Rational Drug Design lecture 4

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Molecular target

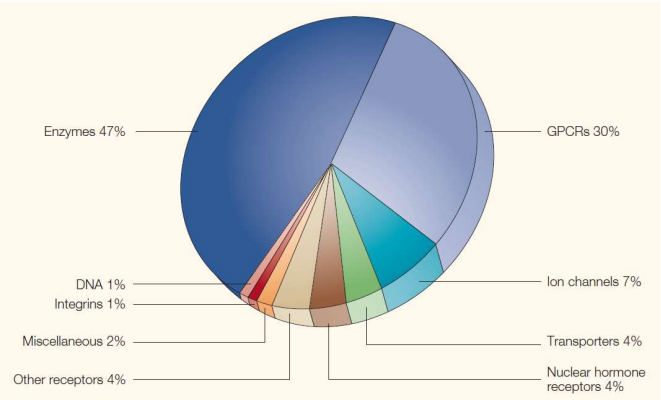
- **Molecular target** – The macromolecule in the living organism that interacts with the drug and the desired therapeutic effect is the result of this interaction.
- Types:
  - Proteins:
    - Enzymes,
    - Receptors,
    - Ion channels,
    - Structural proteins,
    - Membrane proteins,
  - Nucleic acids

Molecular target

- Interactions drug-target can be:
  - noncovalent,
  - Covalent reversible,
  - Covalent irreversible.
- Ligand may cause:
  - Inhibition of function of molecular target without changing the conformation (inhibition of an enzyme, receptor antagonism, ion channel blockage)
  - Change of molecular target function by modification of its conformation (enzyme activation, noncompetitive inhibition of an enzyme, receptor activation, etc.)

Classes of molecular targets

- Main classes are:
  - G protein coupled receptors (GPCR),
  - kinases
  - peptidases



CYP – cytochrome P450, NHR – nuclear hormone-binding receptors, PDE - phosphodiesterases

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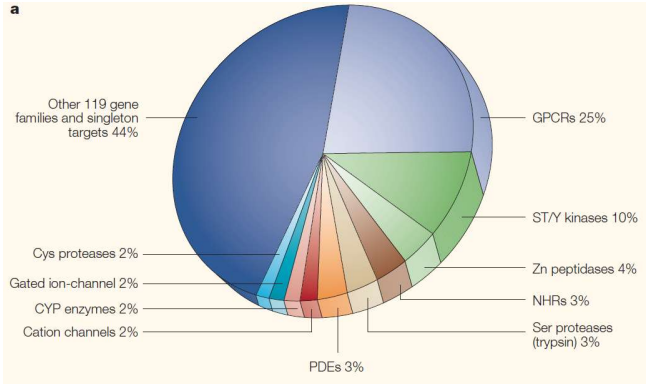
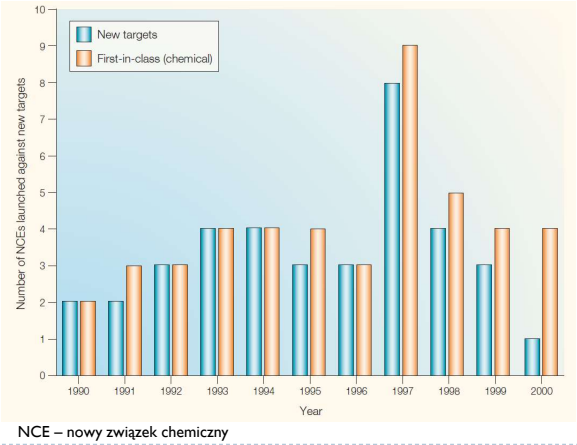


CYP – cytochrome P450, NHR – nuclear hormone-binding receptors, PDE - phosphodiesterases

New drugs – new targets

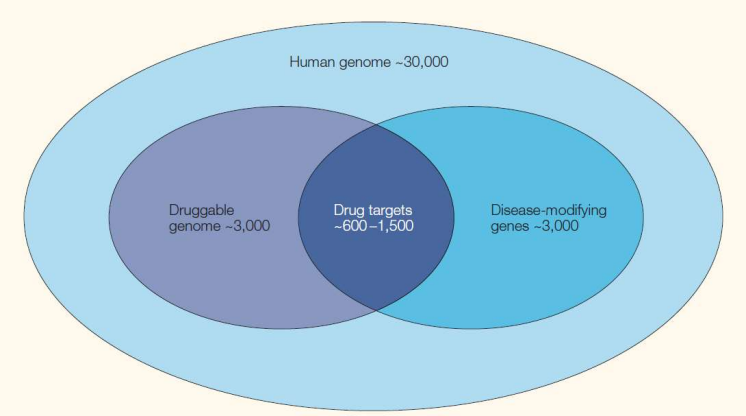
- New targets are continuously explored.



