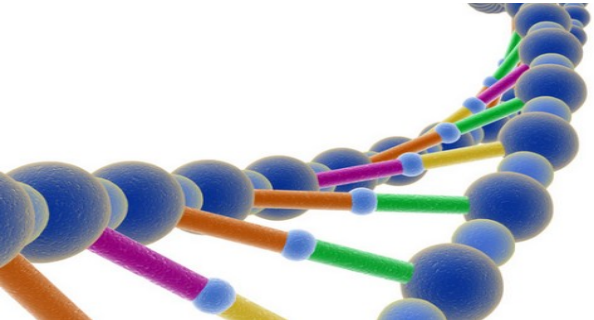
TARGET KNOWLEDGE



Molecular targets can be:

- Proteins
  - Enzymes
  - Transporters
  - Receptors
  - Etc…
- Nucleic acids

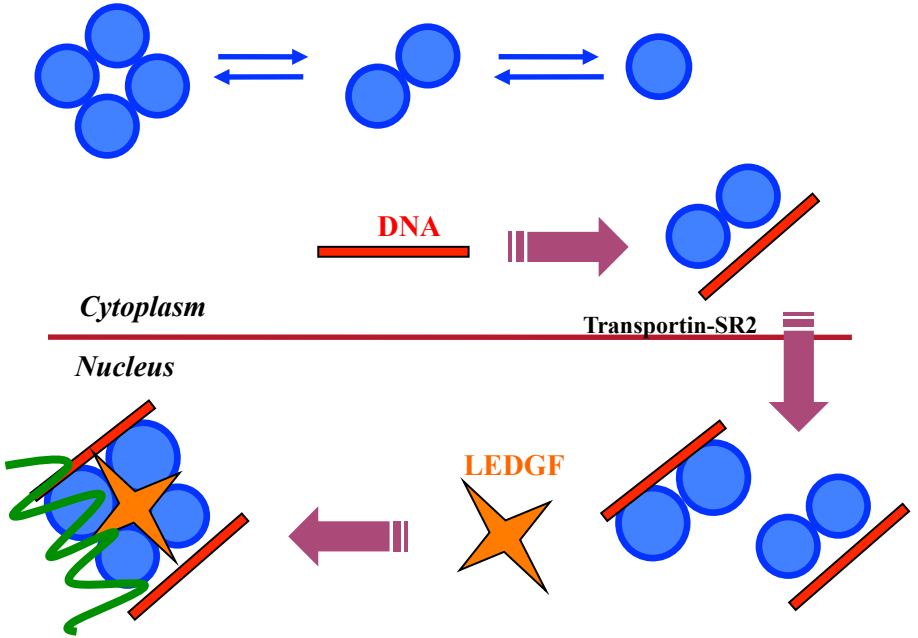


To know the structure of the molecular target is important:

- Understand the mechanism of the activity of the target at the molecular level
- Use this information to design compounds that:
  - Inhibit an undesired effect
  - Enhance a desired effect
- Overcome drug resistance

INHIBITION OF HIV INTEGRASE

- Integrase (IN) is responsible for the **integration** of the **viral DNA** in the cellular DNA
- Monomeric, dimeric, tetrameric and high-order oligomeric states, in equilibrium.
- Dimeric IN binds the viral DNA during the 3′-end processing in cytoplasm.



After nuclear import, two DNA-bound dimers approach each other in the presence of the cellular protein lens epithelium-derived growth factor (LEDGF) and form a tetramer and the integration proceeds to the strand-transfer step.

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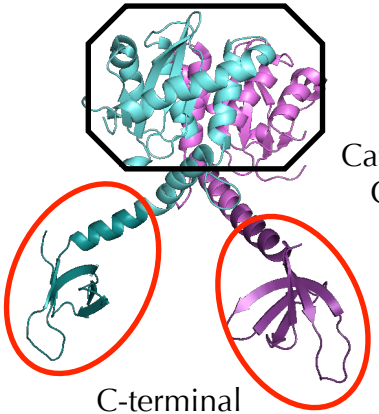
INHIBITION OF HIV INTEGRASE

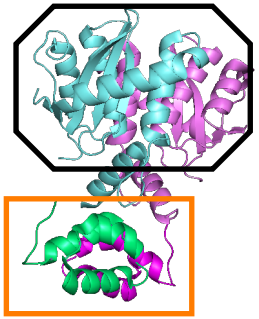
1EX4



Catalytic Core

C-terminal

1K6Y

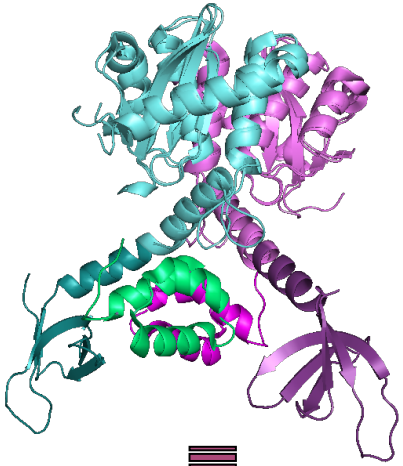


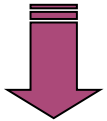
Catalytic Core

N-terminal

Structures assembling

Complete IN dimer





Further refinements:
missing loop residues 47-55 from 1WJD,
missing loop residues 140-149 from 1BL3

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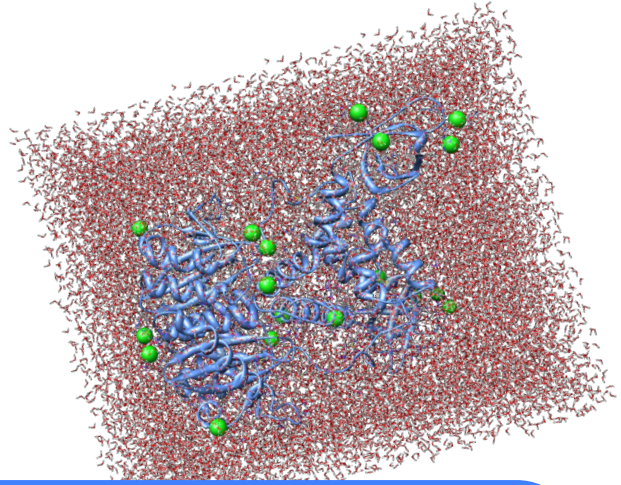
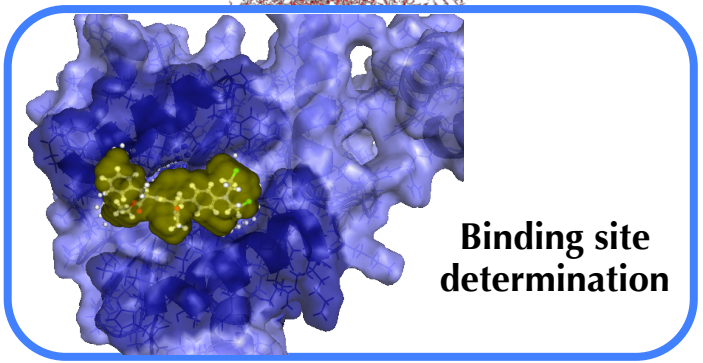
INHIBITION OF HIV INTEGRASE

- The IN dimer model was optimized with a Molecular Dynamics (**MD**) simulation performed with **AMBER10**

- MD trajectory was used for the determination of the most important interacting residues (**hot spots**) through binding free energy estimation with the **MM-GBSA** (Molecular Mechanics-Generalized Born Surface Accessible) approach.





