Experimental Cancer Therapeutics II

Frank Wuest, PhD

Oncologic Imaging, Edmonton PET Centre, CCI
wuest@ualberta.ca

Oncology 520; April 03, 2012

Outline – Specific Topics

- **Introduction to cancer drug design and discovery**

  ➔ **First generation of cancer drugs**
  ➔ **Molecularly targeted small molecule cancer drugs**

- **New approaches to molecular cancer therapeutics**

  ➔ **Process of developing new molecular-targeted therapeutics**
  ➔ **Druggable genome**
  ➔ **Lead generation and lead optimization**

- **Application of Biomarkers**

  ➔ **Role of molecular imaging (PET) in cancer drug development**

Selected References

Cancer drug design and development:

Collins I, Workman P. New approaches to molecular cancer therapeutics. Nat. Chem. Biol. 2006; 2(12):689-700.

Billingsley ML. Druggable targets and targeted drugs: enhancing the development of new therapeutics. Pharmacology. 2008; 82(4):239-44.

van Montfort RL, Workman P. Structure-based design of molecular cancer therapeutics. Trends Biotechnol. 2009; 27(5):315-28.

Hopkins AL, Groom CR. The druggable genome. Nat. Rev. Drug. Discov. 2002; 1(9):727-30.

Biomarkers and molecular imaging:

Carden CP, Sarker D, Postel-Vinay S, Yap TA, Attard G, Banerji U, Garrett MD, Thomas GV, Workman P, Kaye SB, de Bono JS. Can molecular biomarker-based patient selection in Phase I trials accelerate anticancer drug development? Drug Discov. Today. 2010; 15(3-4):88-97.

Pysz MA, Gambhir SS, Willmann JK. Molecular imaging: current status and emerging strategies. Clin. Radiol. 2010; 65(7):500-16.

McCarthy TJ. Positron emission tomography imaging as a key enabling technology in drug development. Ernst Schering Res. Found Workshop. 2007; (62):329-39.

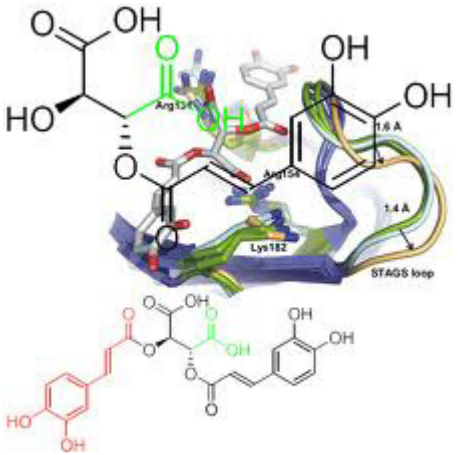
Beloueche-Babari M, Workman P, Leach MO. Exploiting tumor metabolism for non-invasive imaging of the therapeutic activity of molecularly targeted anticancer agents. Cell Cycle. 2011; 10(17):2883-93.

Modern cancer drug design and discovery

Modern cancer drug design and discovery:

Integrating

- ➔ **Targets**
- ➔ **Technologies**
- ➔ **Treatments**



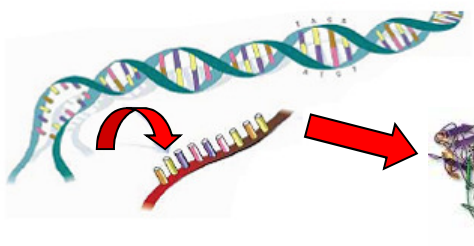
FOCUS: **Opportunities and challenges in the discovery and the design of molecularly targeted small-molecule cancer drugs**

Modern cancer drug design and discovery

1. Molecular targets of contemporary drug discovery projects:

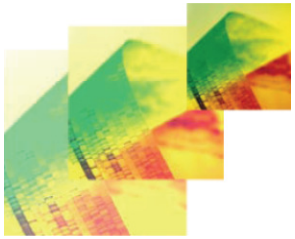
➔ **Reflection of our increasing understanding of genes and signalling pathways responsible for the initiation and malignant progression of cancer**

➔ **How can potential new molecular targets be considered, validated and prioritized?**



Human genome discovery
- `88-`00: Mapped
- `98-`10: Analyzing SNP's

Human proteome discovery
- `00-`20: Discovering protein relationship to genes



Disease target identification

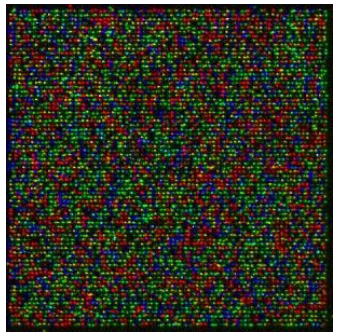
Cancer drug

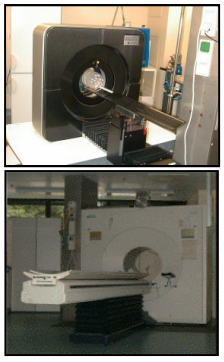
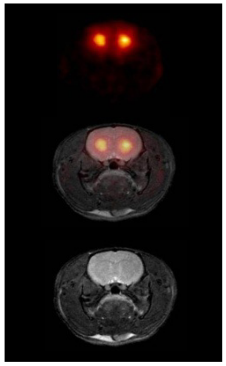
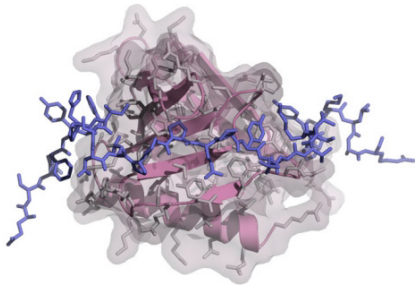
Modern cancer drug design and discovery

2. **<u>Integrated application of a range of powerful drug discovery and drug evaluation technologies:</u>**

- ➔ **Genomics**
- ➔ **High-throughput screening (HTS)**
- ➔ **Molecular imaging**
- ➔ **Structural biology**

<u>GOAL:</u> **Multi-parameter optimization of lead structures towards effective cancer drugs**



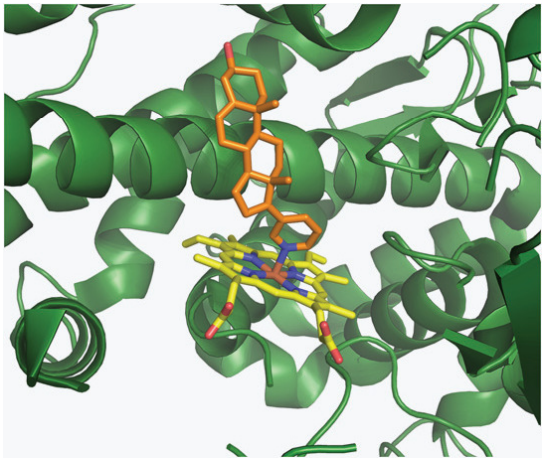




Modern cancer drug design and discovery

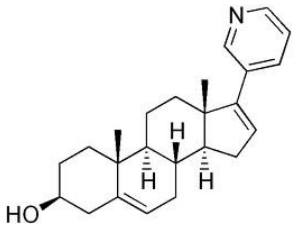
3. Novel treatments:

➔ **Reflection of the success of new mechanism-based molecular therapeutics which act on cancer-causing targets**

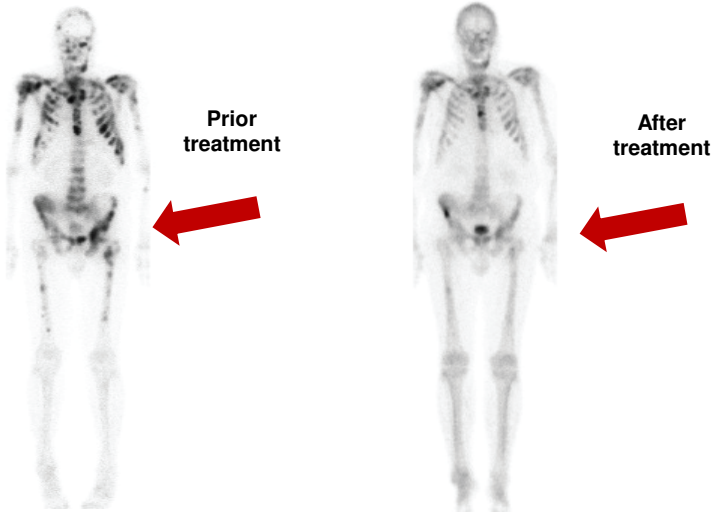
➔ **Novel treatments which benefit from technological innovations in drug design**



Prostate cancer drug abiratone targeting active site of cytochrome P450



abiratone



Effect of abiratone treatment on reducing bone metastases in prostate cancer patients using ^{99m}Tc-MDP SPECT

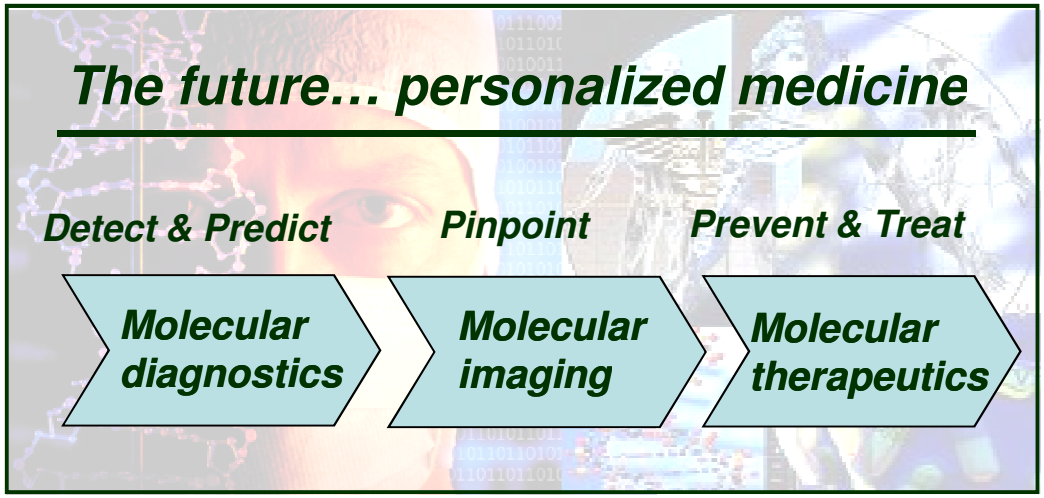
http://www.jointcancerreport.org/discovery-development/cancer-drug-discovery-and-development

CONCLUSION:

Design, discovery and evaluation of cancer drugs are leading the way in the development of personalized molecular medicine

Key: **Identification of biomarkers for:**

- ➔ **Patient selection**
- ➔ **Monitoring treatment effects (Molecular imaging!!!)**



Modern cancer drug design and discovery

Changing times:

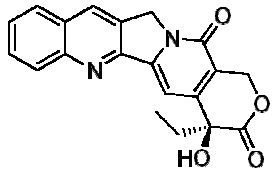
First generation of cancer drugs

➔ **Almost all acted as cytotoxic agents (often based on natural products)**

Mode of action:
- **DNA damage**
- **Inhibition of DNA synthesis**
- **Interference with mechanisms of cell division**

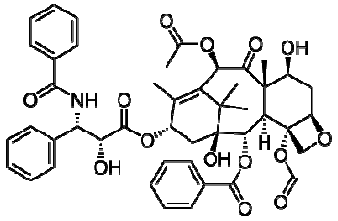
Examples:

➔ **Topoisomerase inhibitors**



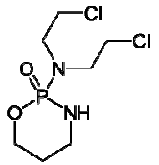
Camptothecin

➔ **Microtubuli-binding drugs**



Paclitaxel

➔ **DNA alkylating agents**

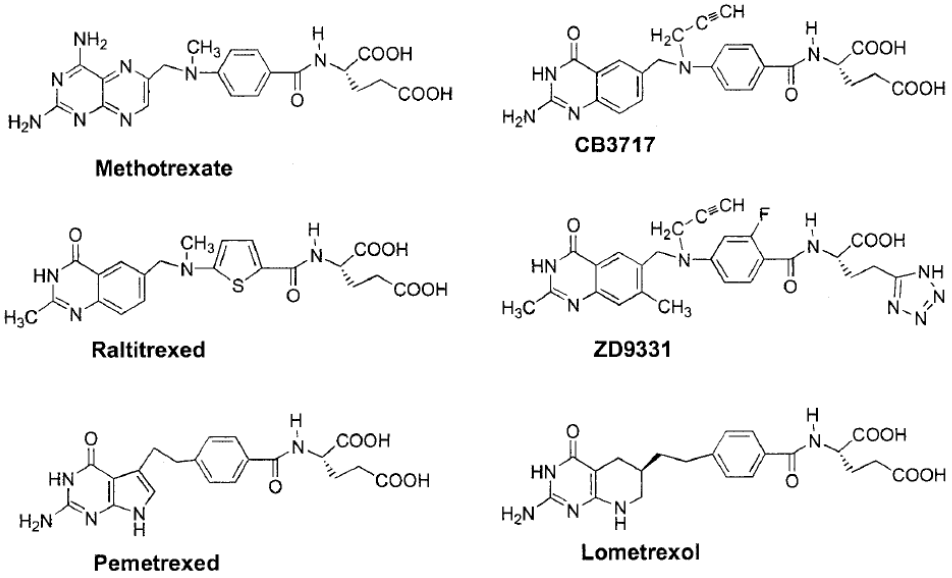


Cyclophosphamide

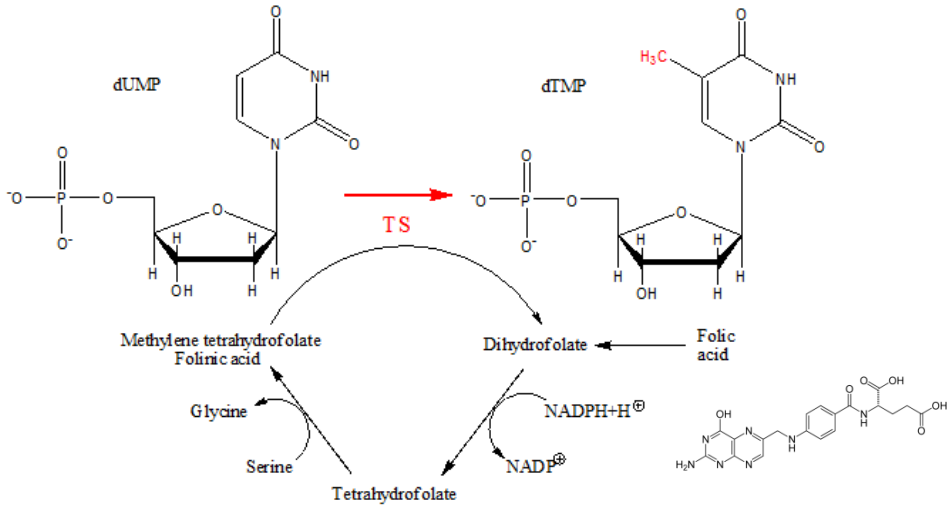
Modern cancer drug design and discovery

Drugs developed in the first, cytotoxic era of cancer drug development were not designed to take advantage of our current knowledge of the genetic and molecular basis of cancer

But: **Many acted according to the "molecular targeting" approach**
Designed according to contemporary medicinal chemistry including SAR and X-ray crystallography
➔ E.g. Antifolate thymidylate synthase inhibitors (anti-metabolites)



Structures of antifolate drugs (anti-metabolites)



Mode of action

http://clincancerres.aacrjournals.org/content/10/3/1080.full

Modern cancer drug design and discovery

Success and limitations:

Notable successes with conventional cytotoxic drug treatment of cancer

But: **Effectiveness of drug treatment varies across the different anatomical, histological, and molecular types of cancer**

☺ **Major improvements in the treatment of leukemias, lymphomas, testicular cancer, and children's malignancies**

☹ **Cancer still second most frequent cause of death in the Western world**
No major improvement through simple fine-tuning of classical cytotoxic agents

---

This view coincidenced with the arrival of novel molecular targets emerging from basic cancer research and genomics