NCE – nowy związek chemiczny

Druggable target

- 'Druggable target' – molecular target which can interact with the drug.



Evaluation of molecular target

- Expression of mRNA transcript in patogenic states.
- Protein found in the research on disease.
- Protein directly related to the disease.
- Chemical compounds interacting with the target in clinical trials.
- Chemical compounds interacting with the target as registered drugs.

Evaluation of molecular target – proof of concept

- It has to be proven that the change of activity of the target is directly related to therapeutic effect.
- Molecular target should be studied at as many levels as possible (studies *in vitro*, cell lines, tissue cultures and *in vivo*).
- Transgenic animal models (gene knockout) are very helpful.
- Effectiveness of potential therapy could be estimated using model compound, antibodies, antisense RNA.

Evaluation of molecular target

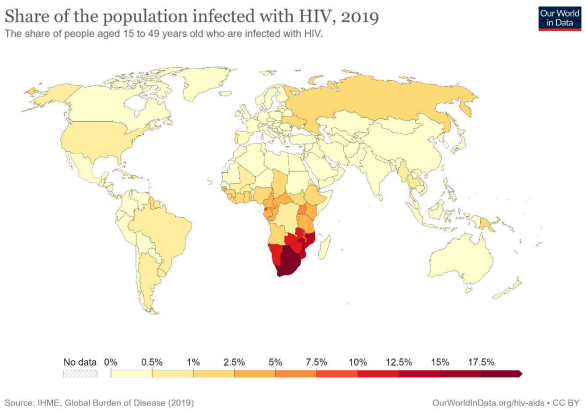
- Information on molecular target important for design and studies of drugs:
  - Function (ligands, substrates, cofactors, products, catalyzed reaction, interactions with other macromolecules)
  - Synthesis/isolation/ occurrence (how to effectively obtain appropriate amount of the protein)
  - Physicochemical properties (solubility, stability, molecular mass, etc.)
  - Structure!!!

HIV

- Human immunodeficiency virus;
- 1983, isolated by Luc Montagnier (Nobel prize 2008) and independently by Robert Gallo
- First specific anti-HIV drugs in the middle of 90-ies of XX c.
- Due to high evolutionary change of the virus cocktails of three drugs are used.

HIV

- HIV is currently very serious problem of many countries.
- Therapies inhibiting the development of infection are available.



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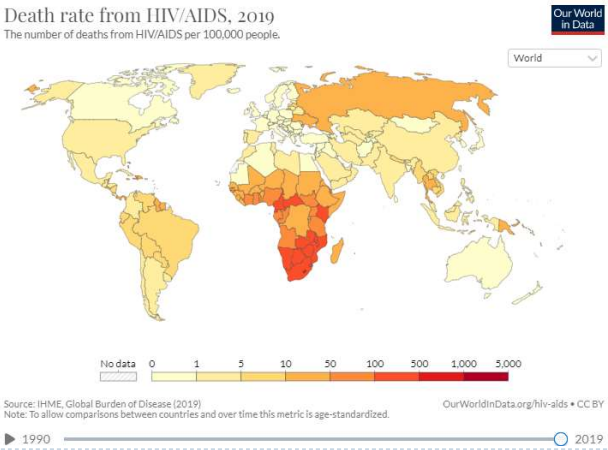

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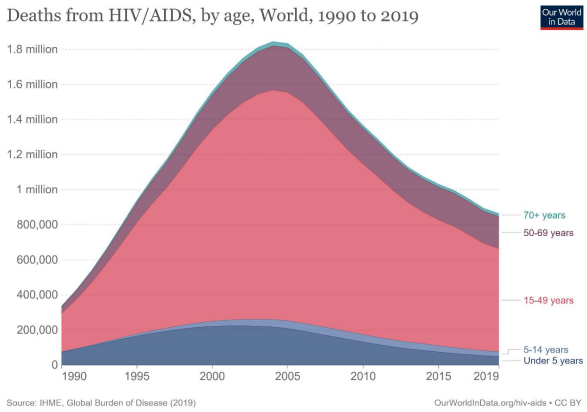
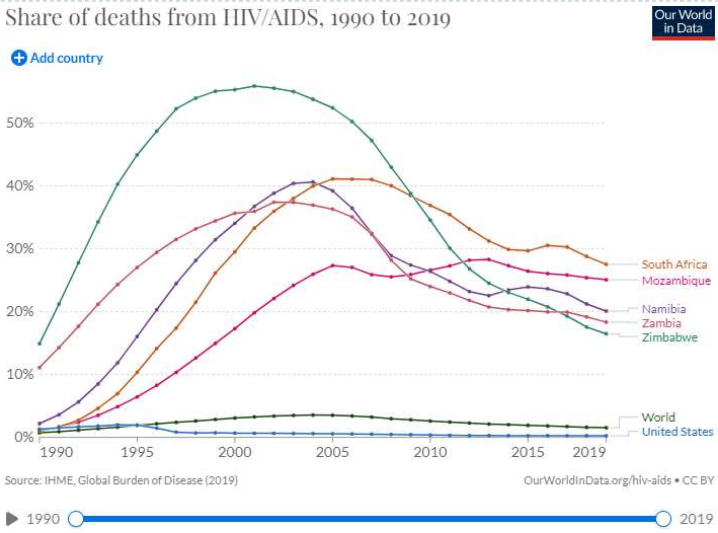
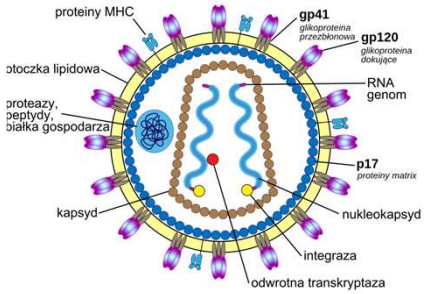