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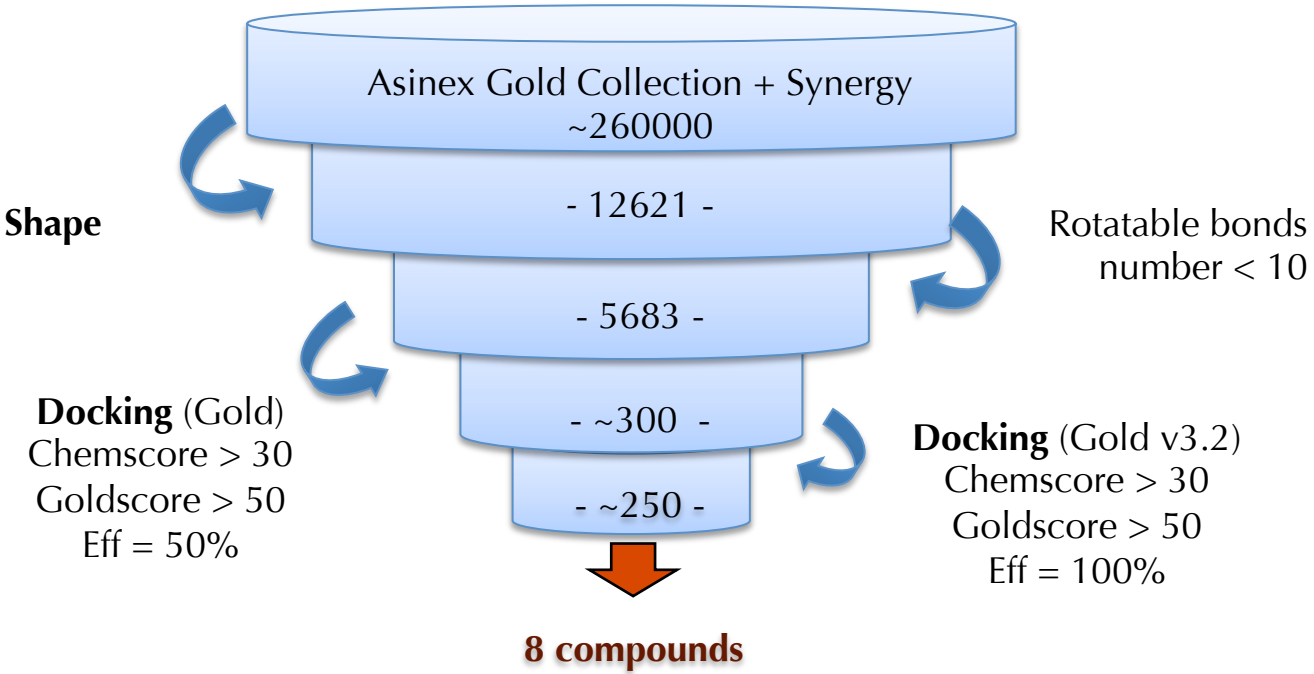
INHIBITION OF HIV INTEGRASE

STRUCTURE-BASED VIRTUAL SCREENING



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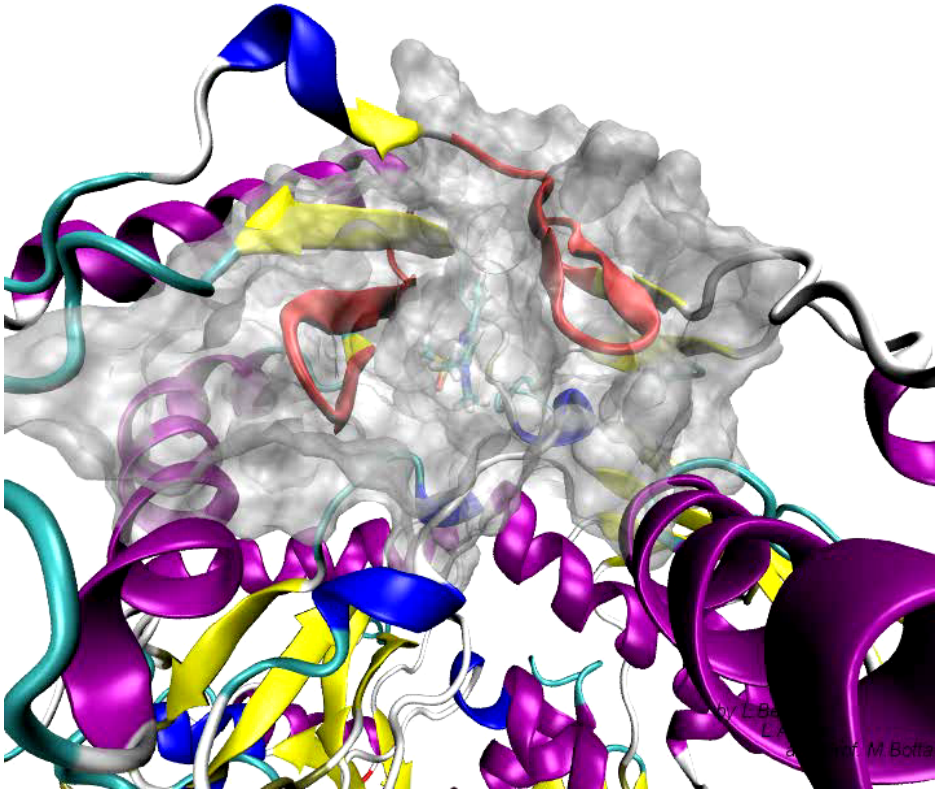
TARGETED MOLECULAR DYNAMICS (TMD)

In TMD, a subset of atoms in the simulation is guided towards the final “target” structure by means of steering forces.

TMD was used to accelarate the migration of DAVP from the NNBP to the x-ray binding pose.