⇒ **Emerging of "molecular cancer therapeutics"**
⇒ **Novel mechanism-based drugs acting on drug targets involved in the molecular causation of cancer**

Modern cancer drug design and discovery

What has changed? - A brief summary -

1. *Today's molecular targets reflect our increasing understanding of the genes and signaling pathways responsible for initiation and malignant progression of cancer*

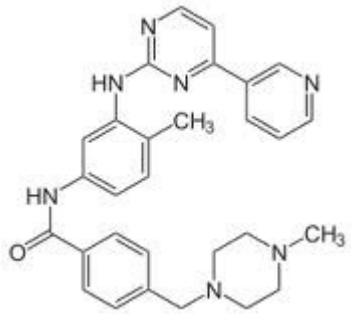
2. *Integrated application of a range of powerful drug discovery technologies*

3. *New treatments which reflect the success of mechanism-based molecular therapeutics*

Novel molecular cancer therapeutics

Agent	Target for agent	Targeted cancer: Solid tumours: NSCLC	Breast cancer	CRC	GIST	Renal cancer	Pancreatic cancer	HNSCC	Haematological tumours: AML	B-cell CLL	CML	B-cell lymphoma	Multiple myeloma
mAbs													
Cetuximab (Erbitux)	EGFR												
Trastuzumab (Herceptin)¶	ERBB2												
Bevacizumab (Avastin)#	VEGF												
Rituximab (Rituxan)**	CD20											✓	
Ibritumomab tiuxetan (Zevalin)*	CD20											✓	
Tositumomab-I^{131} (Bexxar)*	CD20											✓	
Gemtuzumab ozogamicin (Mylotarg)##	CD33								✓				
Alemtuzumab (Campath)	CD52									✓			
Small-molecule inhibitors													
Imatinib mesylate (Glivec)	TKs (BCR-ABL, KIT, PDGFR)				✓						✓		
Gefitinib (Iressa)	TK (EGFR)	✓											
Erlotinib (Tarceva)	TK (EGFR)	✓					✓§§						
Sunitinib (Sutent)	TKs (VEGFR, PDGFR, KIT, FLT3)				✓	✓							
Sorafenib (Nexavar)	Kinases (B-Raf, VEGFR2, EGFR, PDGFR)					✓							
Bortezomib (Velcade)	28S protease												✓

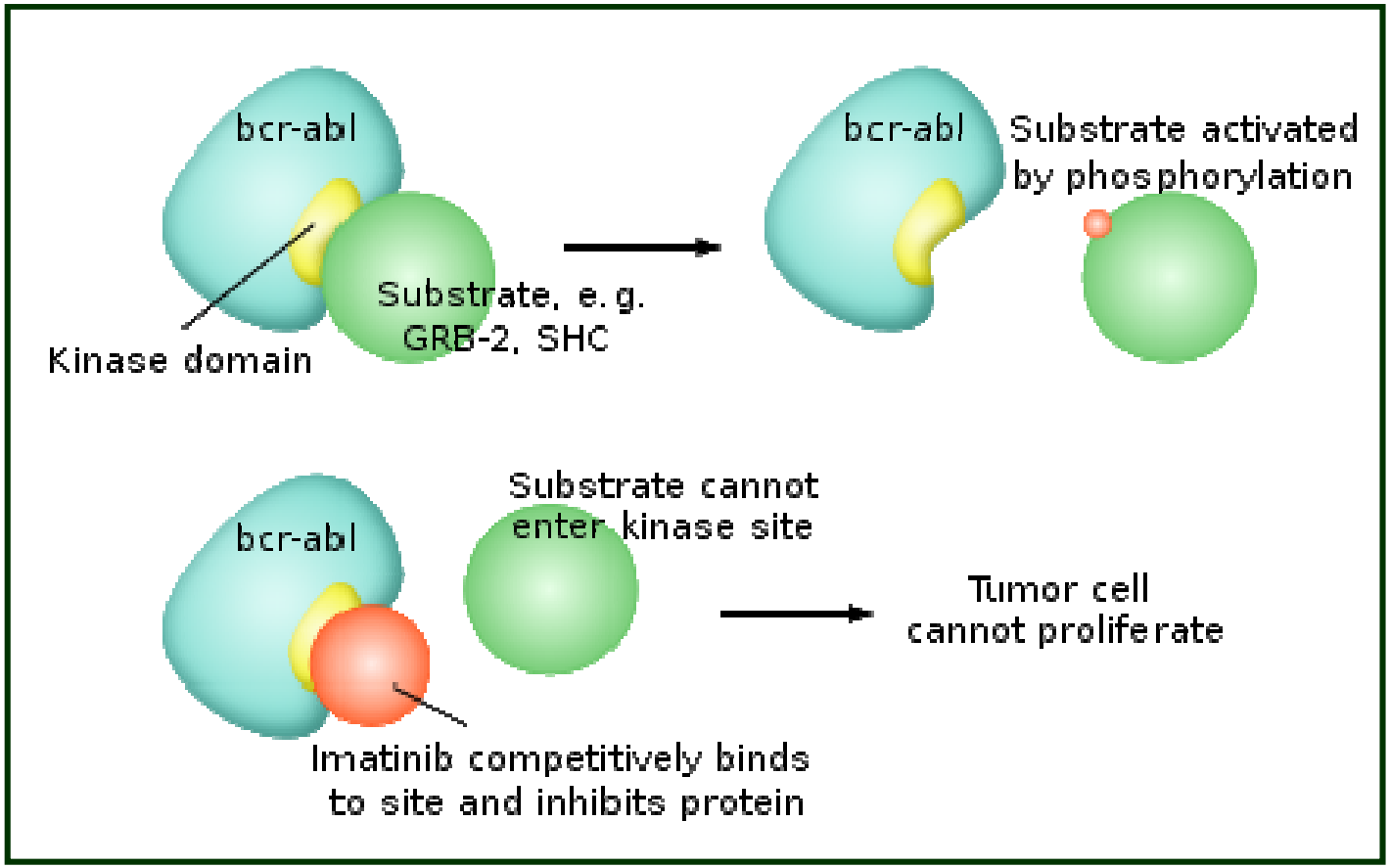
Gleevec:
First successful small molecule for molecularly targeted cancer therapy



Source: Nat Rev Cancer © 2006 Nature Publishing Group

Gleevec: How it works – An example for molecular targeting

Chronic myelogenous leukemia (CML): $30.000 - $100.000 per year
Gastrointestinal stromal tumors (GIST): $65.000 per year



http://www.goshdawnit.com/2008/08/message-from-mike.html

Novel molecular cancer therapeutics

Current challenges of oncology drug discovery and development with respect to small-molecule drug development:

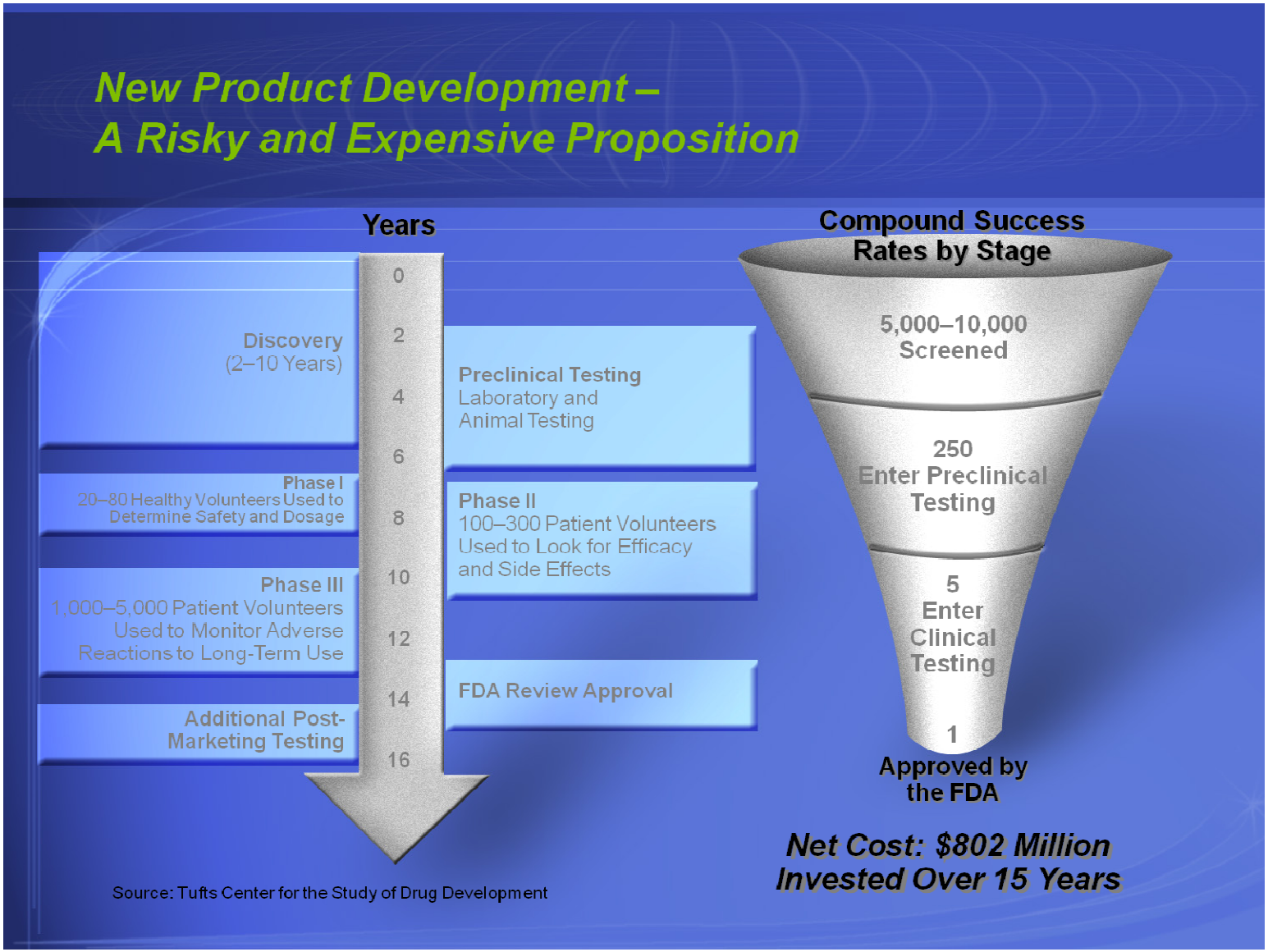
- ***Many of recently approved drugs are mAbs***

- ***Others are not first-in-class agents***

Assessment of overall success rate for oncology drug development:

- ➔ **Failure rates for cancer drugs in clinical trials (1990-2000) were worse than for most other therapeutic areas *(Kola and Ladis, 2004)***
- ➔ **Only 5% of oncology drugs entering the clinic went to gain regulatory approval for marketing (95% failed!!!, other disease fields have 11% success rate)**
- ➔ **Longer development timelines for oncology drugs than for other therapeutic areas**
- ➔ **Large number of cancer drug failures occurred in an advanced stage clinical evaluation (high costs!!!)**

Novel molecular cancer therapeutics



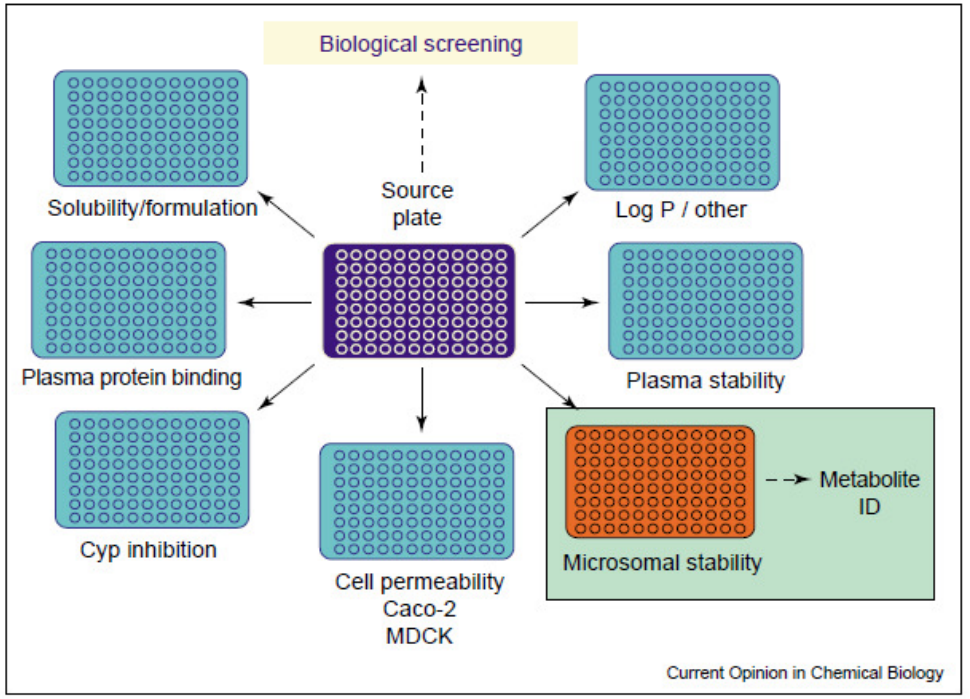
Novel molecular cancer therapeutics

Reasons for failure:

- In early 1990s: ***Poor pharmacokinetics***
Limited bioavailability

Development of a predictive assay (ADME):

Absorption
Distribution
Metabolism
Excretion



Introduction of ADME assay led to a fall in clinical failure rate from 40 to 10% in 2000!!!

Novel molecular cancer therapeutics

Today's reason of attrition of a cancer drug :

➡ ***Insufficient therapeutic efficacy !!!***

<u>How to address the problem:</u>

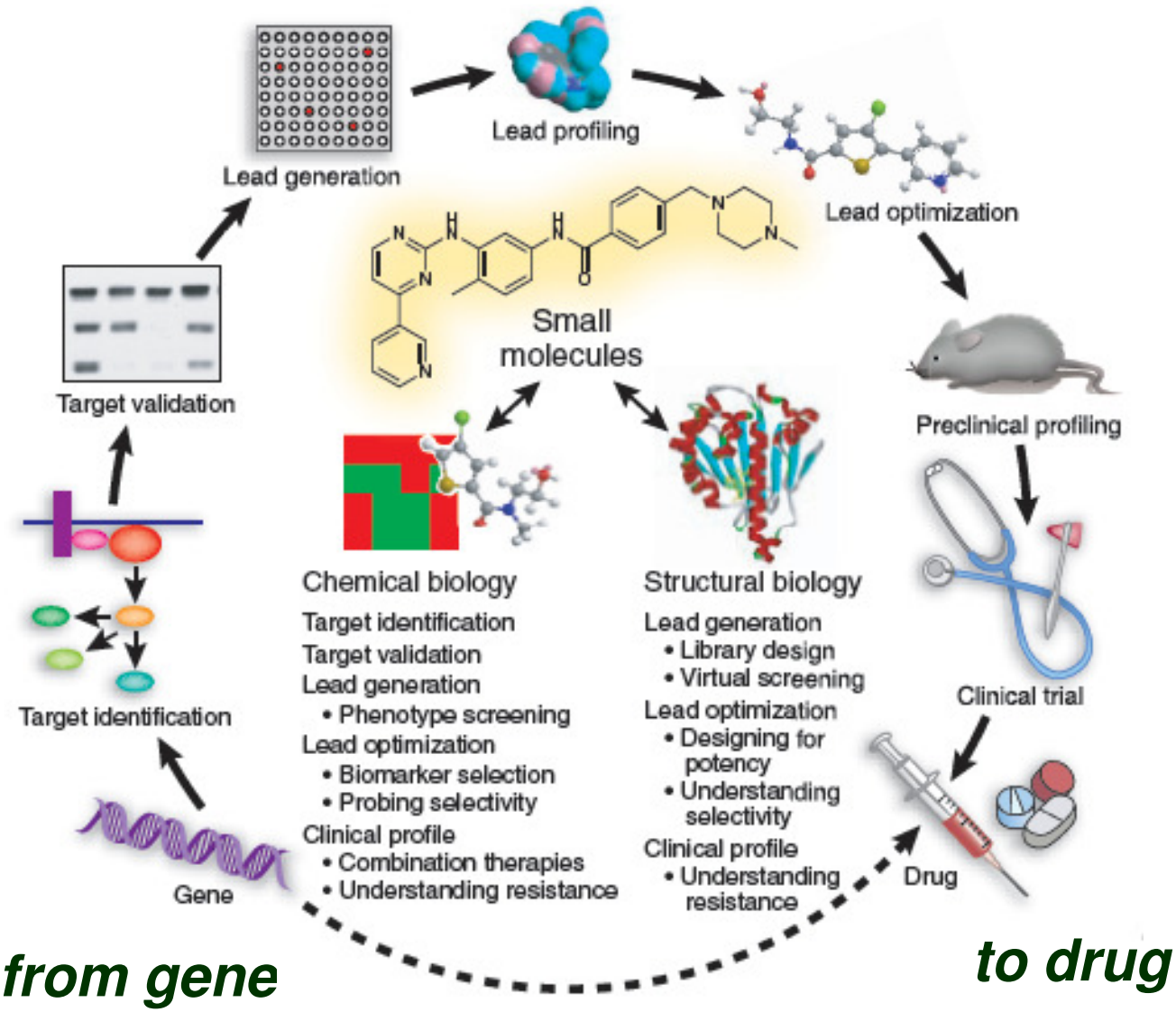
1. ***Selection of the best possible molecular target***
2. ***Use of animal models of human cancers with improved predictive power***
3. ***Better prediction of on-target and off-target toxicity (side effects!!!)***
4. ***Careful selection of biomarkers to identify the most responsive patients and to provide proof-of-concept for the proposed molecular mechanism (➔ molecular imaging)***

Integrated small-molecule drug discovery and development

The process of developing new molecular-targeted drugs:

<u>Interplay between:</u>

- ***Genetics***
- ***Genomics***
- ***Bioinformatics***
- ***Cell and molecular biology***
- ***Structural biology***
- ***Pharmacology***
- ***Tumor biology***
- ***Medicinal chemistry***
- ***Experimental medicine***



Collins I, Workman P. Nat. Chem. Biol. 2006; 2(12):689-700.

