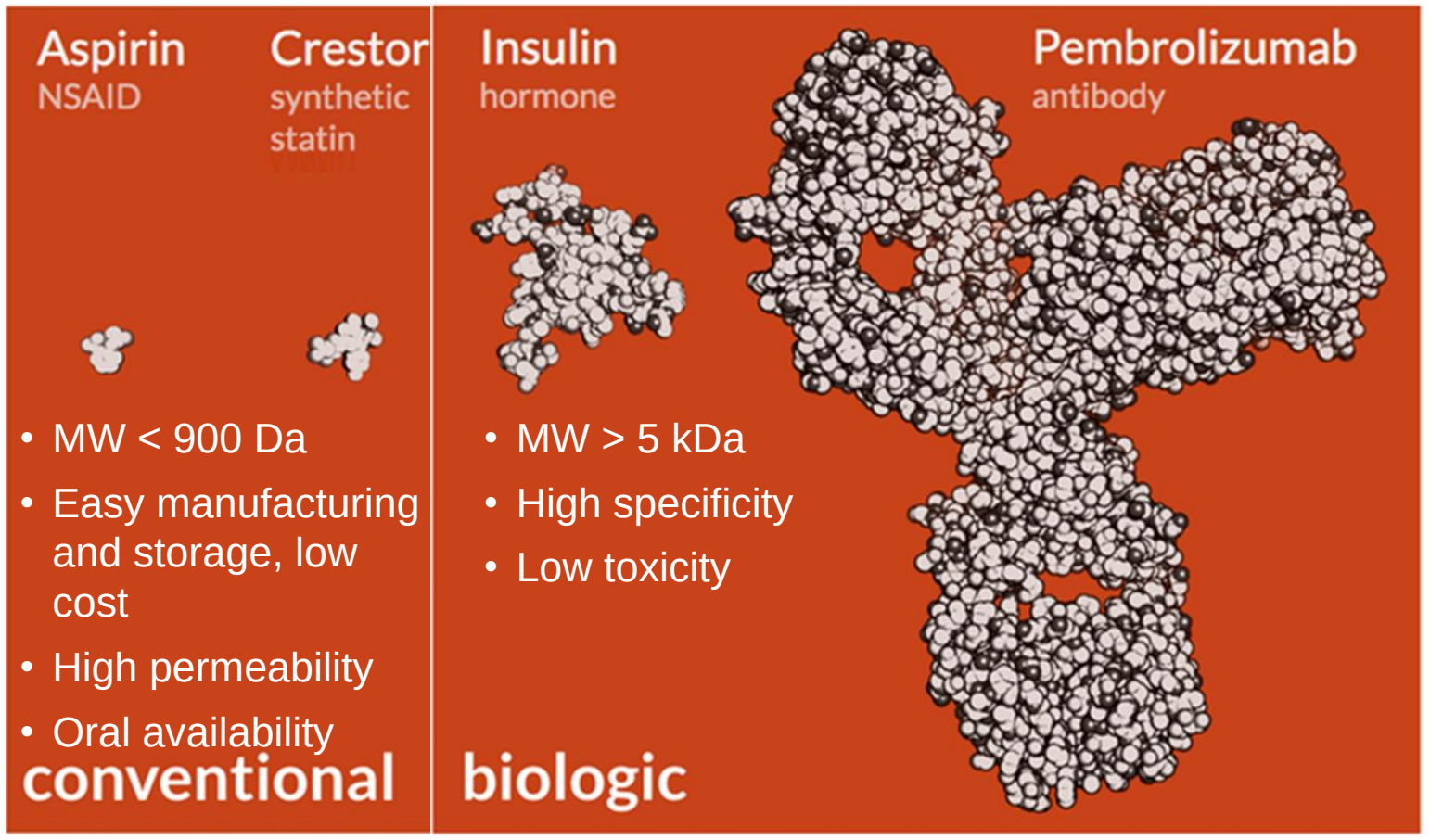
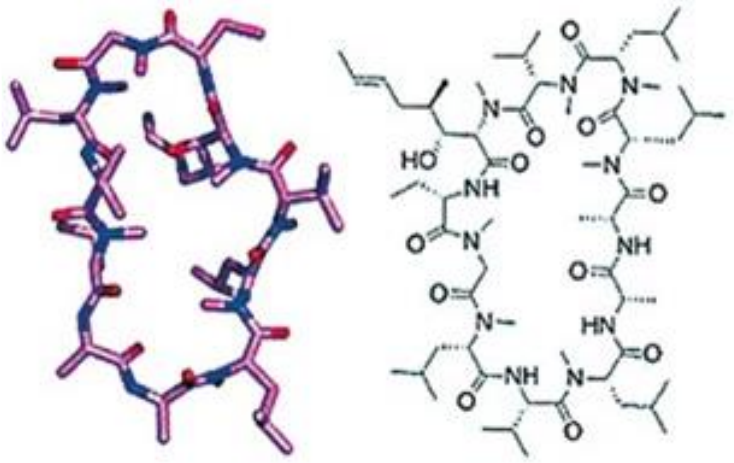
Two general therapeutic categories