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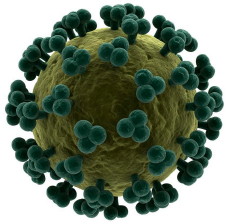
RESEARCH AGAINST HIV



THE FIGHT AGAINST HIV

- Over 25 million deaths
- Over 40 million infected

Current therapy targets viral proteins prone to mutations. This might cause **therapeutic failure**.

Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)
Combivir (zidovudine + lamivudine)
Emtriva (emtricitabine)
Epivir (lamivudine)
Epzicom* (abacavir + lamivudine) *Sold as Kivexa in some countries
Retrovir (zidovudine)
Trizivir (abacavir + zidovudine + lamivudine)
Truvada (tenofovir + emtricitabine)
Videx EC* (didanosine) *Also available generically in the U.S.
Viread (tenofovir)
Zerit (stavudine)
Ziagen (abacavir)

Protease Inhibitors (PIs)
Aptivus (tipranavir)
Crixivan (indinavir)
Invirase (saquinavir)
Kaletra* (lopinavir + ritonavir) *Sold as Aluvia in some countries
Lexiva* (fosamprenavir) *Sold as Telzir in some countries
Norvir (ritonavir)
Prezista (darunavir)
Reyataz (atazanavir)
Viracept (nelfinavir)

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
Edurant (rilpivirine)
Intelence (etravirine)
Rescriptor (delavirdine)
Sustiva* (efavirenz) *Sold as Stocrin in some countries
Viramune XR (nevirapine)
Integrase Inhibitors
Isentress (raltegravir)
Fusion and Entry Inhibitors
Fuzeon (enfuvirtide)
Selzentry* (maraviroc) *Sold as Celsentri in some countries
Single Tablet Regimens
Atripla (efavirenz + tenofovir + emtricitabine)
Complera (rilpivirine + tenofovir + emtricitabine)
Stribild (elvitegravir + cobicistat + tenofovir + emtricitabine)

http://www.aidsmeds.com/articles/DrugChart_10632.shtml

http://questiongene.com/researching-hiv/

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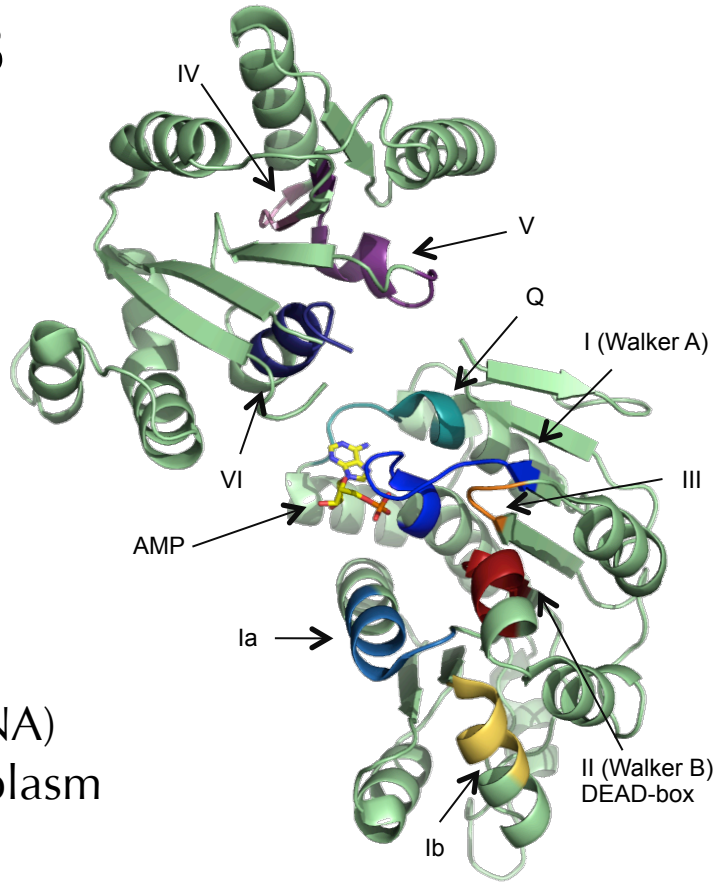
INHIBITION OF DDX3

TARGETING HOST CELLULAR COFACTORS

By targeting host cellular co-factors **essential for HIV** replication drug resistance is less likely to occur.

DDX3 is **essential** for viral nucleic acids (RNA and DNA) shuttling between the cytoplasm and the nucleus.



Hogbom M, Collins R, van de BergS, Jenvert RM, Karlberg T, Kotenyova T, Flores A, Hedestam G, Schiavone L, *J. Mol.Biol* (**2007**), 372 150-159

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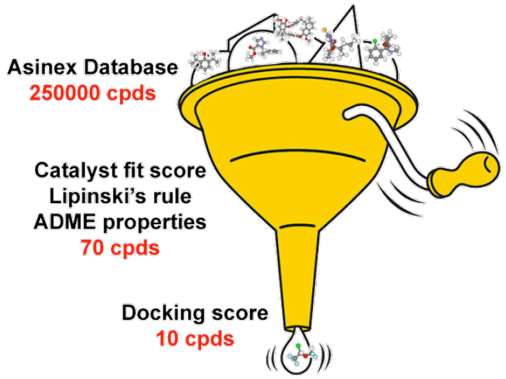
INHIBITION OF DDX3



TARGETING THE ATP BINDING SITE

(Virtual screening)

LA NAZIONE

italianews

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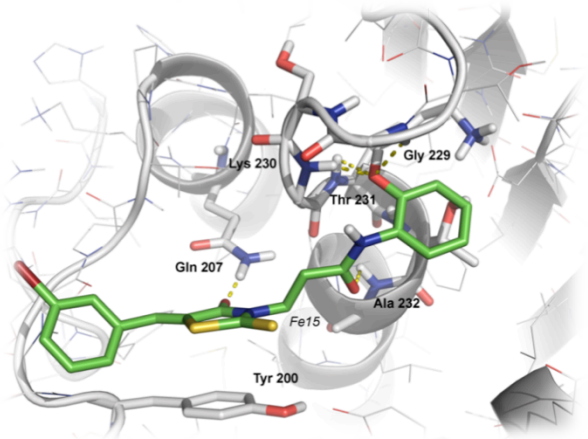
SCIENZA