New molecular targets: The "druggable" cancer genome

Selection of best possible molecular target

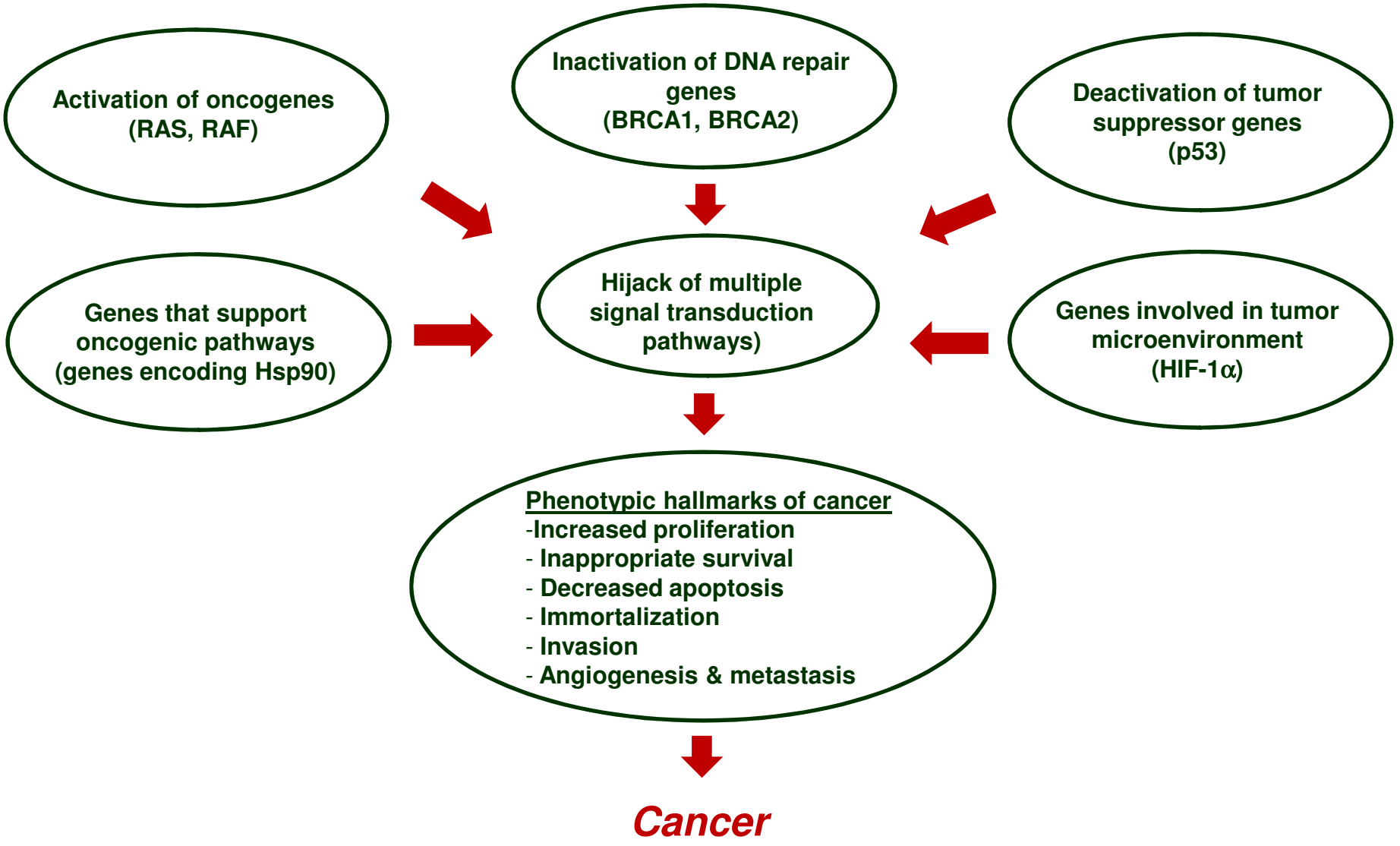
➔ Crucial to the success of drug discovery

Factors influencing the choice of target:

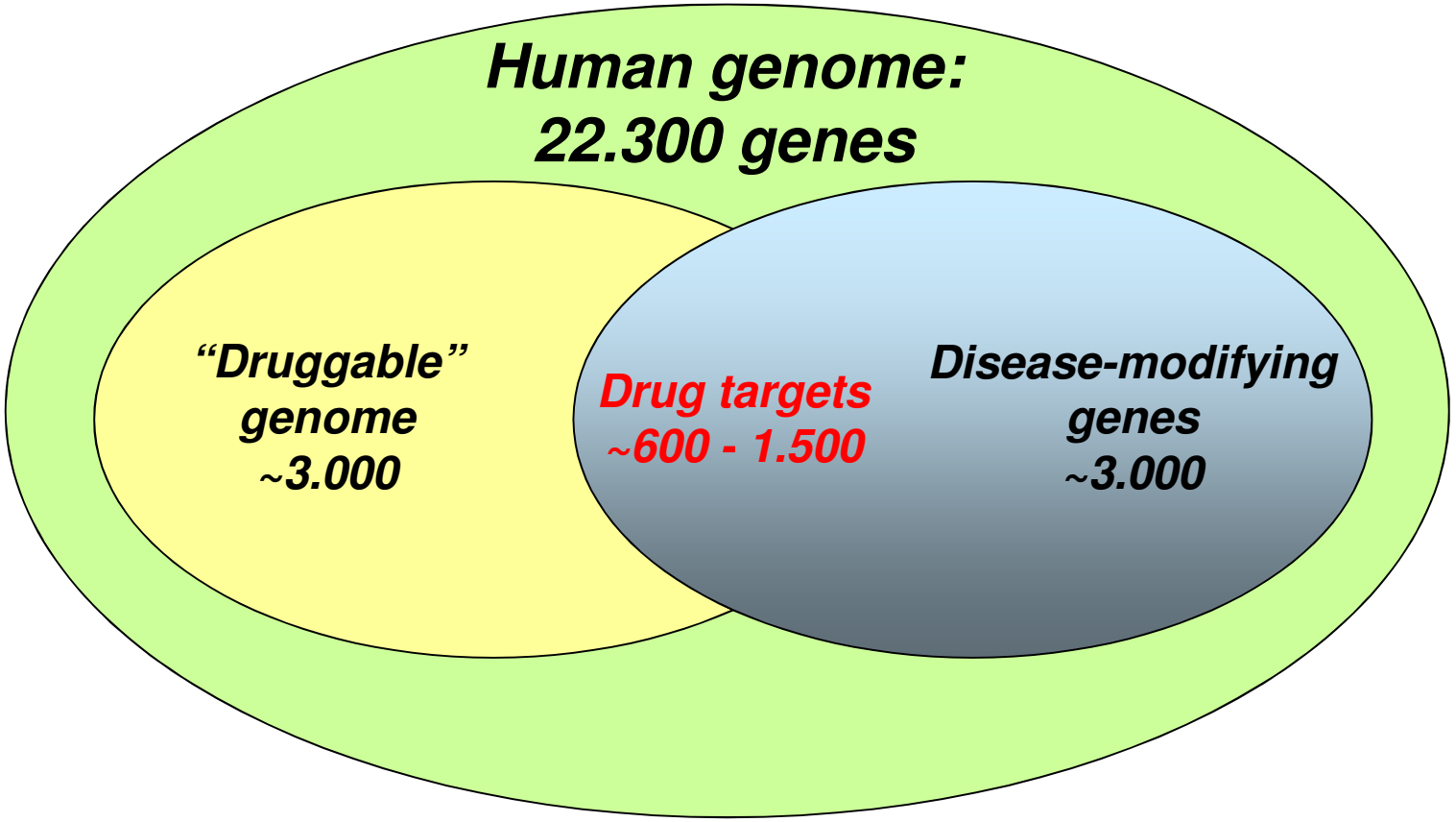
1.Involvement of the target in the initiation and progresstion of cancer
2.Technical feasibility (or "druggability") of the target

Concept of the "druggable" genome (Hopkins and Groom, 2002)

Classes of genes and biological mechanisms involved in cancer



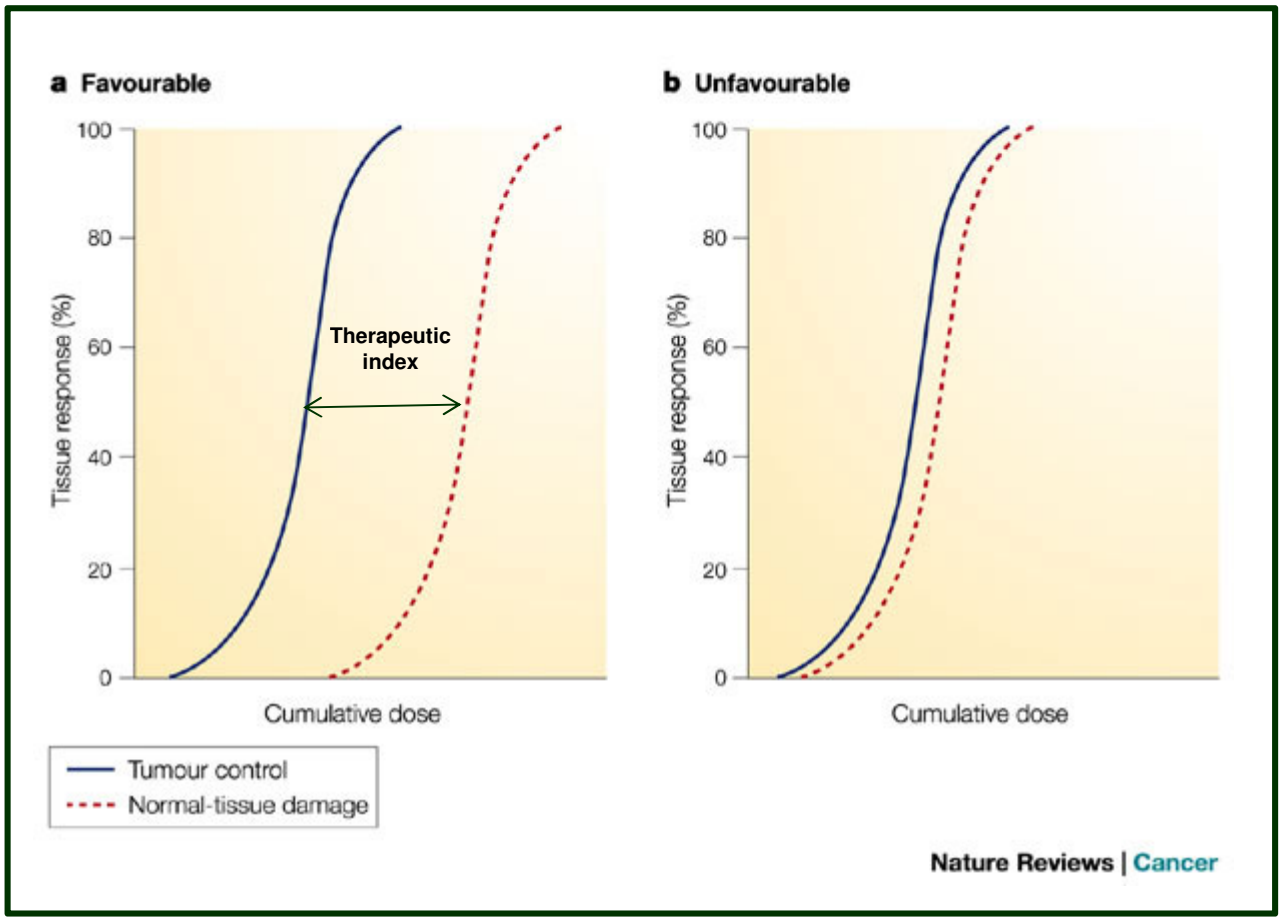
New molecular targets: The "druggable" cancer genome



Quelle: *Drug Discovery Today*, August 2005

New molecular targets

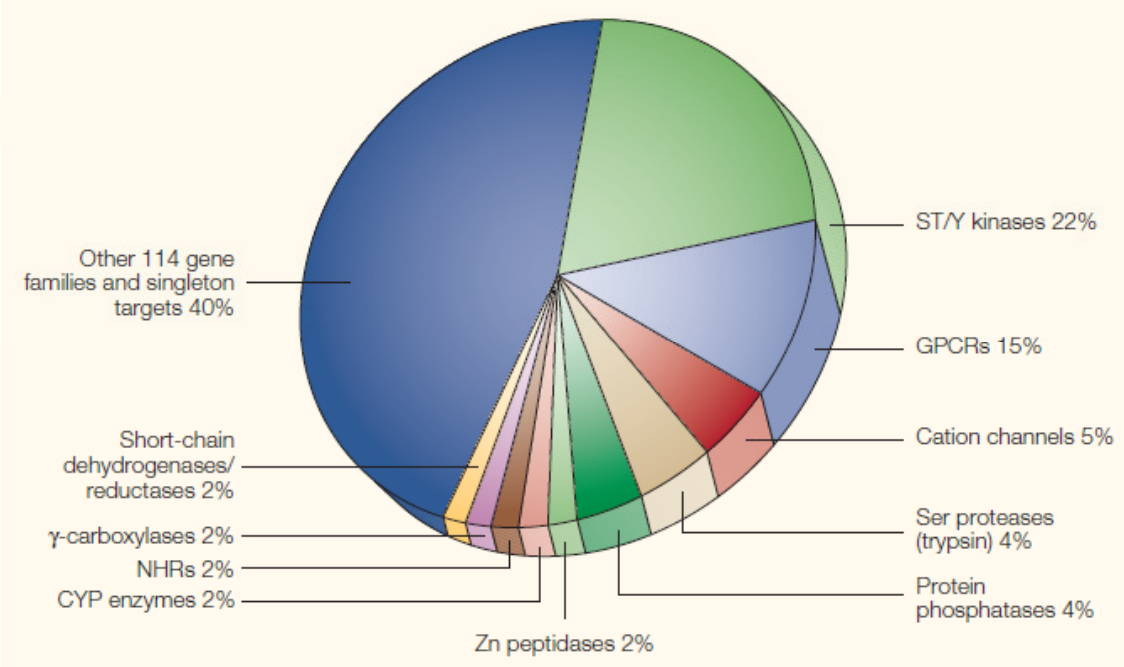
Best molecular targets for drug development of highly effective cancer drugs with minimal side effects will be those that are responsible for major differences between cancerous and healthy cells



Drugs acting on essential function would have a narrower therapeutic index than those that interfere with non-essential functions

New molecular targets: Druggable targets

The druggable genome



Hopkins AL, Groom CR. The druggable genome. Nat. Rev. Drug. Discov. 2002; 1(9):727-30.