Peptide therapeutics



cyclosporin A

- 1 kDa MW < 10 kDa (based on FDA)
- Easy manufacturing and storage, low cost
- Potential cell permeability and Oral availability
- High specificity
- Low toxicity

Growing pipeline of peptide therapeutics by 2021

•Characterized by a rapidly growing pipeline, over 10 product approvals per year, and steady rise in market value, the peptide-based therapies segment represents one of the fastest growing and major drug classes in the biopharmaceutical industry

65+ Approved Products

Already available in markets across various regions of the world

280+ Pipeline Candidates

Presently under development in the clinical and preclinical stages

1,200+ Clinical Trials

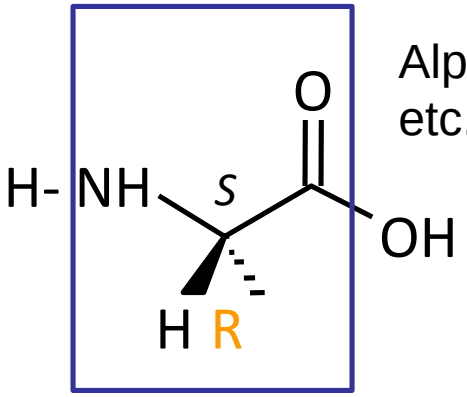
Have either been completed or are currently underway / planned, till date

Definition: peptides

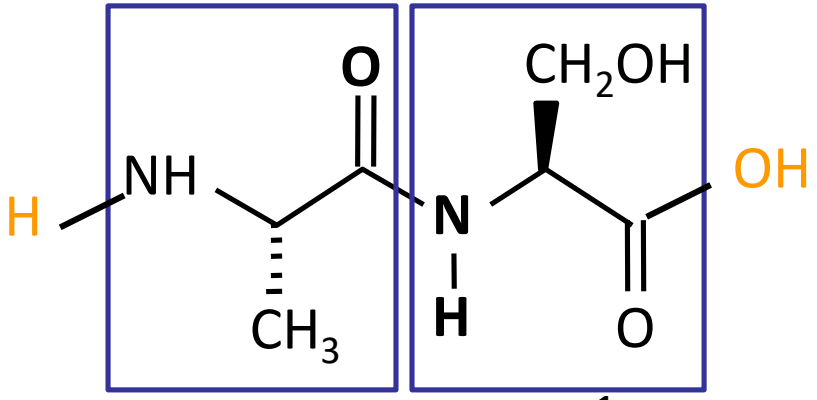
From greek ‘pepsis’ : digestion , oligomer constituted from amino acids

For peptide chemists: Peptide = Protein < 50 amino acids

H-Ala-Ser-OH or H-AS-OH



Alpha, beta, L-, D-, etc.



R = side chain

N-terminus → C-terminus

Amino acid residue
1-letter code : A
3-letters code :
Ala Amino acid :
Ex if R=CH_3 H-Ala-OH

Biosynthesis direction, writing the CONH bond and numbering the amino acids

Chemical synthesis direction C-ter to N-ter

Naturally occurring peptides

- First peptide discovered Insulin (Macleod & Banting, 1923)
  Synthesized only in 1964 (Katsoyannis PG et al. JACS **1964**, 86, 930–932).

- First peptide synthesized: Oxytocin (Vincent Du Vigneaud, 1962)

- Today, more than **7000 natural bioactive peptides** have been identified
- with crucial roles in physiological mechanisms as:

  Hormones : chemical communication and coordination: secreted by **neuroendocrine cells (release in the blood)** -> circulation to stimulate a response on another organ.

  Neuropeptides: hormones **but which are secreted and used in the CNS**. Unlike neurotransmitters, they are not recycled.