Aids, scoperta molecola killer Nuova arma contro l'infezione

mobile email stampa OkNO

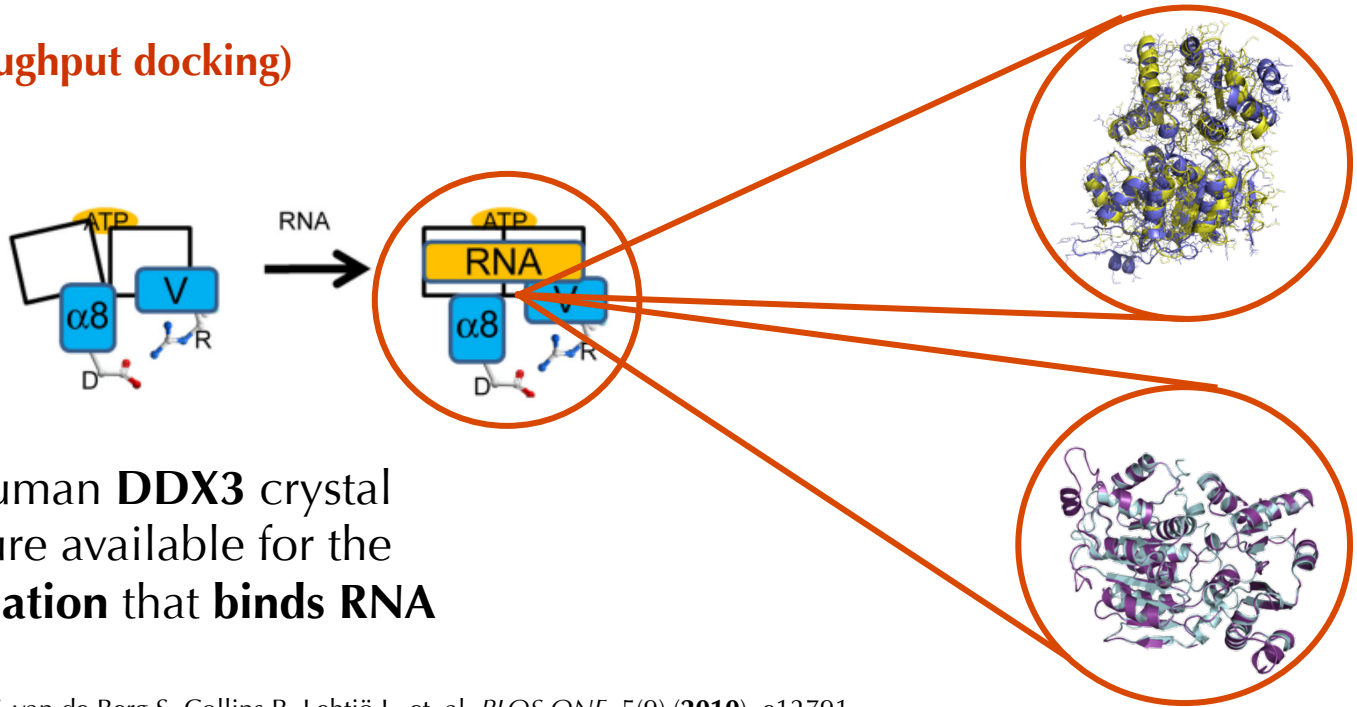
L'Università di Siena ha già depositato due brevetti anti Aids basati su molecole nuove: il professor Maurizio Botta conta di poterle far diventare farmaco completando gli accertamenti preclinici: "Se riusciamo a farle diventare farmaco saranno destinate all'Africa"



INHIBITION OF DDX3: LOOKING FOR SELECTIVITY

TARGETING THE HELICASE BINDING SITE

(High throughput docking)



NO human **DDX3** crystal structure available for the **conformation** that **binds RNA**

Schütz P, Karlberg T, van de Berg S, Collins R, Lehtiö L, et al. *PLOS ONE* 5(9) (**2010**) e12791

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INHIBITION OF DDX3

TARGETING THE ENZYMATIC BINDING SITES

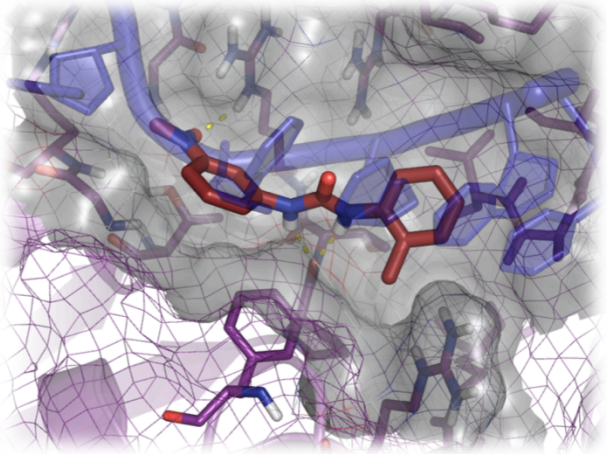
Identification of hit compounds for enzymatic activity

Screening of virtual libraries

Hit optimization

Three compounds families

One family of hybrid compounds



Still **not enough!**

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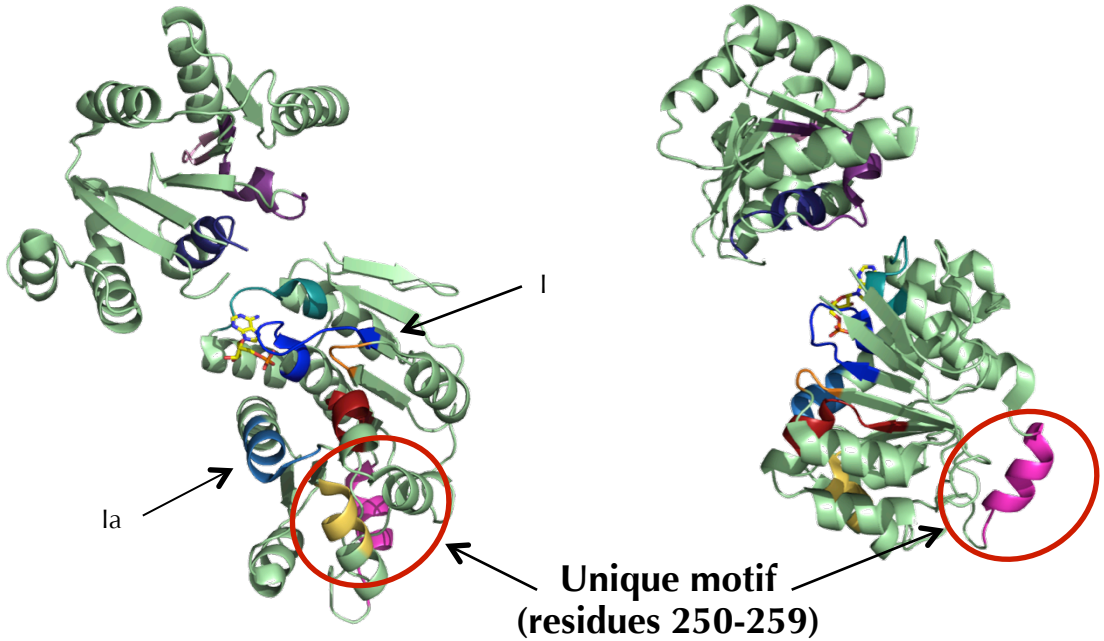
INHIBITION OF DDX3: TRYING TO BE EXTREMILY SELECTIVE

TARGETING THE UNIQUE MOTIF

(High throughput docking)

DDX3 has a **specific insertion** between motifs I and Ia (residues 250 – 259) not generally found in other DExD-box helicases.