High priority to:

Receptors for small endogeneous molecules

Enzymes with well-defind active site (kinases)

Protein-protein interactions involving small domains

Currently not druggable or difficult:

Phosphatases

Large domain-size protein-protein interactions

Mutant RAS G protein, mutant p53

New molecular targets: Druggable targets

Comparison of the druggable genomes of selected eukaryotes

	Homo sapiens	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Saccharomyces cerevisiae*
Total number of predicted genes[8,9,16]	~30,000	13,601	18,424	6,241
Number of proteins in proteome*	21,688	13,849	17,946	6,127
Number of estimated druggable targets	3,051	1,714	2,267	508
Percentage that are predicted druggable targets	~10–14%	12%	12%	8%

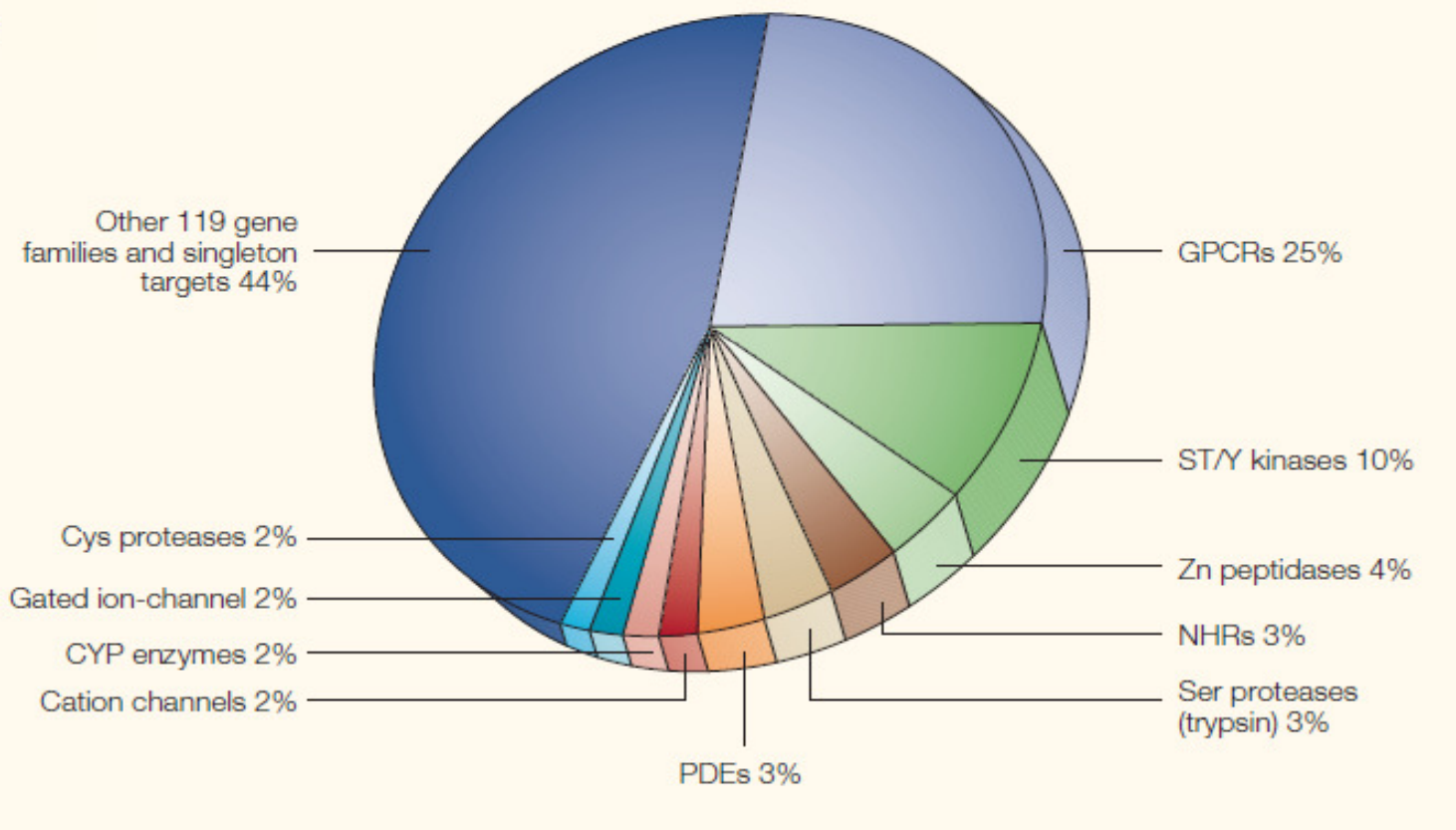
Not druggable targets:

Knowledge of biochemical pathway to allow selection of downstream targets
e.g. no inhibition of RAS ➔ Targeting of downstream MEK kinases

Hopkins AL, Groom CR. The druggable genome. Nat. Rev. Drug. Discov. 2002; 1(9):727-30.

New molecular targets: Druggable targets

Molecular targets of "rule-of-five" compliant drugs



Hopkins AL, Groom CR. The druggable genome. Nat. Rev. Drug. Discov. 2002; 1(9):727-30.

Lipinski's "Rule-of-Five"

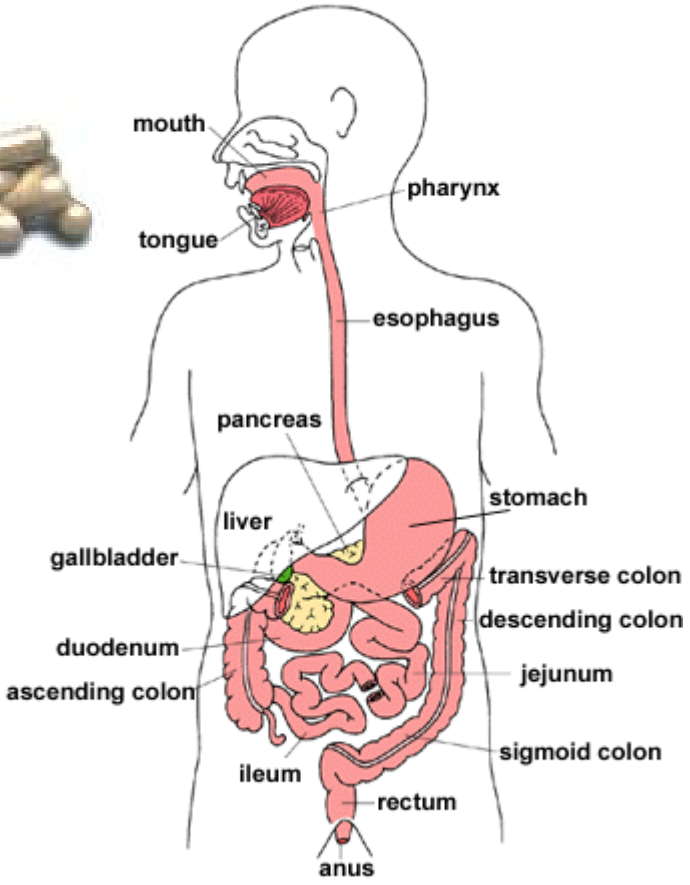
The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active. ***Poor ADME, when:***

Molecular mass: >500

Lipophilicity (logP): >5.0

>5 H-bond donors (e.g. OH und NH)

>10 H-bond acceptors (e.g. N und O)



Absorption
Distribution
Metabolism
Excretion

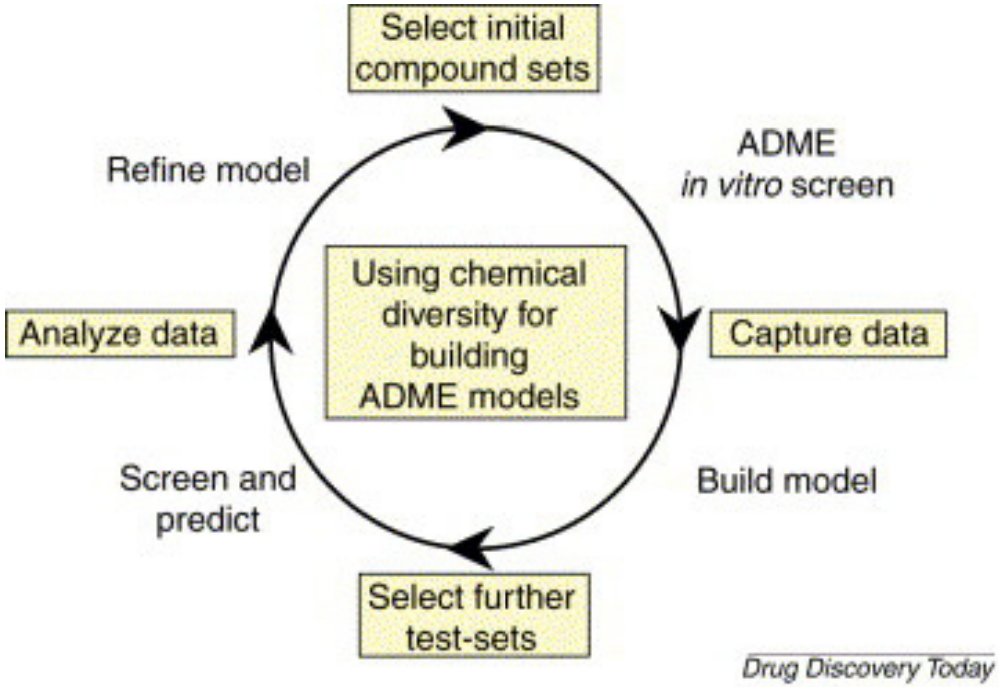
} ***Pharmacokinetics Bioavailability***

From drug target to development candidate

After target selection ➔ Generation of lead compounds

The heart of small molecule drug discovery is the iterative cycle of chemical synthesis and biological evaluation

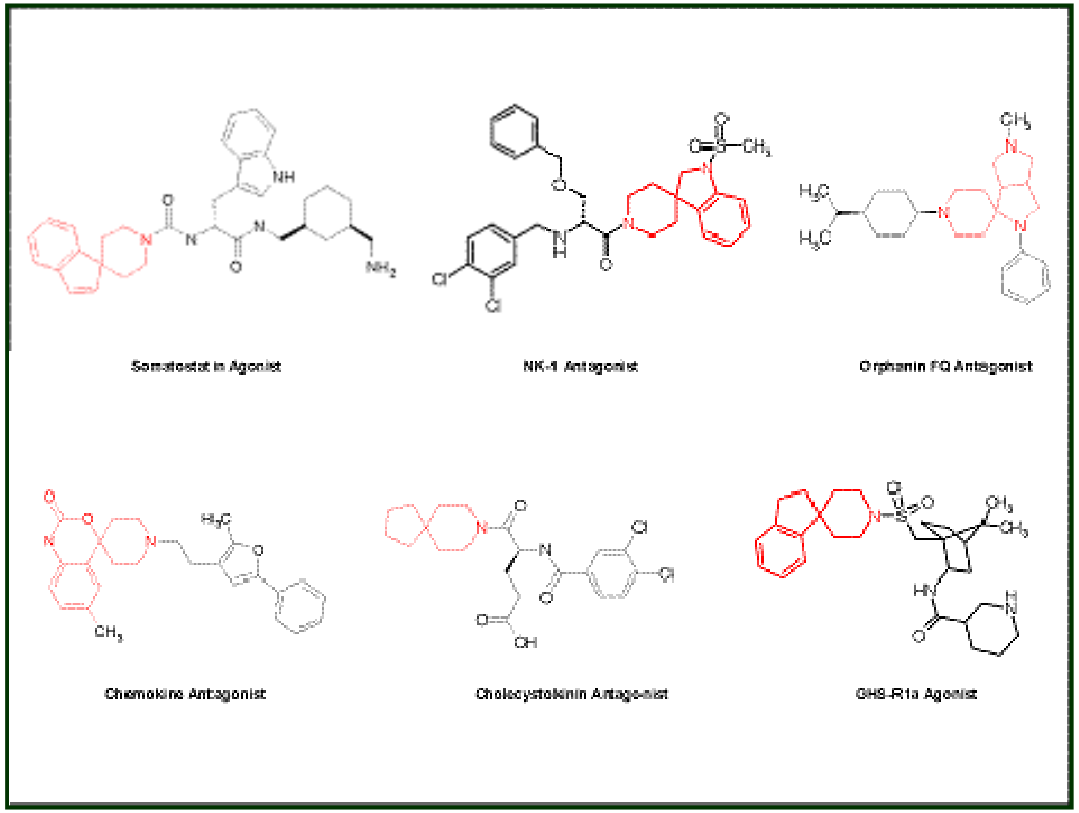
Natural products vs. synthetic compounds



From drug target to development candidate

<u>Natural products</u>: Pre-optimized through selective forces of evolution

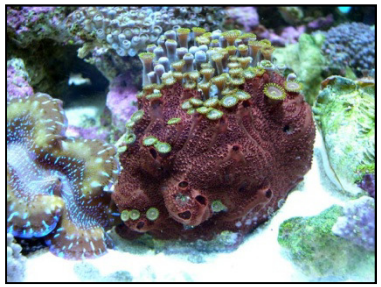
➔ Priviledged structures



Reported GPCR ligands containing spiropiperdines as recognition motives

From drug target to development candidate

Challenge to use natural products



Bioactive concentrate

Bioassay-based Separation and purification

Purified bioactive compound

Structure elucidation

New compound

SAR
Synthesis of derivatives
Scale-up synthesis

Potential lead

Medicinal chemistry

Drug development

Known Compound?