  Growth and differentiation factors, Ion channel ligands , anti-infectious, transporters of substances through membranes

- As natural products: antibiotics, immunosuppressants, etc.

Review Keld Fosgerau, Torsten Hoffmann
Peptide therapeutics: current status and future directions.

Two main classes of naturally occurring peptides

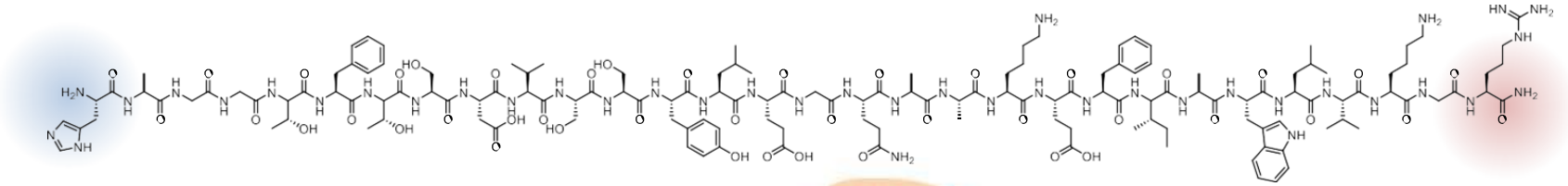
Ribosomal peptides

- synthesized mRNA translation
- modifed by proteolytic enzymes from propeptides (longer peptides chains) to yield their active form.
- Subjected to multiple posttranslational modifications (phosphorylation, hydroxylation, palmitoylation, glycosylation, disulfide bon formation…)

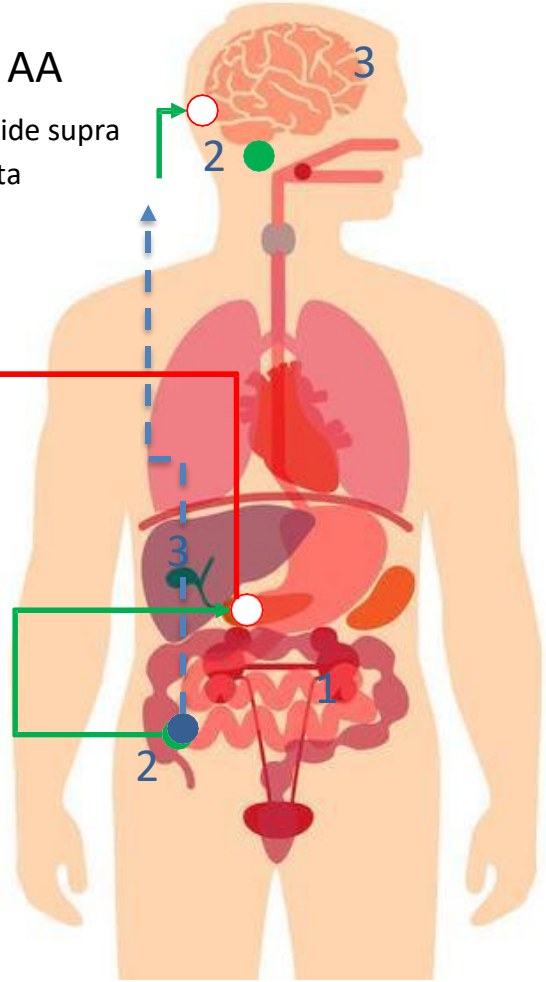
Non-ribosomal peptides

- synthesized by non-ribosomal peptide synthetases independant of mRNA, very
- often produced by microorganisms (bacteria, fungi)
- High structural diversity: linear, cyclic, branched

Glucagon-like peptide 1 GLP1: anorexigenic and antidiabetic



Main active forms 30 and 31 AA
GLP1 7-Gly^{37} et 7-Arg ^{36} NH_2 vide supra
Secreted by intestine and medulla oblongata