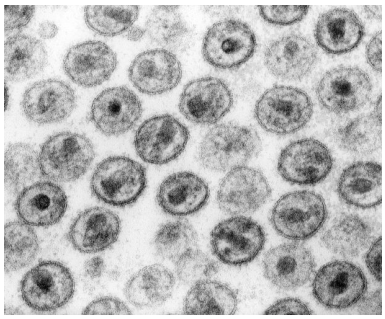
HIV/AIDS



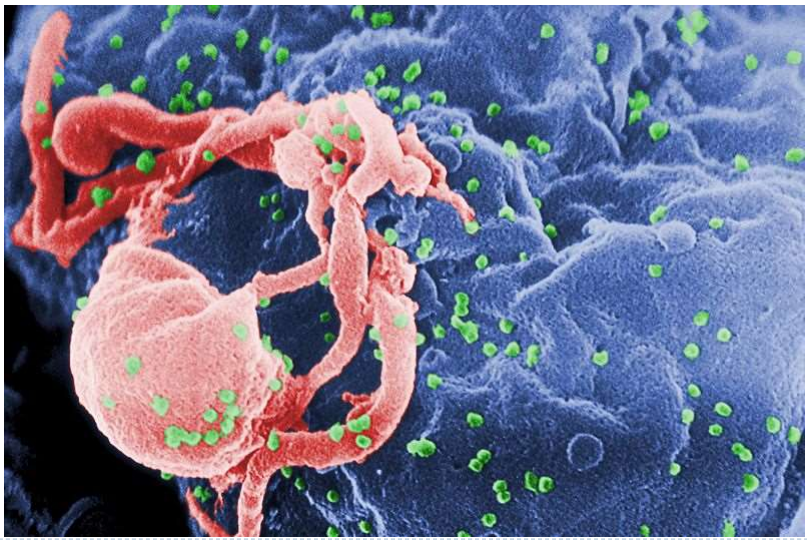
HIV - construction

- retrovirus
- Ball-shaped
- diameter120 nm

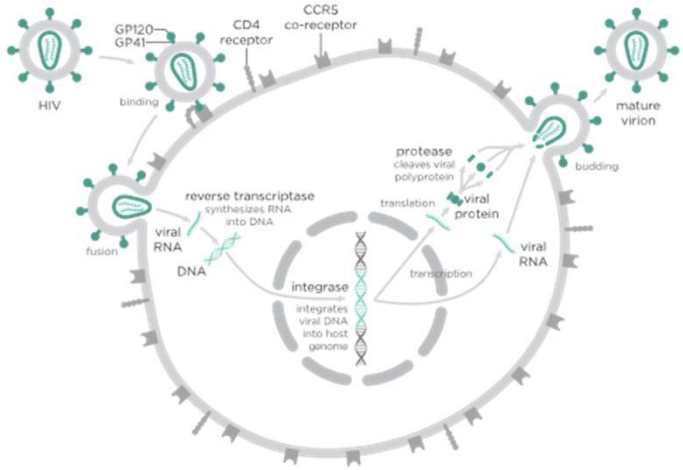




HIV – limphocyte T

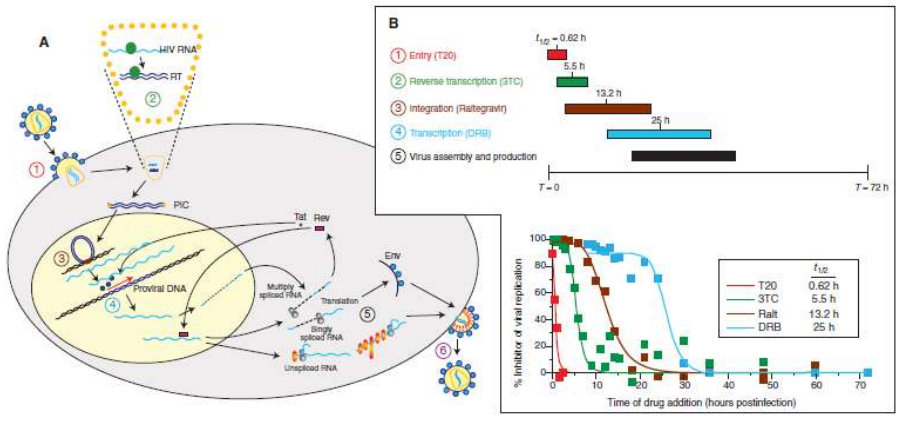


Replication cycle

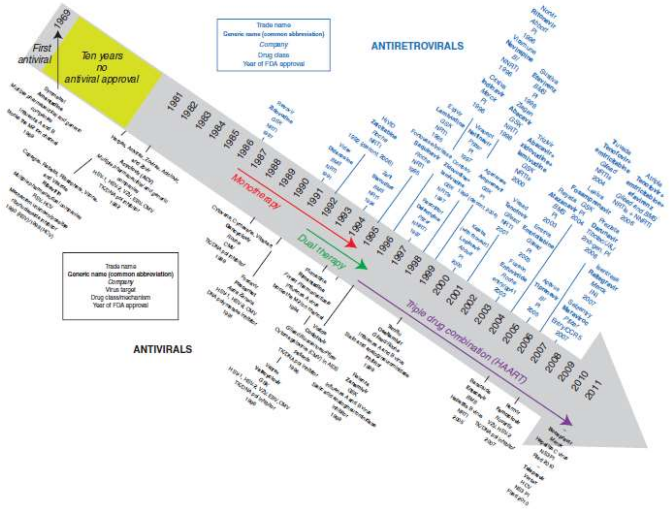


Replication cycle

Replication cycle and molecular targets

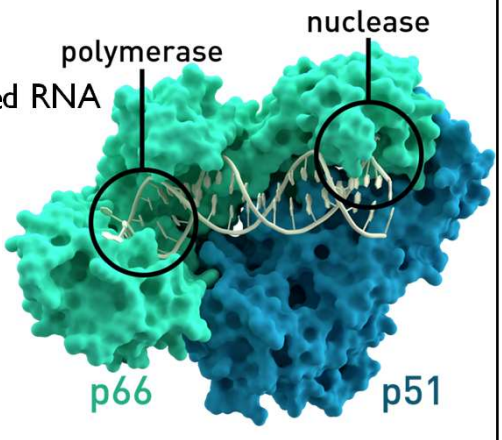


Introduction of anti-HIV drugs



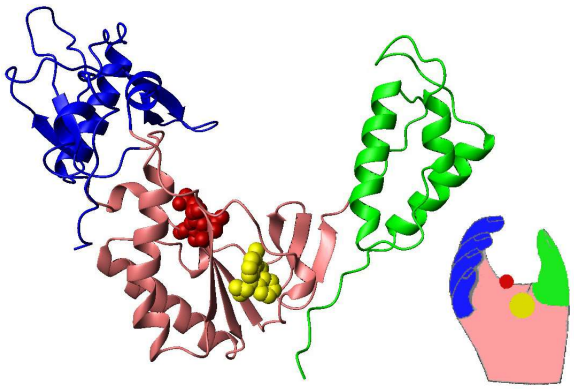
HIV reverse transcriptase

- Synthesis of complementary DNA strand on the basis of RNA (reverse transcription)
- Double-stranded DNA is obtained from one-stranded RNA
- RNA is hydrolyzed
- Enzyme has two subunits: p66 (560 aa), p51 (440 aa).
- P66 subunit contains active sites (polimerase and nuclease).



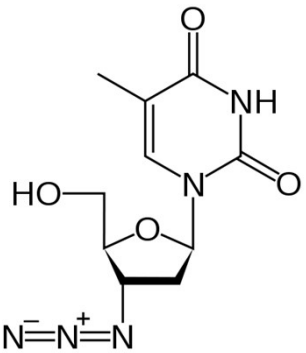
HIV reverse transcriptase

- The structure of P66 subunit is analogous to hand.
- Active site is located in the center part (in red)
- Non-nucleotide inhibitors bind to the region marked in yellow