Required Amount of compound

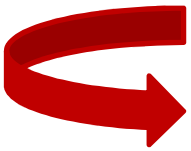
1-10 µg

1-10 mg

From drug target to development candidate

Challenge to use synthetic compounds

Small molecule compounds: Very rarely clinical candidates



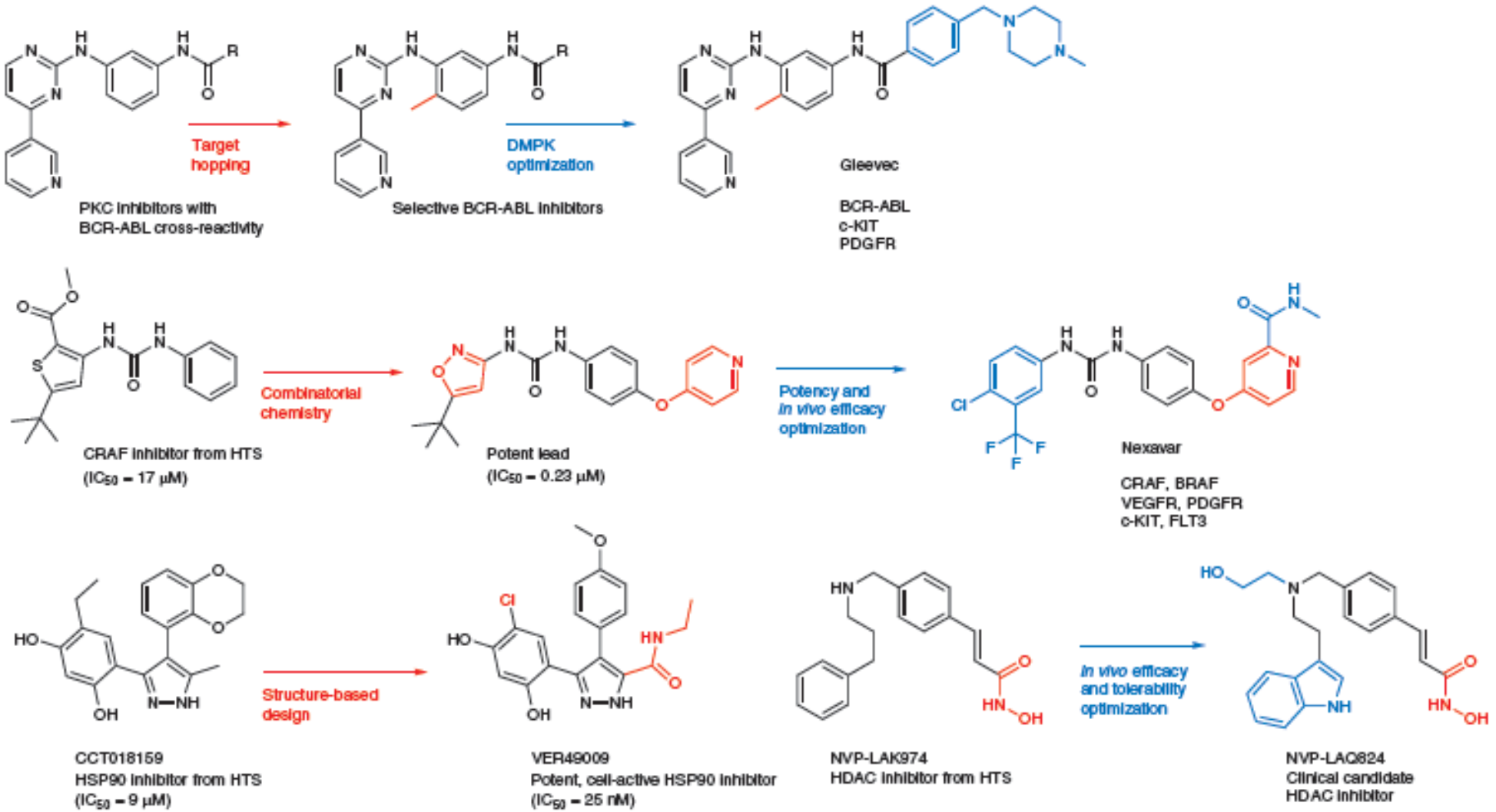
Need for substantial structural modifications

Chemical starting points: - Structure of endogeneous biological ligands
- **Existing drugs**
- **Natural product pool**

Alternative: Compound libraries from HTS

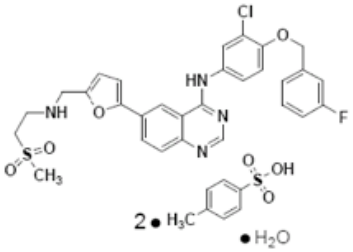
➔ **Biological HTS of 100.000 to 2.000.000 chemically diverse small compounds**

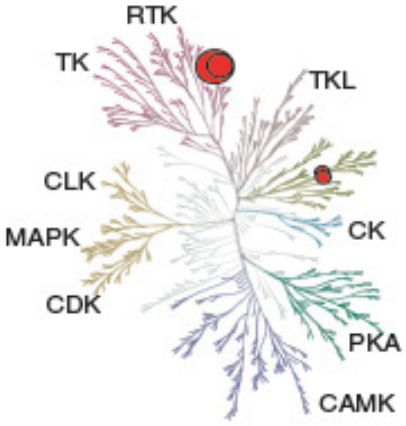
Selected histories of lead generation and lead optimization



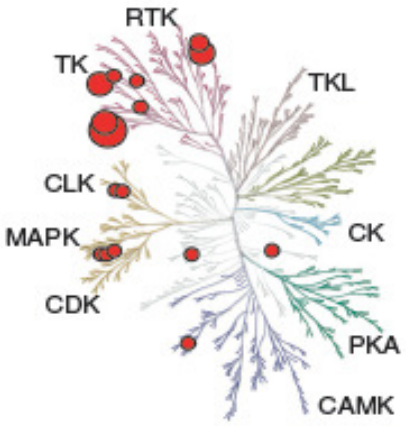
http://www.nature.com/nchembio/journal/v2/n12/images/nchembio840-sc1.jpg

Kinase binding selectivity for inhibitors using the human kinome

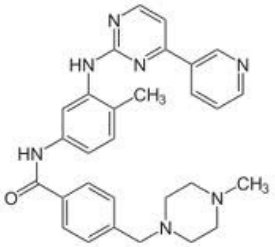


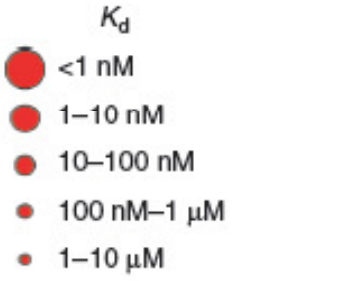


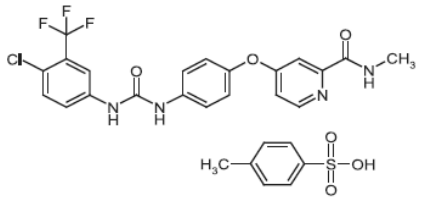
Tykerb

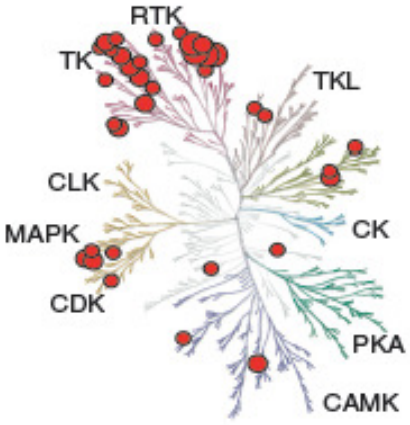


Gleevec

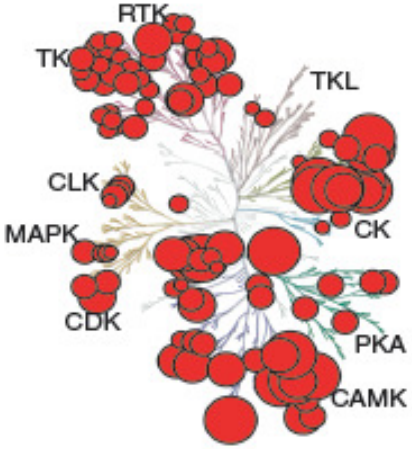




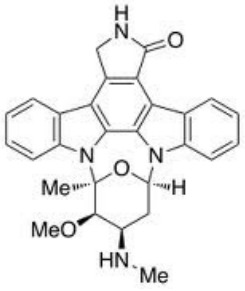




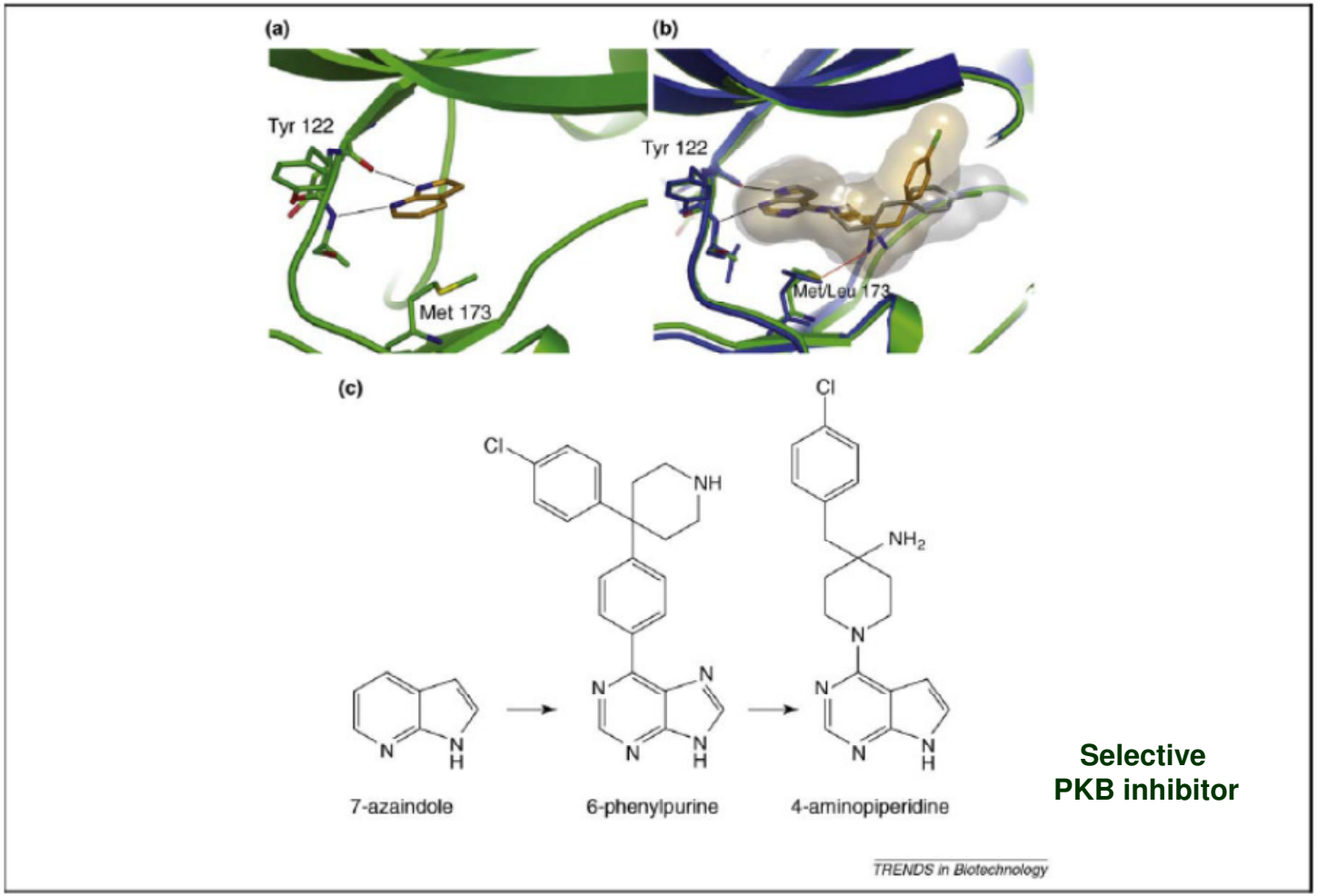
Nexavar



Staurosporine



Fragment-based drug discovery



‘Rule of three‘ for fragments:

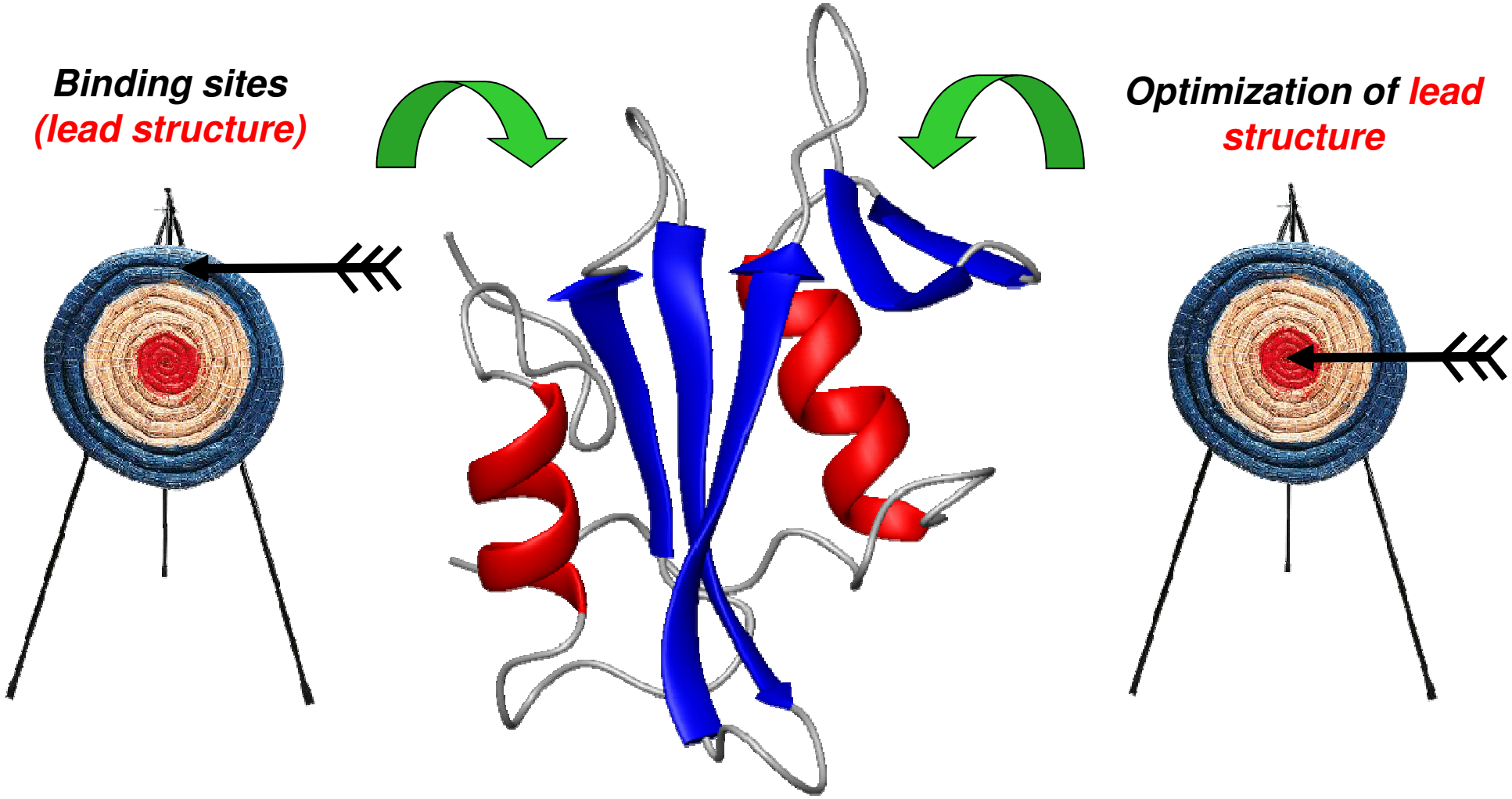
MW < 300, logP < 3, number of H-bond donors and acceptors each should be < 3

Typical physicochemical and biological properties

Property	Fragment	Lead	Drug
Molecular weight	<300	<400-450	<500
Lipophilicity (logP)	<3	<4	<5
H-bond donors	≤3	≤4-5	≤5
H-bond acceptors	≤3	≤8-9	≤10
Polar surface area	N/A	N/A	≤140-150 A^2
Chemically reactive groups	N/A	None present	None present
Target activity (IC_{50}; K_i)	>>10^{-5}-10^{-6} M	10^{-6}-10^{-6} M	10^{-8}-10^{-9} M
Structure-Activity-Relationship (SAR)	NMR or X-ray	Useful SAR established	Full SAR understood