3) Activation of GLP1R receptors of neurons : anorexigenic effect

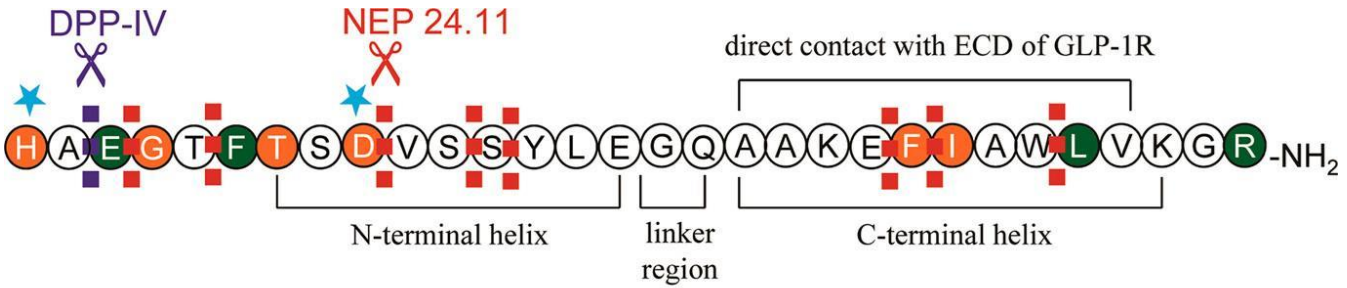
2) Production of GLP1 by Medulla Oblongata

3) Activation of GLP1R of pancreas: insulin secretion, inhibition of glucagon production
Sugar level decrease

2) Production of GLP1
By Ileum

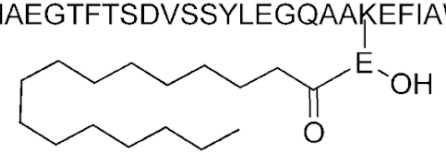
1) High sugar levels: glucose absorbed by intestine:
Signal sent to CNS

GLP1 analogues



Exenatide HGEGTFTSDLSKQMEEEAVRLFIEWLKDGGPSSGAPPPS-NH_2 **1**

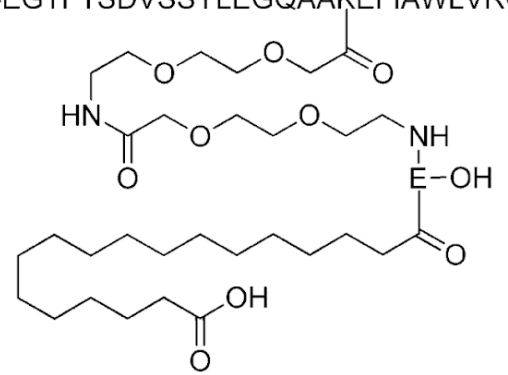
Liraglutide HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG **2**



Taspoglutide H-Aib-AEGTFTSDVSSYLEGQAAKEFIAWLVK-Aib-AR-NH_2 **3**

Lixisenatide HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPSKKKKKK-NH_2 **4**

Semaglutide H-Aib-EGTFTSDVSSYLEGQAAKEFIAWLVRGRG **5**

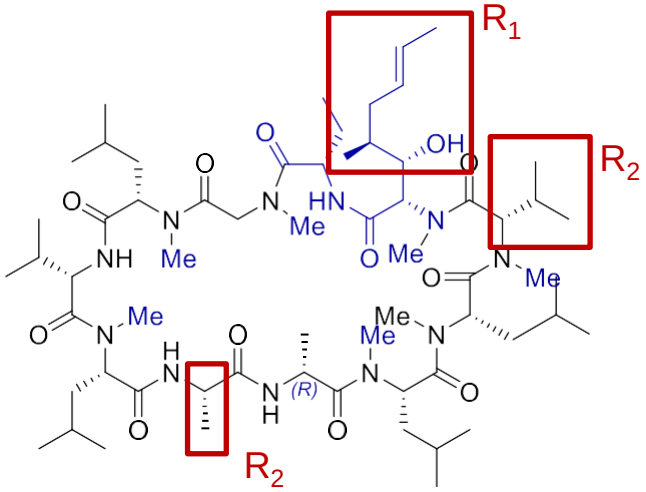


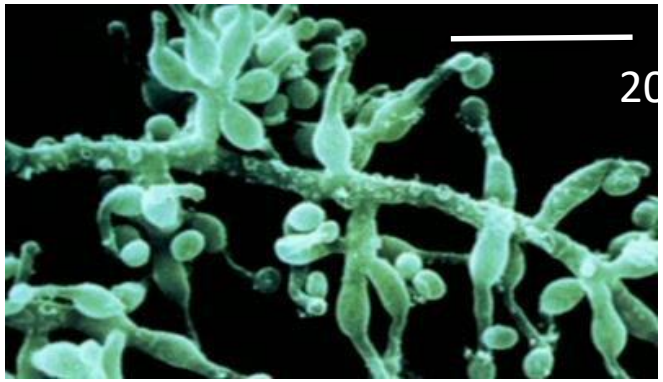
Market size:
$22.4B in 2022

JMC, 2015, 58, 1020

Cyclosporin: immunosuppressant

- 11 AA, Immunosuppressant, used to treat auto immune diseases and graft rejection
- Synthetized by a non ribosomalpeptide synthethase.
- Isolated from a microscopic fungi (1976), *Tolypocladium inflatum* from soil samples. Inhibits an enzyme (calcineurin) and T cell activation