Garbelli A., Beermann S., Di Cicco G., Dietrich U., Maga G, *PLoS One*, **2011,** 6(5):e19810

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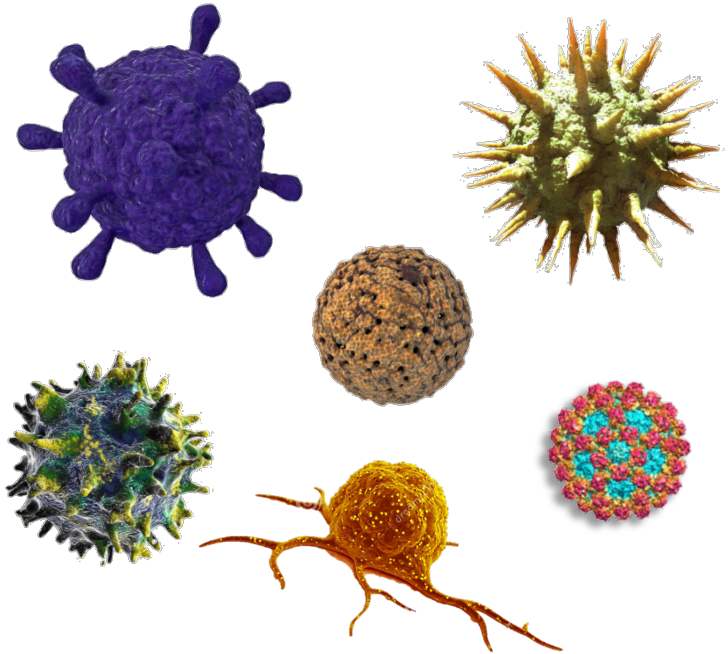
THE SAME INHIBITOR FOR SEVERAL VIRUSES… AND CANCER!!!

During this work it was demonstrated that **DDX3** is also **involved in several other diseases.** Compounds were not cytotoxic, but active against:



- ***HCV:*** interacts with HCV core protein which is used by the virus to build its nucleocapsid.
- ***Japanese Encephalitis V:*** binds the viral RNA during viral replication.
- ***Poxvirus:*** binds K7 protein, essential to overcome the IFN-mediated cellular response.
- ***West Nile V, Dengue V:*** unknown mechanism
- ***Aggressive cancers*** *(lung cancer, prostate cancer, breast cancer)****:*** unknown mechanism

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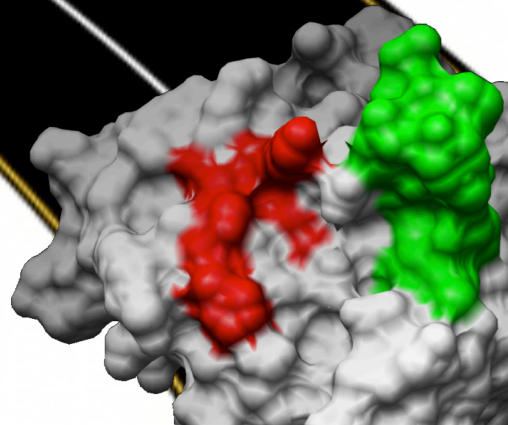
ADMET – GETTING TO THE TARGET

Solubility
(e.g. plasma concentration, etc…)

Lipophilicity
(e.g. plasma protein interaction, organ distribution, etc…)

Acidity/basicity
(e.g. ionization state in solution, etc…)

Permeability
(e.g. crossing cell membranes, crossing BBB, etc…)



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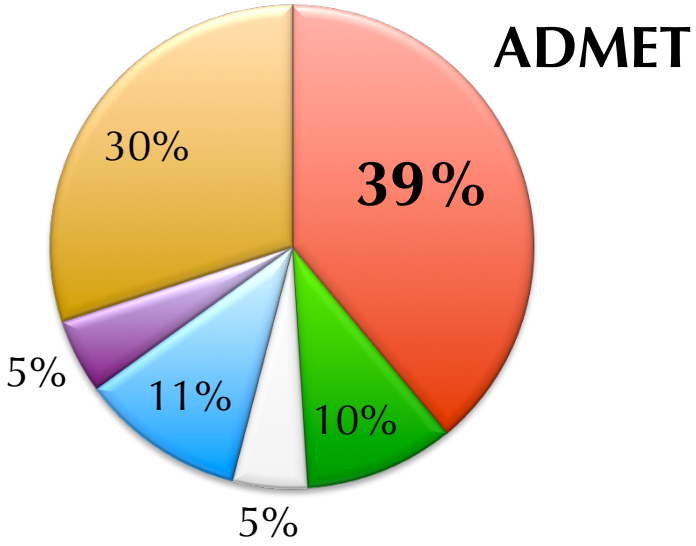
DRUG LIKENESS (ADMET) DETERMINATION

- **39%** of drug candidates drop because of **ADMET** problems
- These problems are discovered **late** in the drug development process
- Great **loss of investments**