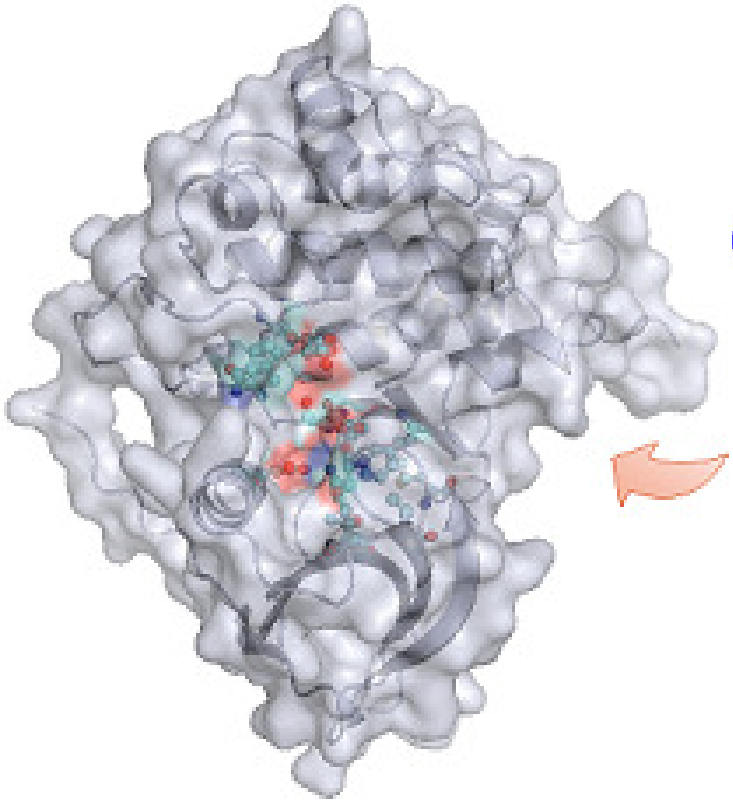
Hit to lead generation

„we do better with structure...“

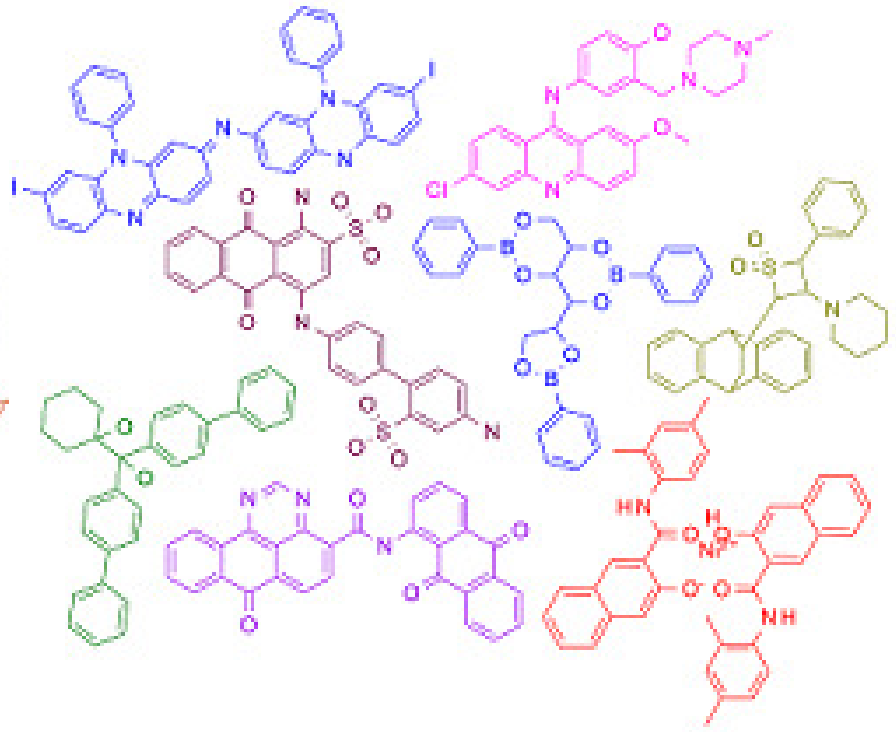


Hit to lead generation

„we do better with structure...“



EGFR-TK, ball & stick
refers to binding residues



8 hits from NCI diversity database

Summary – Conclusions:

➔Shift of paradigm

Focus on mechanism-based drugs acting on drug targets involved in the molecular causation of cancer

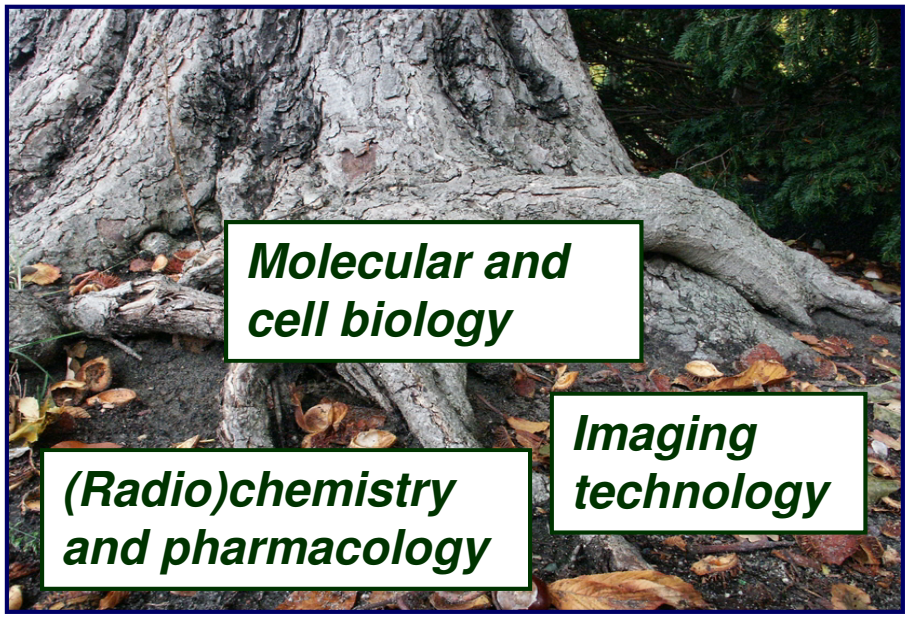
Careful selection of molecular target (”Druggable” cancer genome)

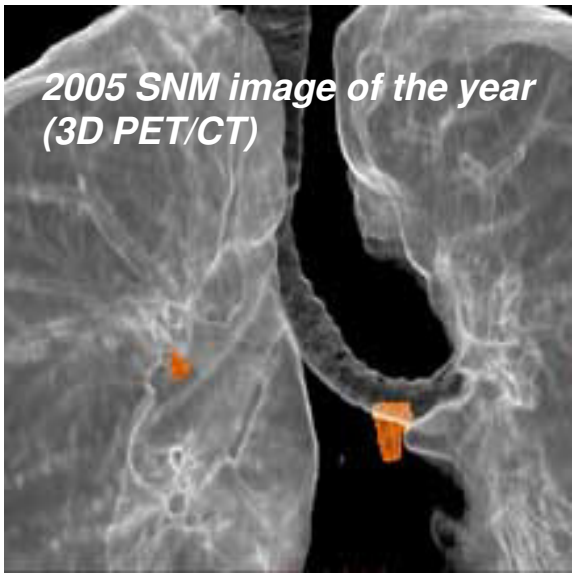
Interplay between chemical biology and structural biology (we do better with structure)

Complex hit to lead to drug developing process

Biomarkers and molecular imaging

Non-invasive assessment of dynamics of a given biological process at the cellular and molecular level in the physiological intact organism over time



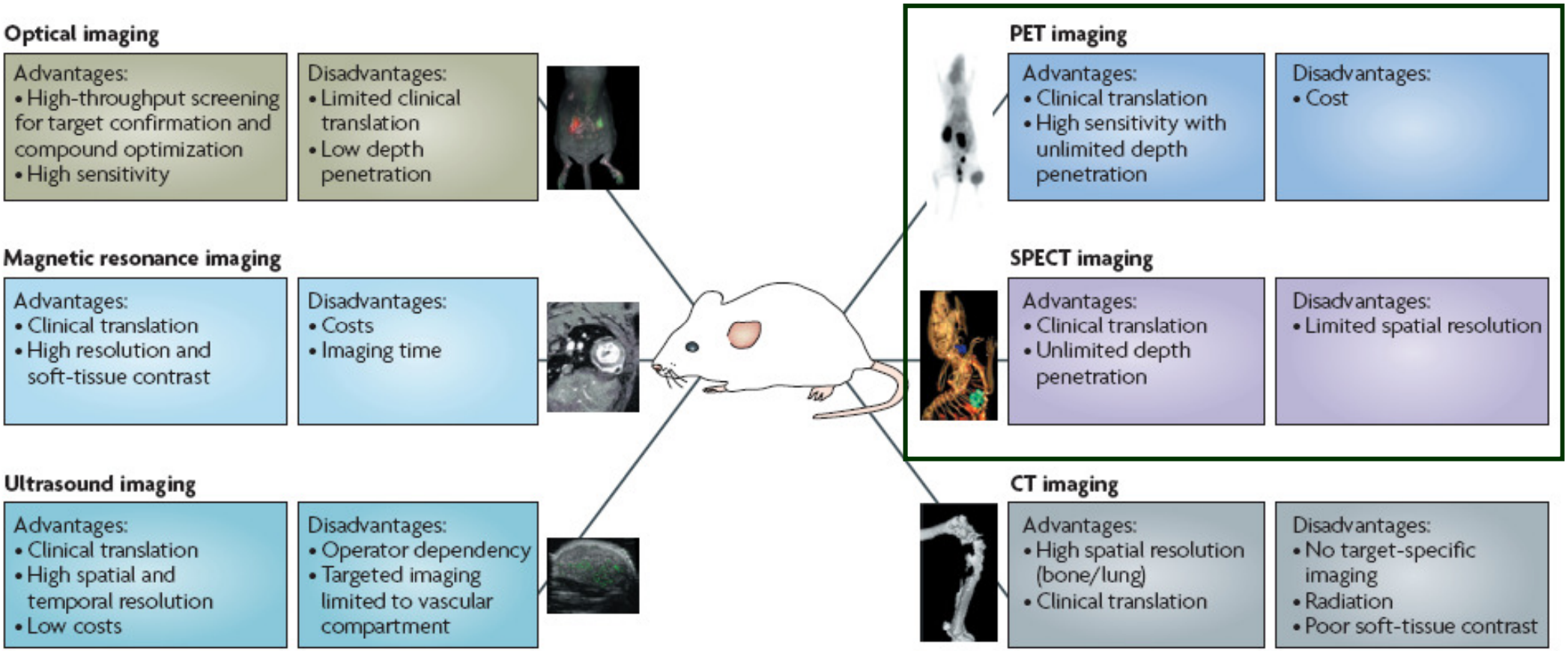


Impact on:

- ➔ Diagnosing and staging of cancer
- ➔ Monitoring therapy
- ➔ Functional/anatomical imaging
- ➔ Drug development & evaluation

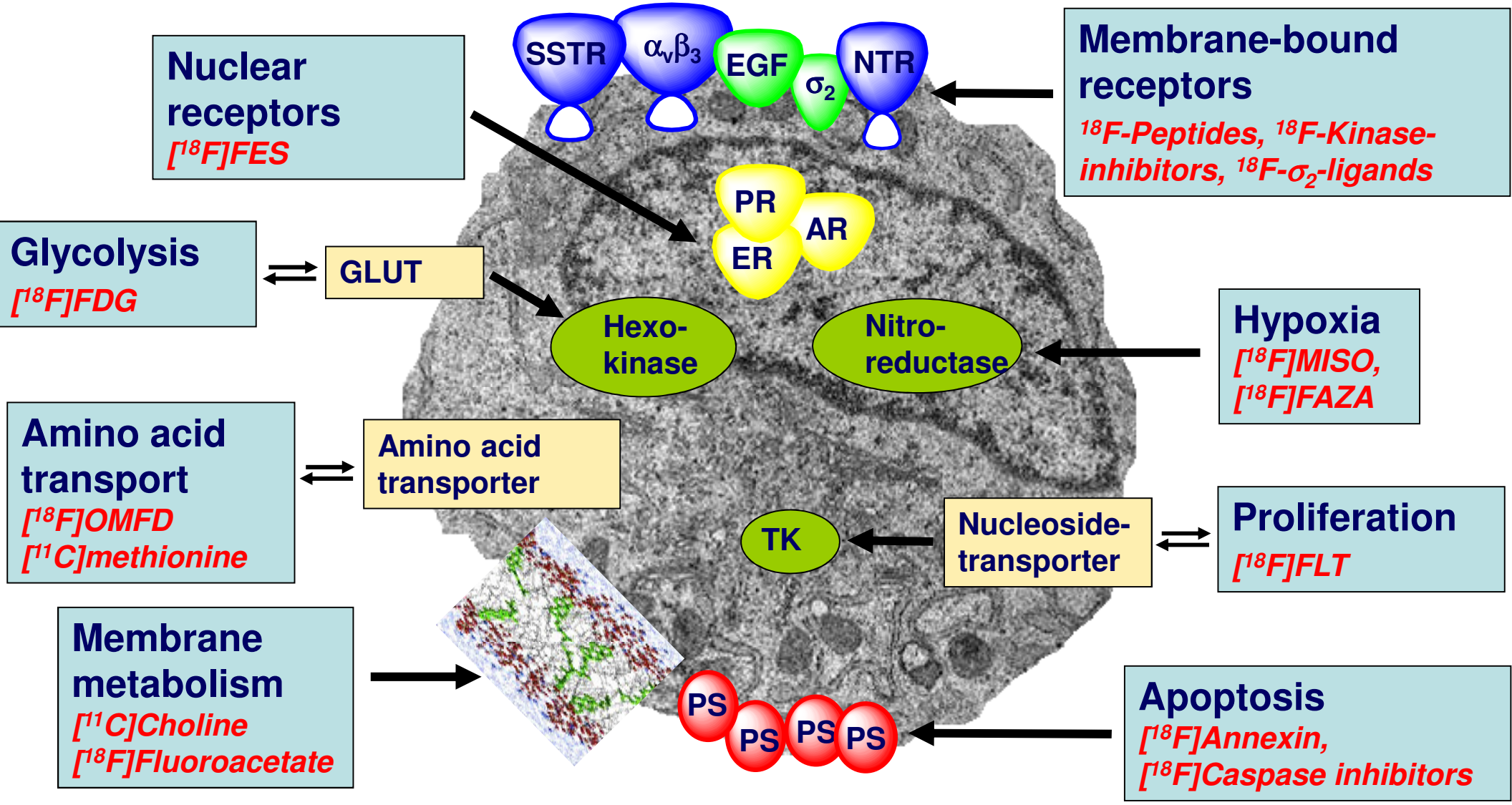
Molecular imaging ➔ Molecular medicine

Molecular imaging methodologies



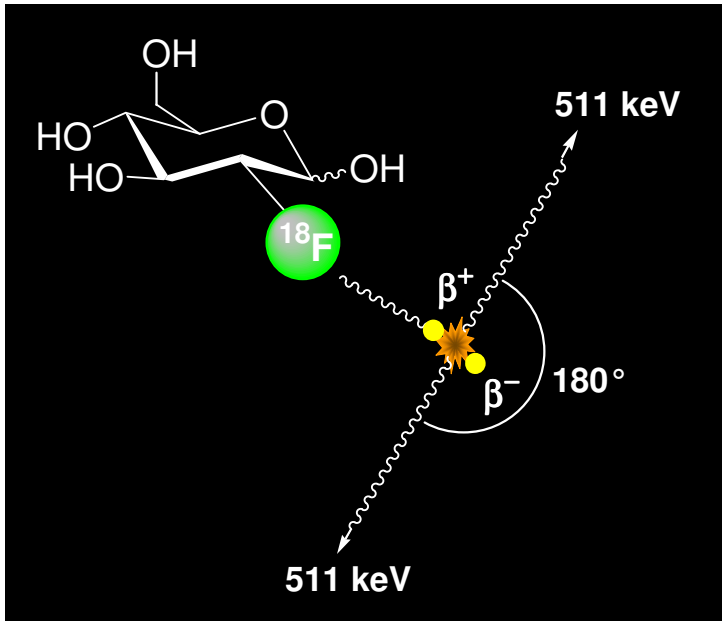
Willmann et al., Nature Drug Discovery. 2008.