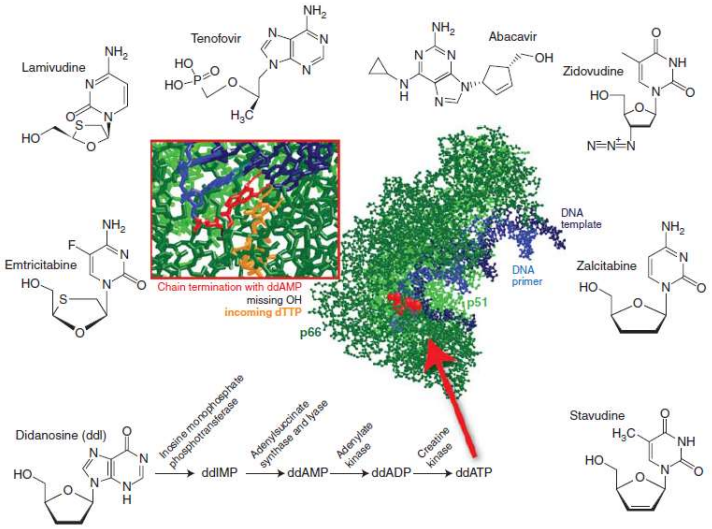
HIV reverse transcriptase

- HIV reverse transcriptase was the first molecular target for anti-HIV drugs.
- Inhibitors of reverse transcriptase are important anti-HIV drugs
- Retrovir (azidotimidine, AZT) was the first anti-HIV drug.
- Introduced to the market in 1987 as a result of accelerated procedure (25 months).
- AZT is converted to triphosphate, which is used by reverse transcriptase and inhibits the polimerase reaction.



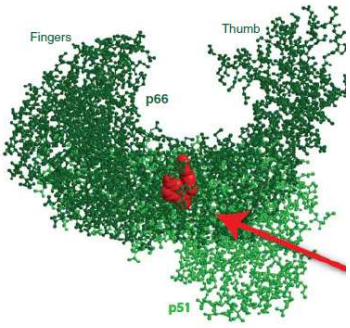
Dallas Buyers Club

Nucleotide inhibitors of reverse transcryptase



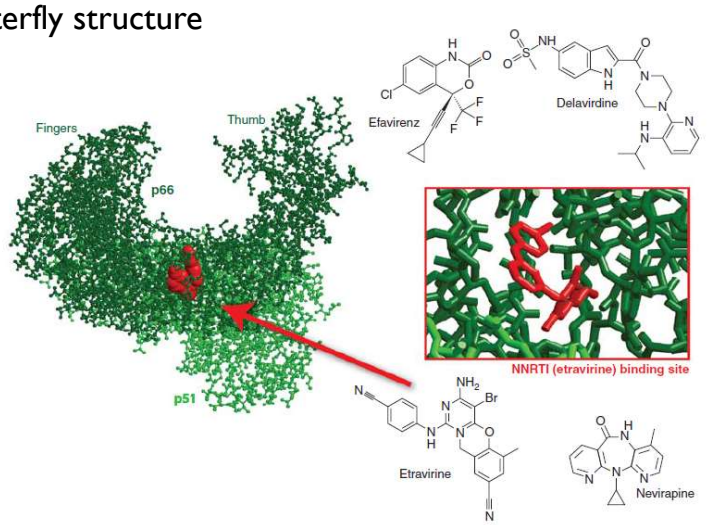
Inhibitors of reverse transcryptase

- First Non-Nucleotide Inhibitors of Reverse Transcriptase (NNRTI) was introduced to the market in1996.
- All NNRTI binds to the same place – ca. 10 Å from active site.
- Inhibitor binding causes conformational changes of the enzyme.



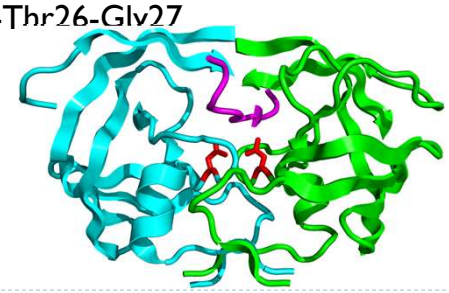
Inhibitors of reverse transcryptase

- Butterfly structure

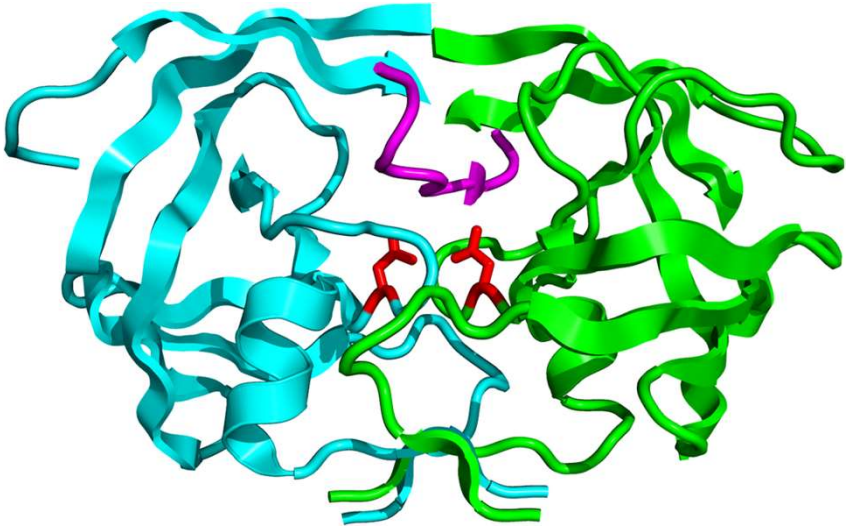


HIV protease

- Aspartate protease
- It cleaves polypeptide chain in order to obtain proteins necessary to construction of mature virus.
- Dimer, two chains 99 amino acid residues
- C_2 symmetry
- Two catalytic triads Asp25-Thr26-Gly27
- Most effectively cleaves Phe-Pro or Tyr-Pro