- **ADME determination**
- Toxicology profile determination

H. van de Waterbeemd, E. Gifford, Nature Rev. D,D 2003, 2:192-204

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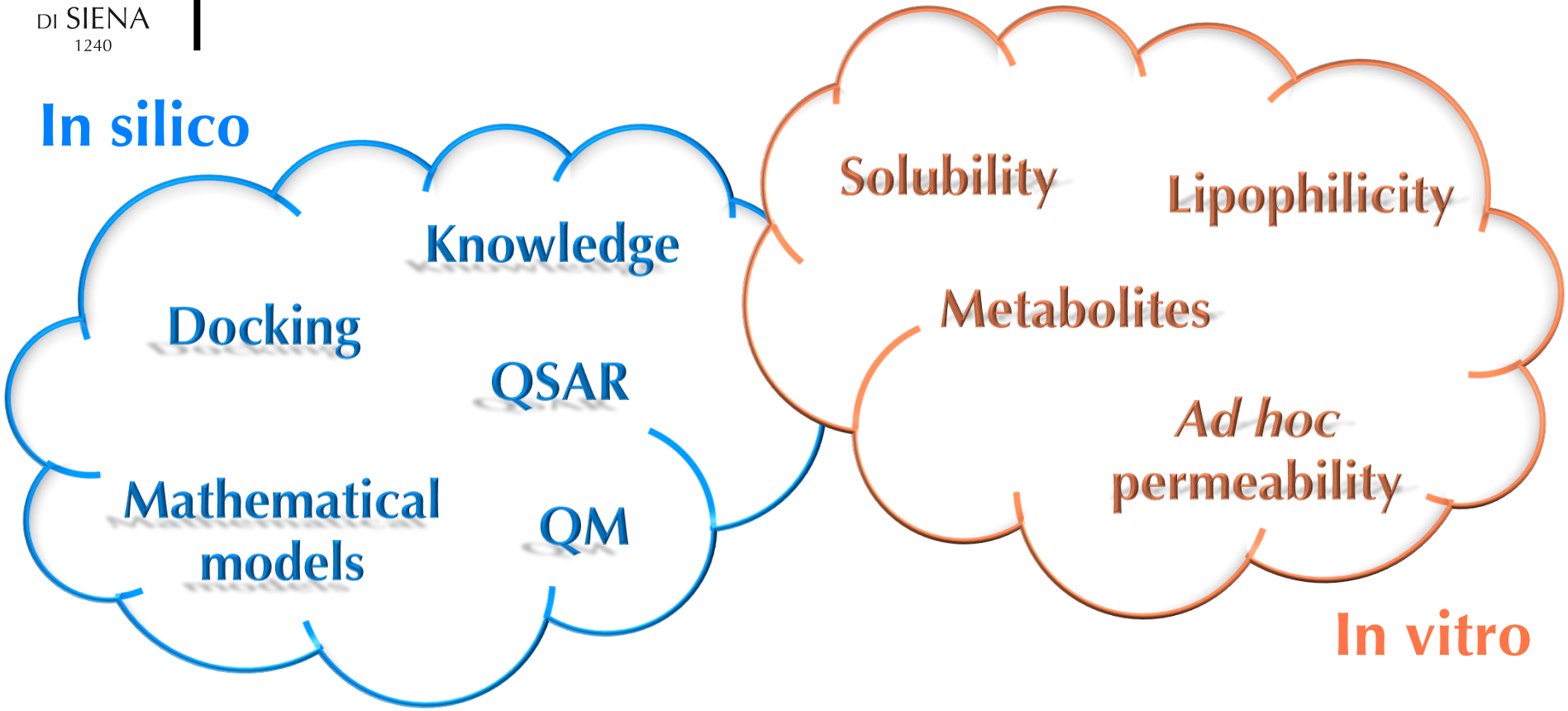
ADMET PREDICTIONS



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ADMET PREDICTIONS

It is important to PREDICT:

- The ability of the designed compou
  reach the target tissue or organ
- The metabolic products of the compounds
- The toxicity of the compounds and
  metabolites

BEFORE reaching the preclinical stag

Design and synthesize compounds with the **desired Pk profile**

Design **drug delivery options**

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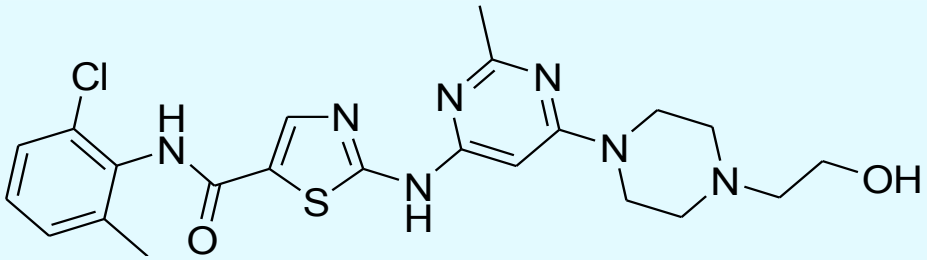
DUAL C-SRC/ABL INHIBITORS

Src and Abl share a **significant sequence homology** and a **remarkable structural resemblance.** For this reason ATP competitive compounds originally developed against Src, showed to be potent Abl inhibitors as well.

Dasatinib:

- First dual Src/Abl inhibitor
- Approved in 2006 by US-FDA for the treatment of imatinib resistant CML
- Currently in several clinical trials for the treatment of different solid tumors



Dasatinib
(Bristol-Meyer-Squibb)

Schenone, S. et al. Expert Opin Investig Drugs 2010, 8, 931

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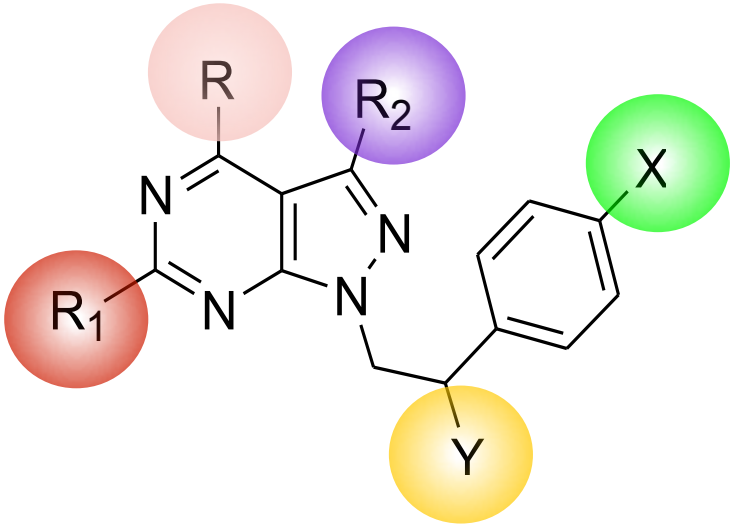
DUAL C-SRC/ABL INHIBITORS

Drug BBB Permeability (BBB PAMPA assay)



Very good activity:
(e.g. SI306)

- K_i = 0.04 μM
- IC_{50} = 0.7 μM
- In vivo (50 mg/Kg for 60 days) = 50% tumor growth reduction
- Metab. Stability = 95%
- Papp = 5.27×10^{-6} cm/sec
- **BBB Papp** = 7.10×10^{-6} cm/sec

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DUAL C-SRC/ABL INHIBITORS

*Unfortunately TK inhibitors possess **poor pharmacokinetic properties**, especially low water solubility.*

Biochemical Properties

1. Metabolism (phase I and II)

Physical-Chemical Properties

2. Permeability

3. Aqueous solubility
   - Cyclodextrines
   - Liposomes
   - Prodrugs

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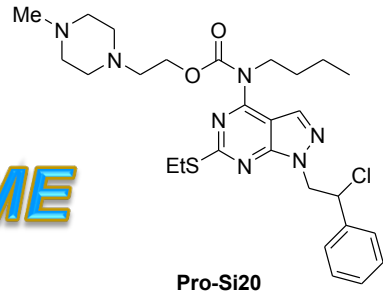
DUAL C-SRC/ABL INHIBITORS