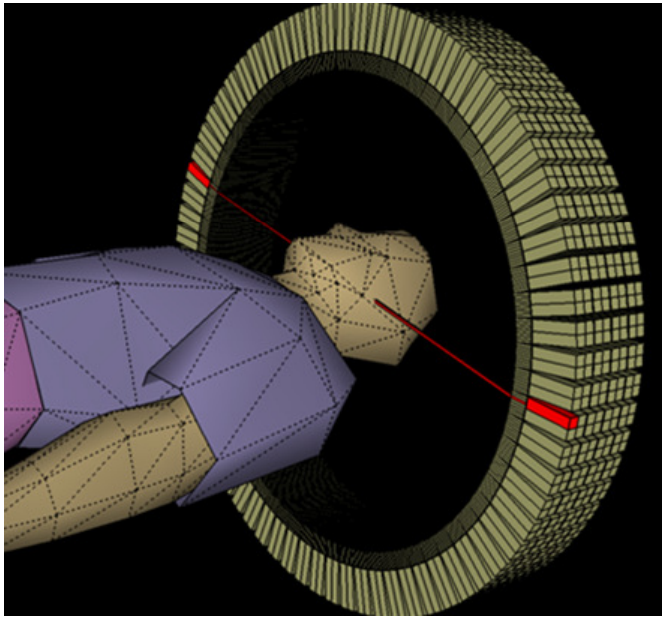
What to image: Selected PET radiotracers in oncology

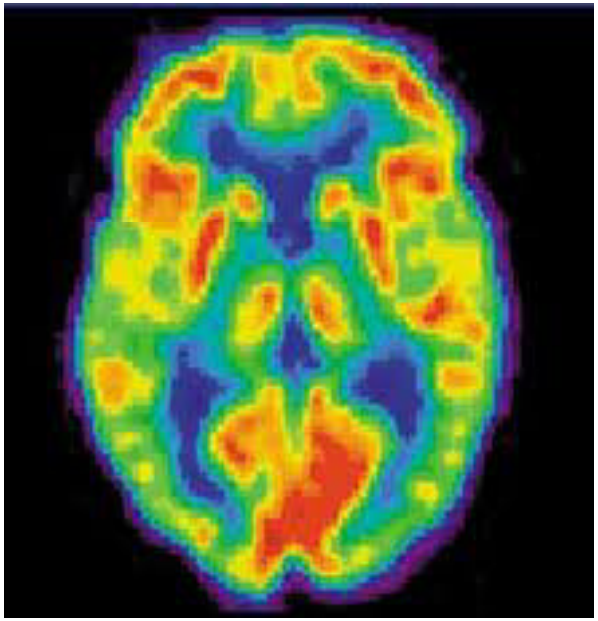


Positron emission tomography (PET)

PET: A multidisciplinary approach



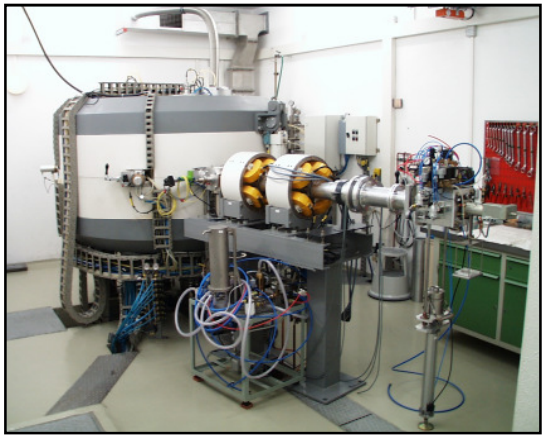




PET – A multidisciplinary approach

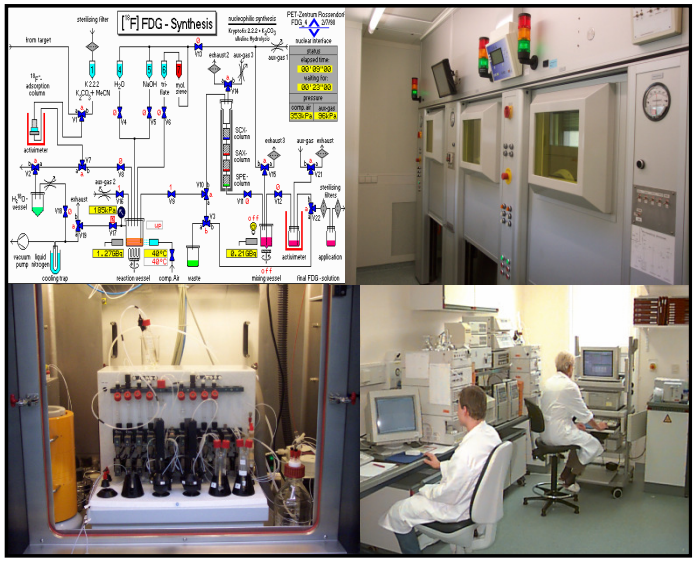
Radionuclide production

Radionuclide	Half-life
^{11}C	20.4 min
^{13}N	9.96 min
^{15}O	2.03 min
^{18}F	109.8 min
^{64}Cu	762 min
^{68}Ga	68.3 min
^{76}Br	966 min
^{120}I	88 min
^{124}I	4.15 d

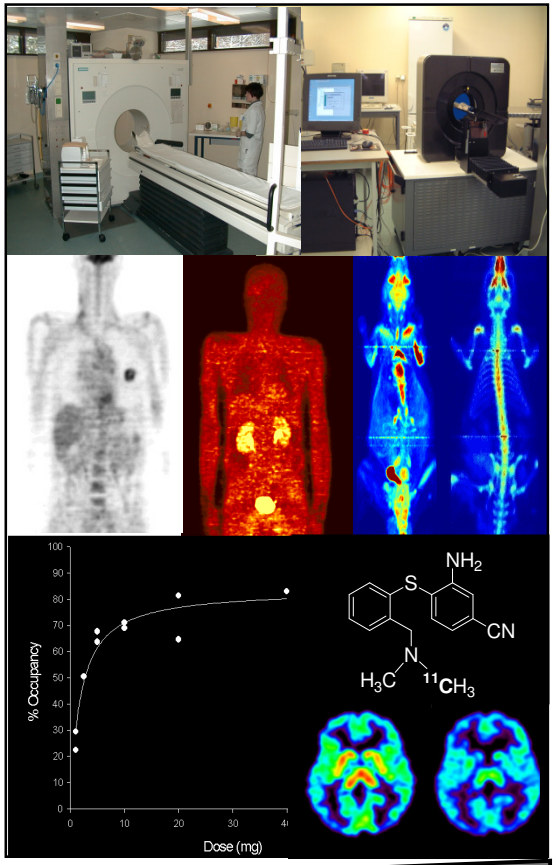


"Smart" radiotracers

Design
Synthesis (automation if possible)
Quality control

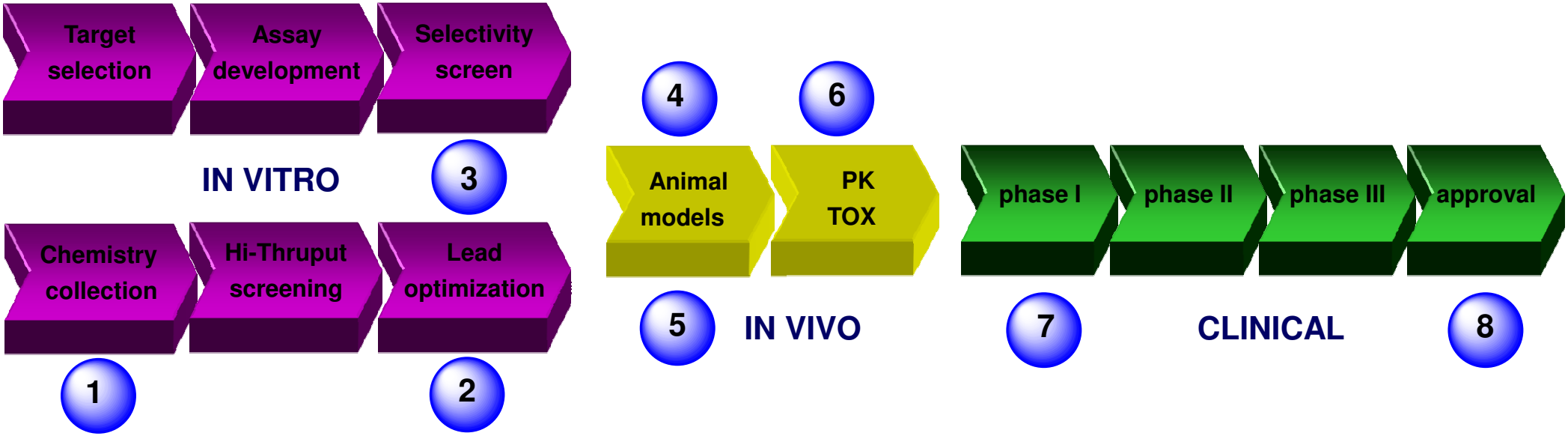


Nuclear medicine Drug research



0 ——— **Time** ——— **max. 3 x $t_{1/2}$**

PET in drug development



1. Optimization chemical collection using μ-PET data
2. Radiolabeled lead compounds (radiotracers) for in vivo studies
3. In vitro selectivity
4. Non-invasive disease surrogates
5. Early PK analysis
6. Complete PK (humans, μ-dosing?)
7. Human PK (dosing, receptor occupancy, metabolites…)
8. Patient selection, diagnosis, response. Images as marketing tools

Application of PET Imaging in Drug Development

Determination of the relation between administered dose and clinical outcome

GOAL: Understanding the events related with

- **Drug administration**
- **Drug adsorption**
- **Drug distribution**
- **Drug metabolism**
- **Drug excretion**
- **Drug response to target organs and biochemical effector systems**

PET as a general *in vivo* tracer method allows:

- **Performance of non-perturbed observations *in vivo* (research animals & man)**
- **Studies at very low concentrations (< 10^{-9} M; tracer concept, *PET-microdosing)***

Drivers for change

Ability to make rational decisions in development

- **Go/No go decisions with confidence**

Use clinical technologies to better understand

- **Proof of Target**
- **Proof of Mechanism**
- **Proof of Efficacy**

Can we make these decisions sooner?

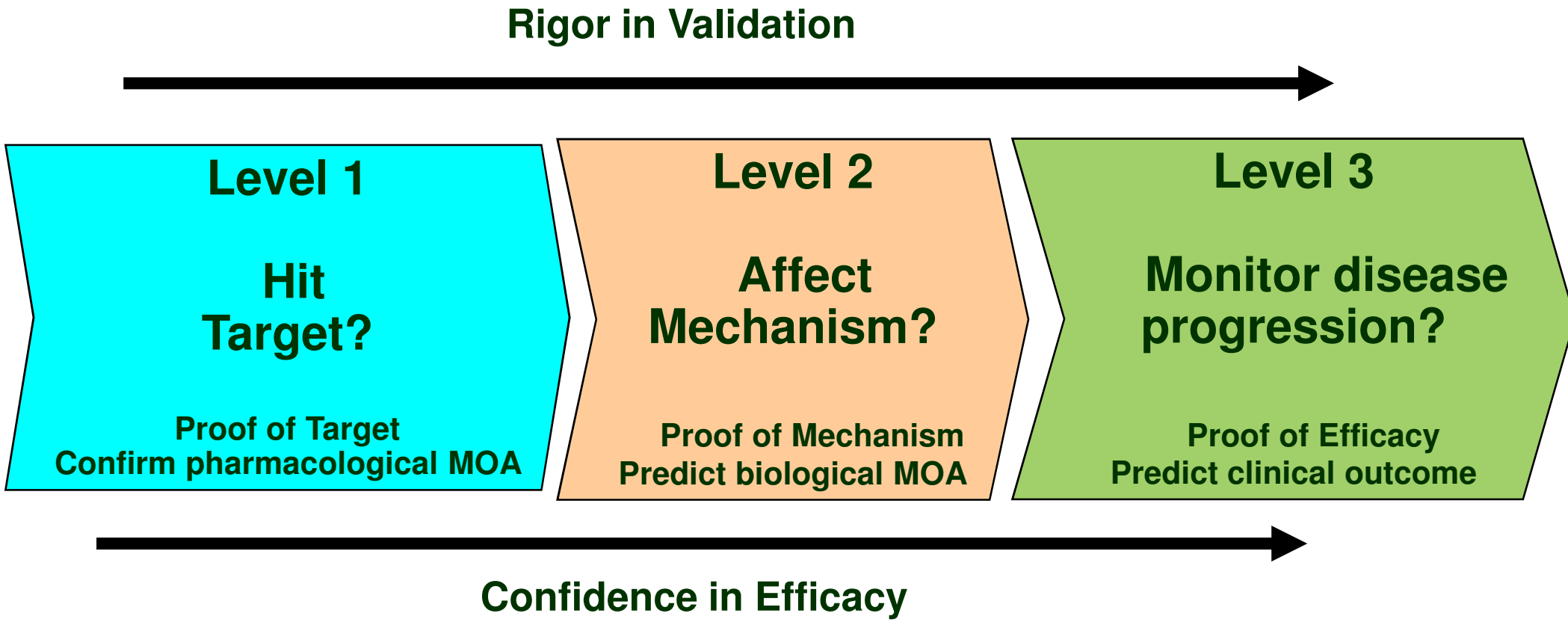
- **Impact of translational imaging**

Longer term impact to human healthcare

- **Individualized medicine**

Application of biomarkers in therapeutic drug development

Three definitions of biomarker (PET radiotracer) levels to describe the particular application of a biomarker



Level One Biomarkers -- Proof of Target

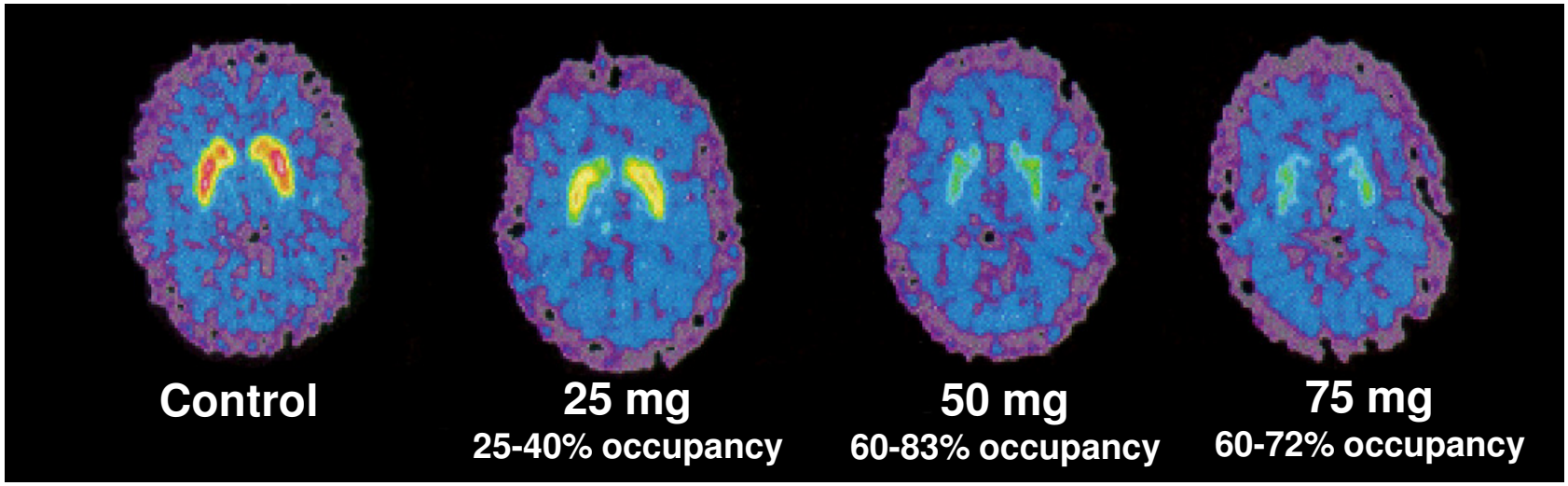
Level 1

Hit Target?