HIV protease – active site



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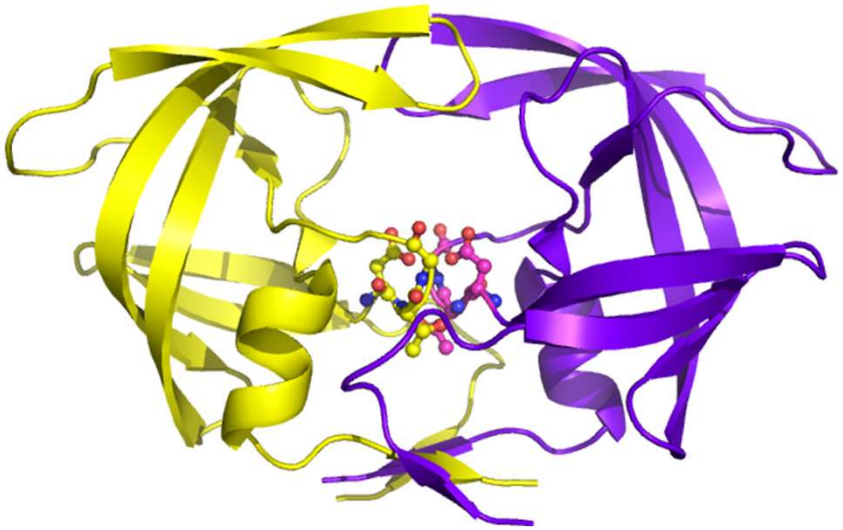

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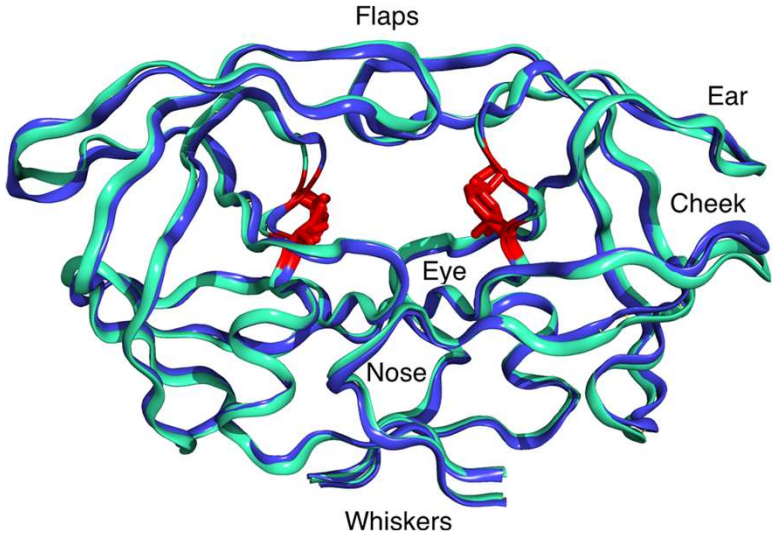
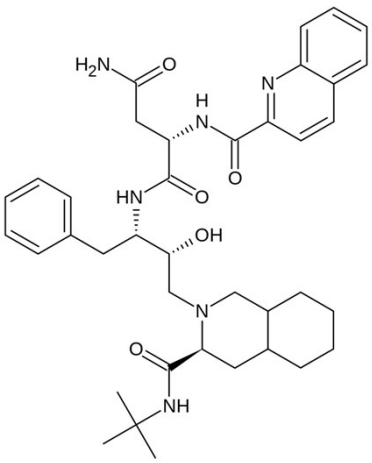
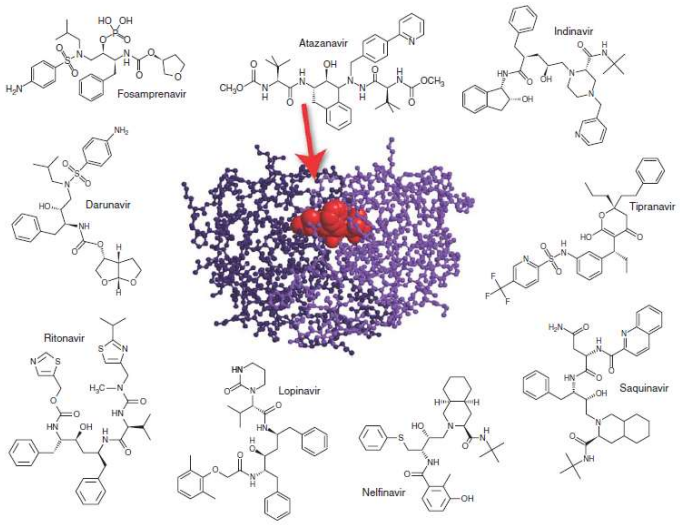