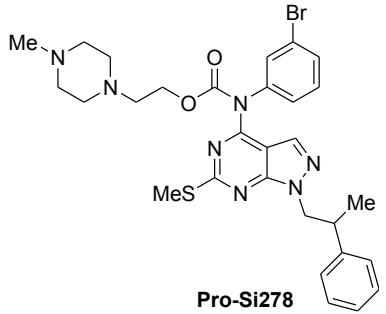
Aqueous Solubility (Prodrugs)

Cpd	H_2O solubility (μg mL^{-1})	Stability				Metabolic stability (%)
		H_2O $T_{1/2}$	PBS pH 7.4 $T_{1/2}$	MeOH $T_{1/2}$	Human Plasma $T_{1/2}$	
SI20	0.05	ND	ND	ND	ND	91.5
SI278	0.01	ND	ND	ND	ND	95.1
Pro-SI20	1.91[b]	30 mins	63mins	125 mins	28 mins	ND
Pro-SI278	6.47	>48 hrs	>48 hrs	>48 hrs	193 mins	99.9

ADME



Pro-Si20



Pro-Si278

Activity Assays

Cpd	K_i μM		IC_{50} (SD) μM	
	c-Src	c-Abl wt	32D-p210	32D-T315I
SI20	0.60	0.32	3.5 (0.8)	6.7 (1.2)
SI278	0.018	1.07	6.2 (0.8)	5.8 (0.9)
Pro-SI20	NA	NA	1.2 (0.1)	2.4 (0.1)
Pro-SI278	NA	NA	2.8 (1.6)	2.6 (0.2)

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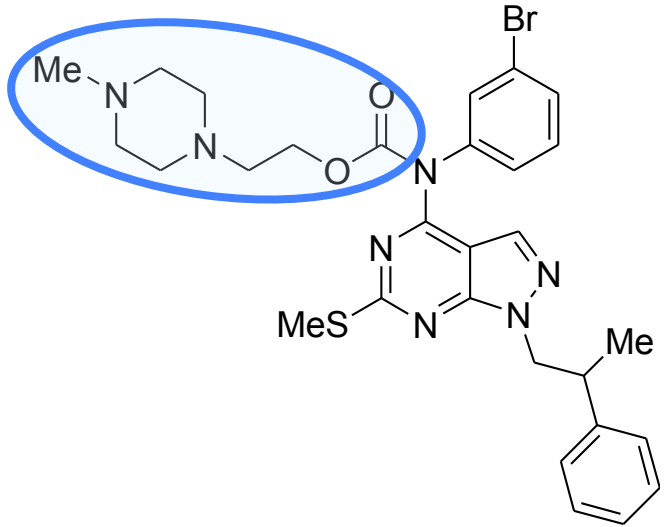
TOWARDS PERSONALIZED THERAPY

Directing the compound to the target (Prodrugs)

- ✓ The prodrug approach was a **success** in increasing compound solubility



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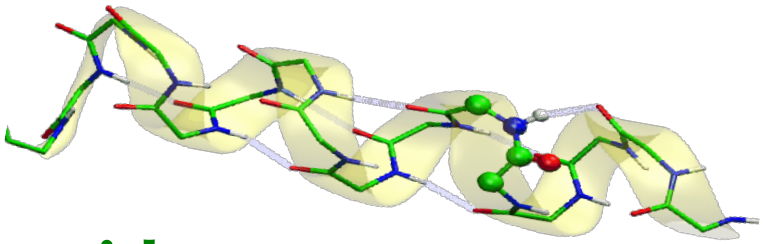
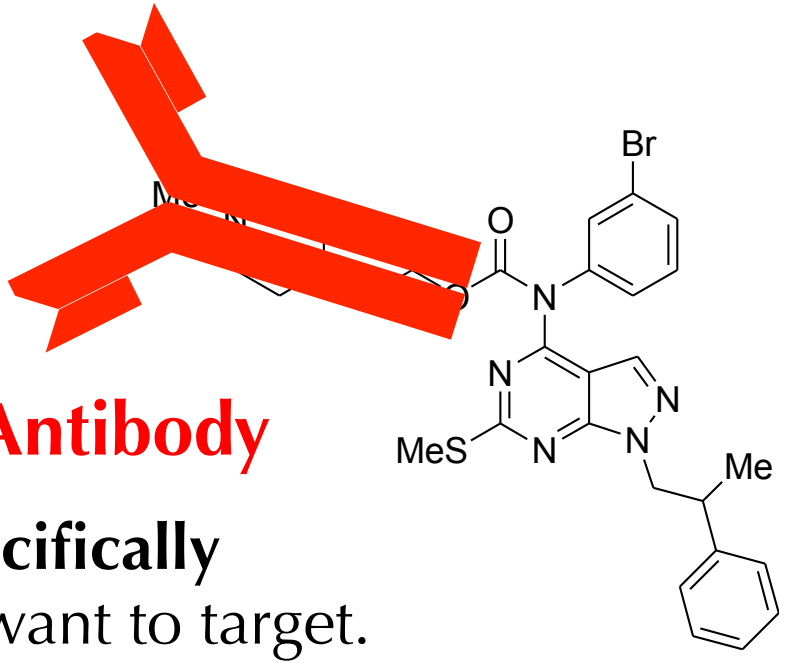
TOWARDS PERSONALIZED THERAPY

Directing the compound to the target (Prodrugs)



Peptide



Antibody

IDEA: attach something that is **specifically recognized** by the cell we want to target.