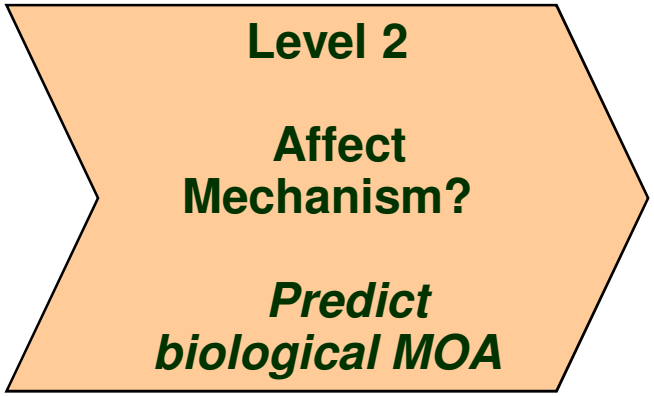
Confirm pharmacological MOA

PET receptor occupancy to assist in a No/Go decision

➔ Very popular for neuroreceptor mapping



Level Two Biomarkers -- Proof of Mechanism



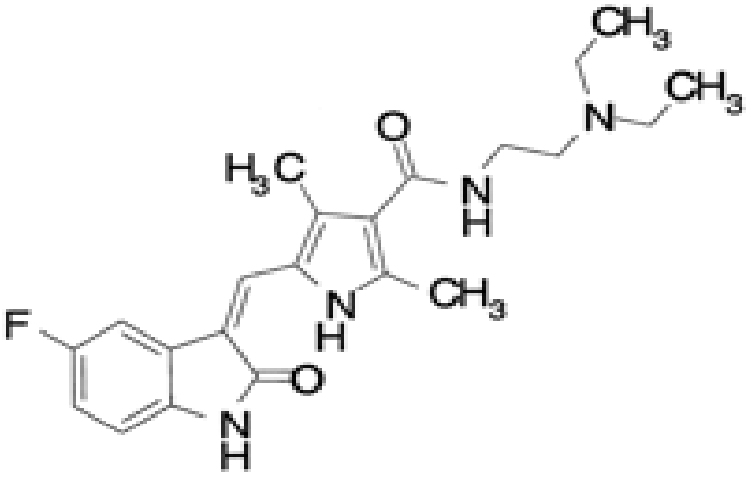
PET imaging in oncology drug development

FDG	*metabolism*
FLT	*proliferation*

- **<u>Early</u> Response -- Faster than tumor shrinkage?**
- **<u>Effective</u> Response -- Affect mechanism?**

<u>Predictive</u> response of therapeutic or clinical benefit?

SU11248, sunitinib maleate (Sutent)



- **Selective multi-target inhibition of:**
  - **PDGFR**
  - **VEGFR**
  - **cKIT**
  - **FLT3**
- **Antitumor and anti-angiogenic activity**

- ***What types of information can PET provide to the exploratory development phase of novel targeted cancer therapies?***

Two Phase 1 trials of SU11248 used PET Imaging

- **A Phase 1 study of SU011248 in the treatment of patients with malignant Gastrointestinal Stromal Tumor (GIST) who are intolerant of, or with disease progressing on imatinab mesylate (Gleevec)**

- **Pilot study of PET imaging to assess biological response to SU011248 L-malate salt**

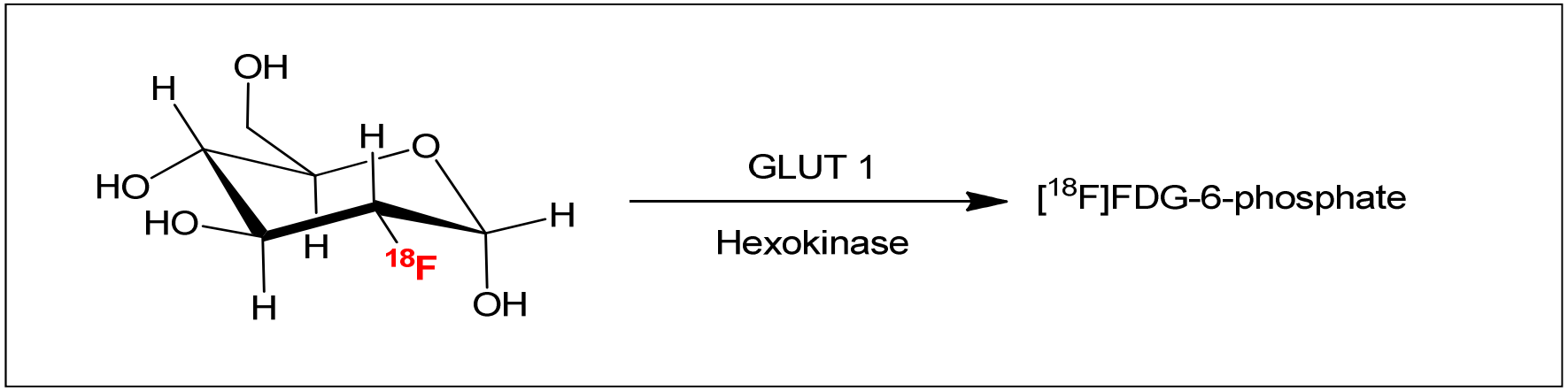
A quick word about Phase 1 trails in oncology

- **Primary endpoint of any Phase 1 study is safety & tolerability**
  - **Dose escalate to target**
  - **Normal volunteers**

- **Oncology – patients with end stage disease**

- **In oncology Phase 1 studies**
  - **Clinical benefit is not expected**
    - **Not necessarily the target patient population**
    - **However tumor size assessment by CT is conducted**

[^{18}F]FDG – marker of tumor metabolism



- **FDG reveals metabolic shutdown**
- **Widely available**
- **PET/CT has opened a new window on cancer imaging**

SU11248 (Sutent) in GIST

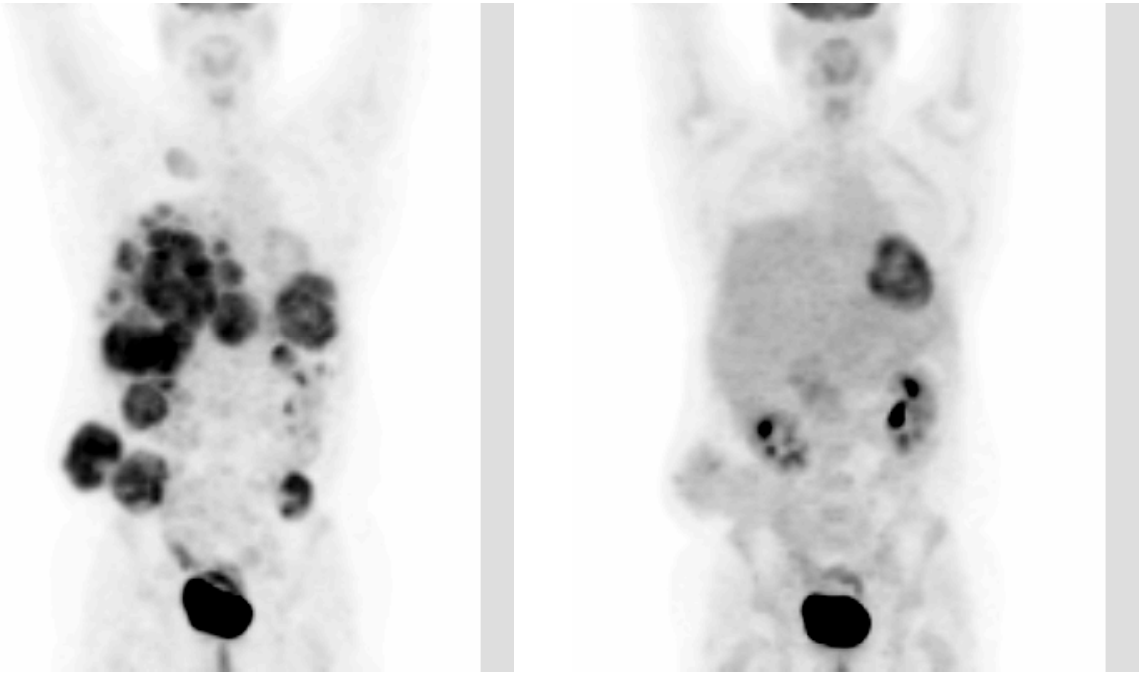
Metastatic GIST FDG-PET response to SU011248 in a patient resistant to Gleevec™

50 mg/day (2 wks on 2 wks off)

Link metabolic response to

- plasma levels (PK)
- serum and biopsy markers (PD)

ASCO 2003 Abstract #3273



Baseline

After treatment

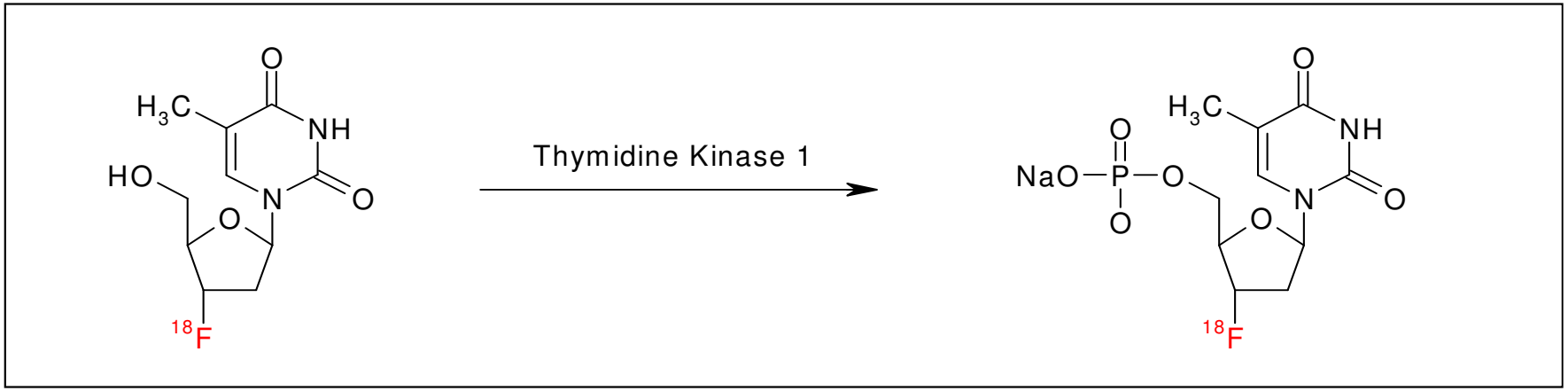
Is the PET response predictive of clinical outcome?

- ***For SU11248 (Sutent) it is too early to say, but…***

- ***For Gleevec in GIST***
  - **FDG PET response is highly correlated with cKIT genetic variants**
    - **Exon-11 (70%) -- major effect**
    - **Exon-9 (15%) -- minor effect**
    - **Wild-type -- no effect (PDGFR-α drives disease)**

Source: G. Demetri NCI FDG-PET Workshop Washington Jan 2005

[^{18}F]FLT – a marker of tumor proliferation

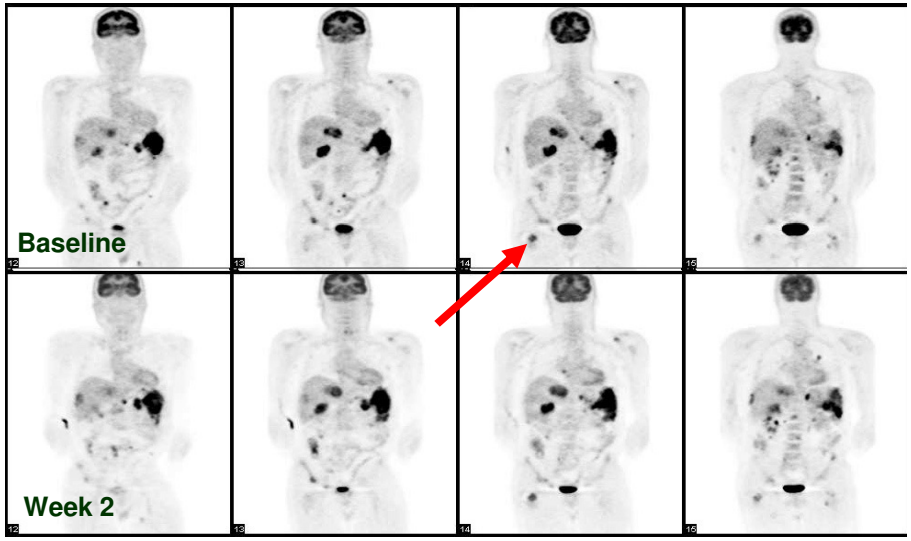


- **FLT reveals proliferative response**
  - **Cells are not dead, but cannot divide**
  - **Cells will still be metabolically active**
    - **Will it be an earlier response indicator than FDG?**

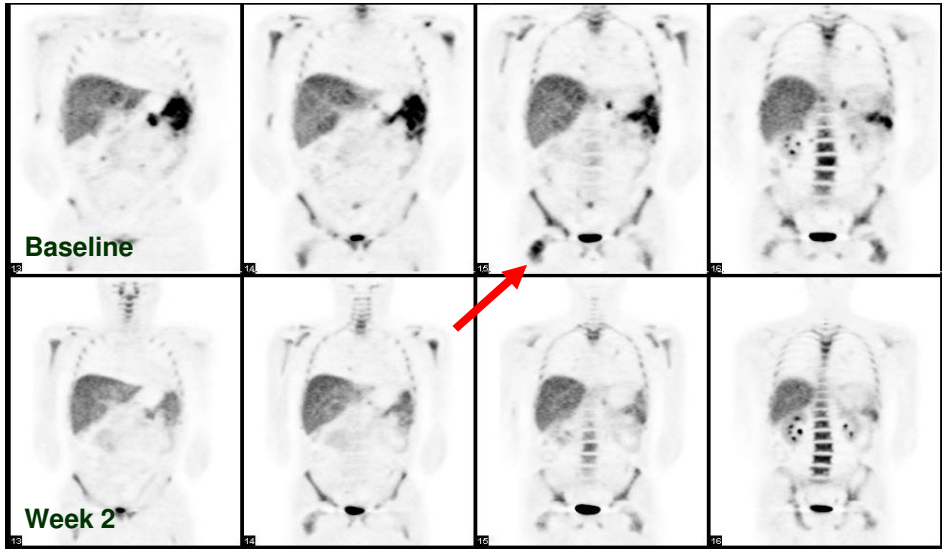
FDG/FLT PET after SU11248 (Sutent) treatment

Metastatic malignant melanoma

FDG



FLT



Ongoing lessons from Oncology

- PET provides rapid whole body assessment of tumor burden
  - Quickly identify heterogeneous responses
  - With PET/CT – guide tumor biopsy?
- FDG PET provides rapid feedback for early metabolic response
- FLT provides similar information on proliferation response
  - Combination of the two provides significant amounts of information within a subject
- For SU11248 (Sutent) -- *Value of PET is significant*
  - PET data increased the confidence around the biological activity
    - Allowed to make faster decisions around going into Phase II

Monitoring of metabolic changes after treatment

Other examples

Targeted probe/drug	MRS observed metabolic alterations	PET observed metabolic alterations
HSP90 inhibitors (17-AAG, CCT018159)	(+)GPC, (+)PC, (+)PME, (+)PE (cells), (-)NTP (tumor xenografts)[48,49,88]	(-)FDG, (+)^{11}C-choline, (-)^{11}C-methionine uptake (cells)[89]
BRAF-MEK1/2 inhibitors (U0126, PD0325901, PLX4032)	(-)PC (cells)[52]	(-)^{18}F-choline uptake (tumor xenografts),[53] (-)FDG uptake (tumor xenografts)[54,55]
PI3K-AKT-mTOR inhibitors (LY294002, wortmannin, PI-103, PX-866, everolimus)	(-)PC, (-)total choline (cells),[51,57,58] (-)pyruvate-lactate exchange (cells and tumor xenografts, DNP analysis)[59]	(-)FDG uptake (tumor xenografts)[60,61]
HDAC inhibitors (LAQ824, SAHA)	(+)PC (cells and tumor xenografts), (+)PME, (+)PE, (+)choline, (-)GPC, (-)GPE, (-)PCr, (-)NTP, (+)Pi, (-)Glucose (tumor xenografts).[72]	(-)FDG uptake (patient tumors)[90]
BCR-ABL, PDGFR inhibitor (imatinib)	(-)PC, (-)lactate, (-)glucose, (+)NTP (cells),[73,74] pyruvate-lactate exchange (tumor xenografts, DNP analysis)[75]	(-)FDG uptake (patient tumors)[91,92]

The signs (+) and (-) signify an increase and a decrease in metabolite levels, respectively.