HIV protease inhibitors

- HIV protease is one of the most explored molecular targets for drugs.
- PDB database contains ca. 470 crystal structures with various ligands and mutants, while PubMed database contains ca. 17000 articles on this protein.
- First HIV protease inhibitor - saquinavir was introduced to the market in 1995.
- Saquinavir considerably enhanced the anti-HIV therapy.

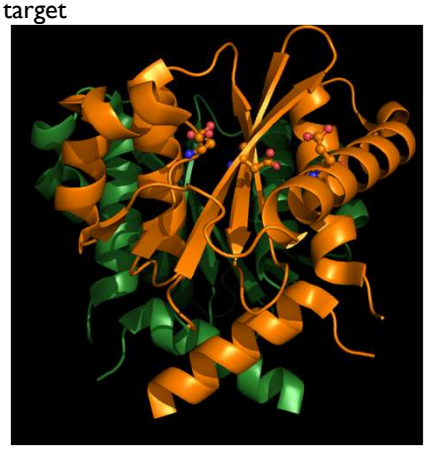


HIV protease inhibitors



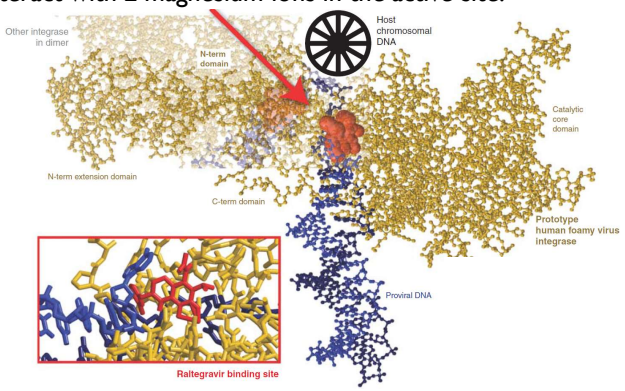
Integrase

- Enzyme integrating genetic material of virus with host DNA.
- Integrase is also very good molecular target for anti-HIV drugs.
- First integrase inhibitor was introduced to the market in 2007 (Raltegravir).



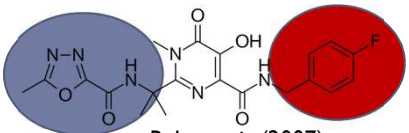
Integrase

- Known integrase inhibitors inhibit transfer of DNA strand (integrase *strand transfer inhibitors, InSTI*).
- Inhibitors bind exclusively to integrase- virial DNA complex
- Inhibitors interact with 2 magnesium ions in the active site.

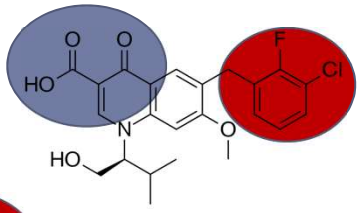


Integrase Inhibitors

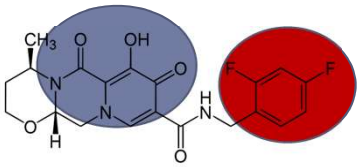
- Integrase Inhibitors consist from two parts:
  - Metal binding domain
  - Aromatic group (DNA intercalating)



Raltegravir (2007)



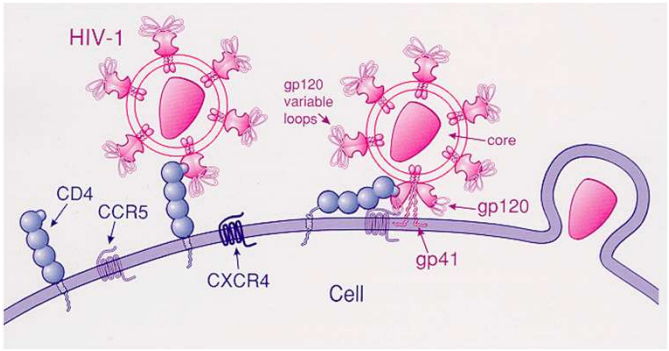
Elvitegravir (2012)



Dolutegravir (2013)

entry inhibitors

- Compounds inhibiting interaction between HIV and lymphocytes T and block virus entry to the cell.
- Inhibitors can interact with proteins on virus surface (gp41 and gp120) as well as on host cell surface (CD4 i CCR5)