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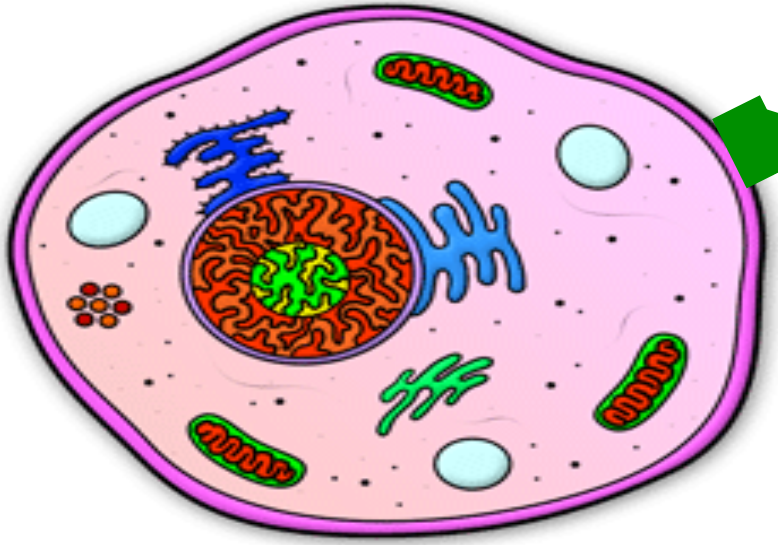
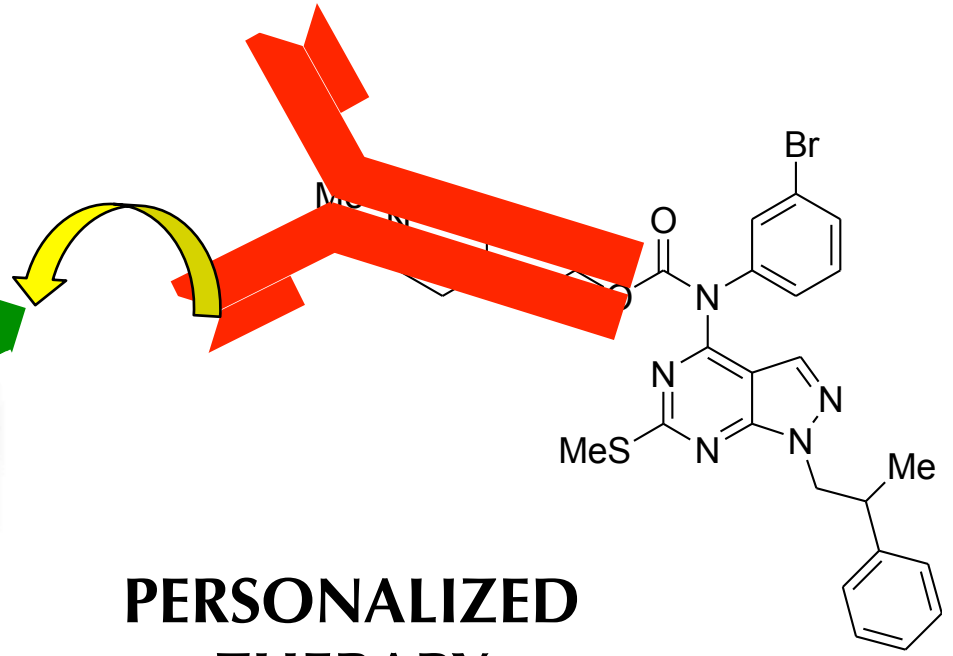
TOWARDS PERSONALIZED THERAPY

Directing the compound to the target (Prodrugs)





PERSONALIZED THERAPY

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CONCLUSIONS

Novelty can improve future Drug Discovery:

- Increase of knowledge of the target:
  - ✓ More rational design
  - ✓ Personalized therapy
- In silico drug design:
  - ✓ Less compounds synthesized and tested
  - ✓ Get quicker and cheaper to drug candidate
- Early ADMET prediction/determination:
  - ✓ Aid the choice about a compound destiny earlier in the process
    - ➢ Drop lead candidates earlier in the process
    - ➢ Design appropriate drug delivery solutions

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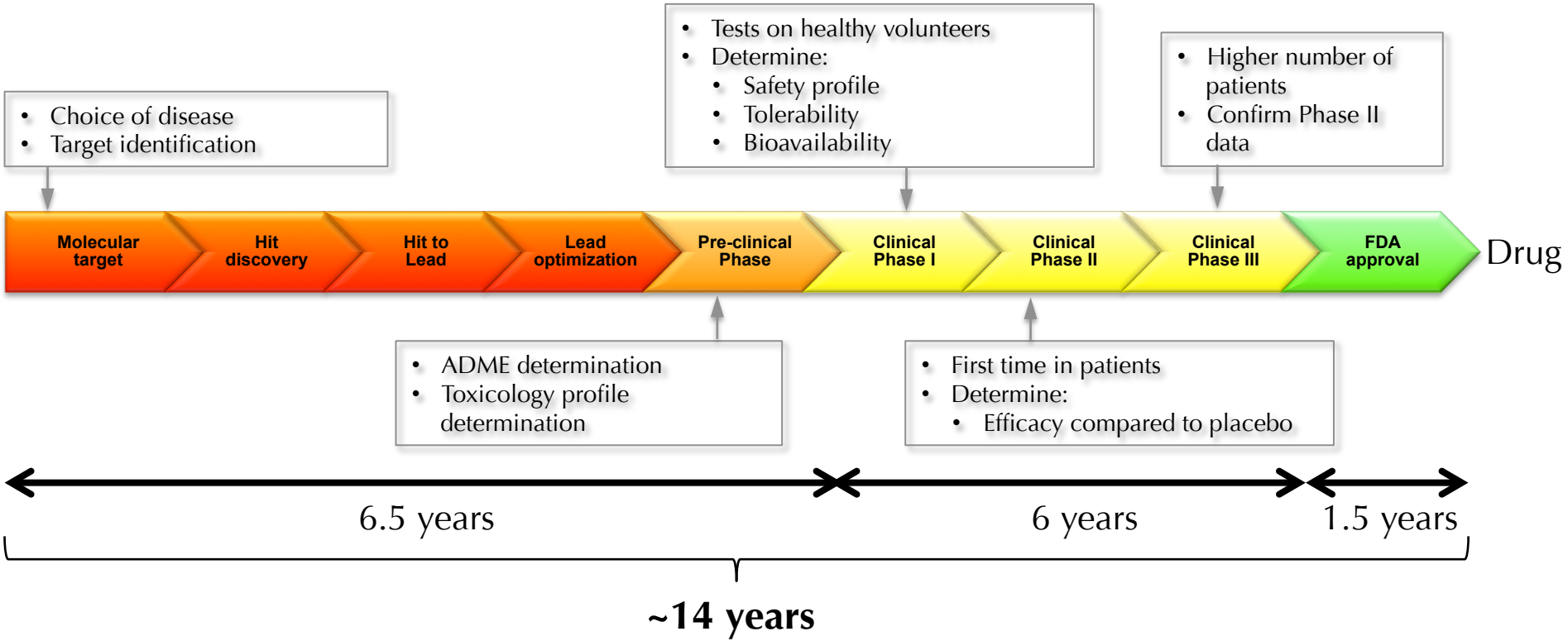
.....BUT THE JOURNEY TO MAKE A DRUG IS STILL VERY LONG AND WINDING....



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"Finding a drug is a complex combination of many disciplines such as structural biology, molecular biology, synthetic chemistry, computational chemistry, pharmacology and medicine.

Only a good collaboration can drive the work to the final goal."

Maurizio Botta

Iuni Trist