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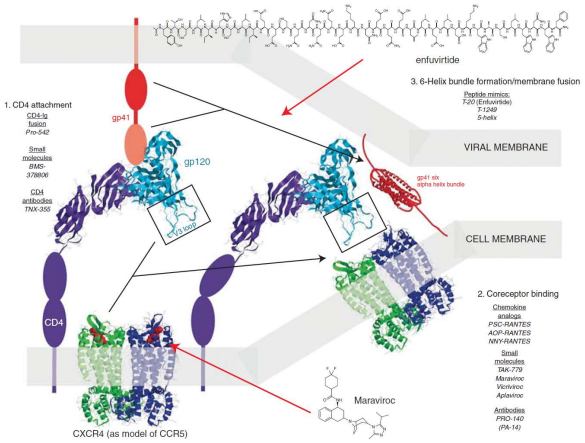
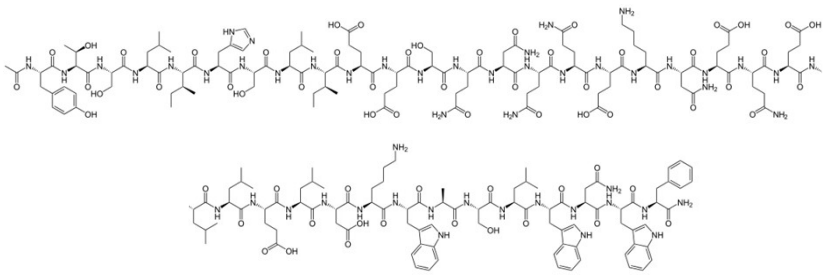


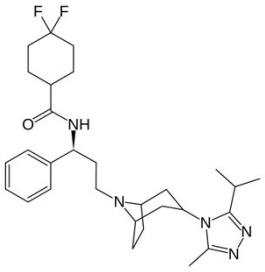
Entry inhibitors

- Fuzeon, peptide entry inhibitor (36 aa) binding to gp41 protein.
- Used in the case of failure of other therapies.
- High cost of the therapy (ca. 25 000 USD).



Entry inhibitors

- Malaviroc, CCR5 receptor antagonist. It inhibits CCR5 - gp120 interaction.
- Binds to hydrophobic cleft of CCR5 and changes the conformation extracellular loop.
- Tropism – some viruses are using CCR5 receptors (R5 viruses), while others CXCR4 (X4 viruses), or both (X4R5 viruses).



Malaviroc

Variability of the HIV

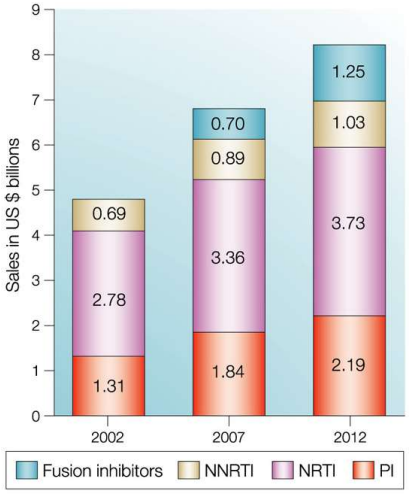
- Infected human has ca. 10 000 – 100 000 viruses /mL
- 1 mutation for 1 000 – 10 000 nucleotides
- HIV genome contains 10 000 nucleotides.
- Each replication cycle can produce1-10 mutations!!!
- There could be many virus mutants (also in one patient) with various drug resistance.

HAART

- Typically, a combination of 3 drugs is used to counter virus mutants at a similar level.
- *HAART - highly active antiretroviral therapy*
- Typically, a mixture of two nucleoside reverse transcriptase inhibitors and a non-nucleoside RTI or protease inhibitor is used.
- Monotherapy results in the selection of drug resistant viruses.
- HAART allows replication to be inhibited to undetectable levels and rebuilding of T cells.

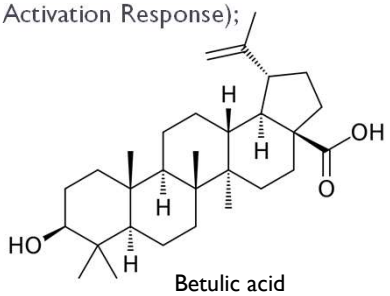
Revenue

- Revenue from HIV medicines continues to grow.



Summary HIV

- Anti-HIV drugs are directly related to the different phases of virus replication :
  - Entry inhibitors;
  - Reverse transcriptase;
  - integrase;
  - Interaction of regulatory protein Tat (Trans-activator of transcription) with RNA – TAR (Trans Activation Response);
  - protease;
  - Maturation inhibitors.



Betulic acid

Summary

- Choosing a molecular target is a key element to the success of a pharmaceutical project.
- Basic research on molecular targets dramatically increases the potential for drug design.
- The combination of several drugs with different molecular targets gives you the ability to treat pathogens with high variability.