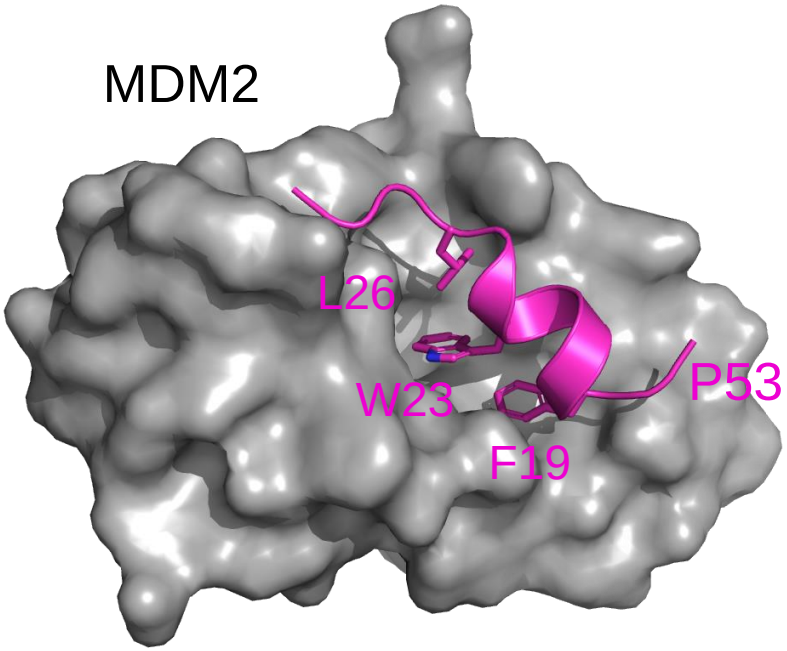


c[MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-DAla-MeLeu]

Abu= aminobutyric acid
MeBmt = Butenyl-methyl-L-threonine
Sar= sarcosine (i.e. N-Methyl Glycine)

cyc[MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-Dala-MeLeu]

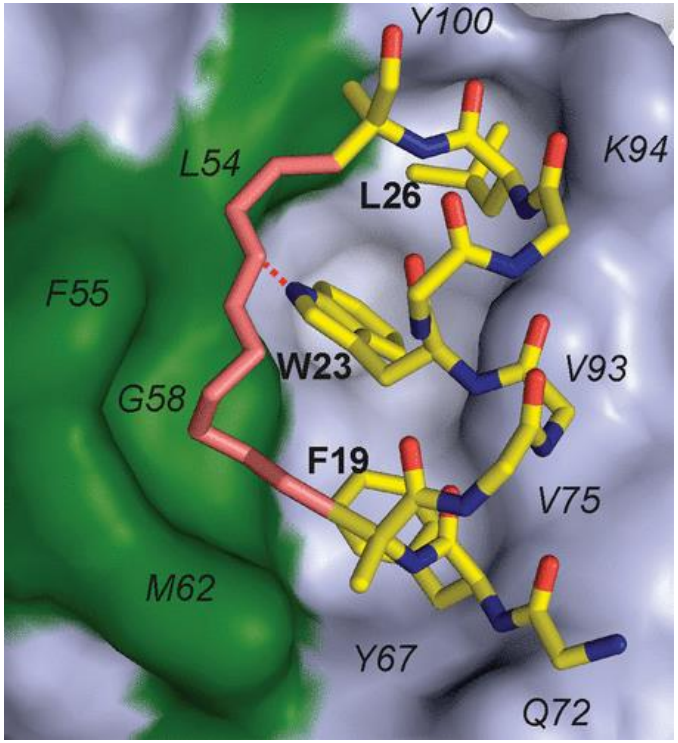
Peptides derived from proteins as PPIs



P53-MDM2 complex

(K_d: 700 nM)

Stapling



(K_d: 55 nM)

PPI: protein-protein interaction inhibitor

JACS, 2012, 134, 103

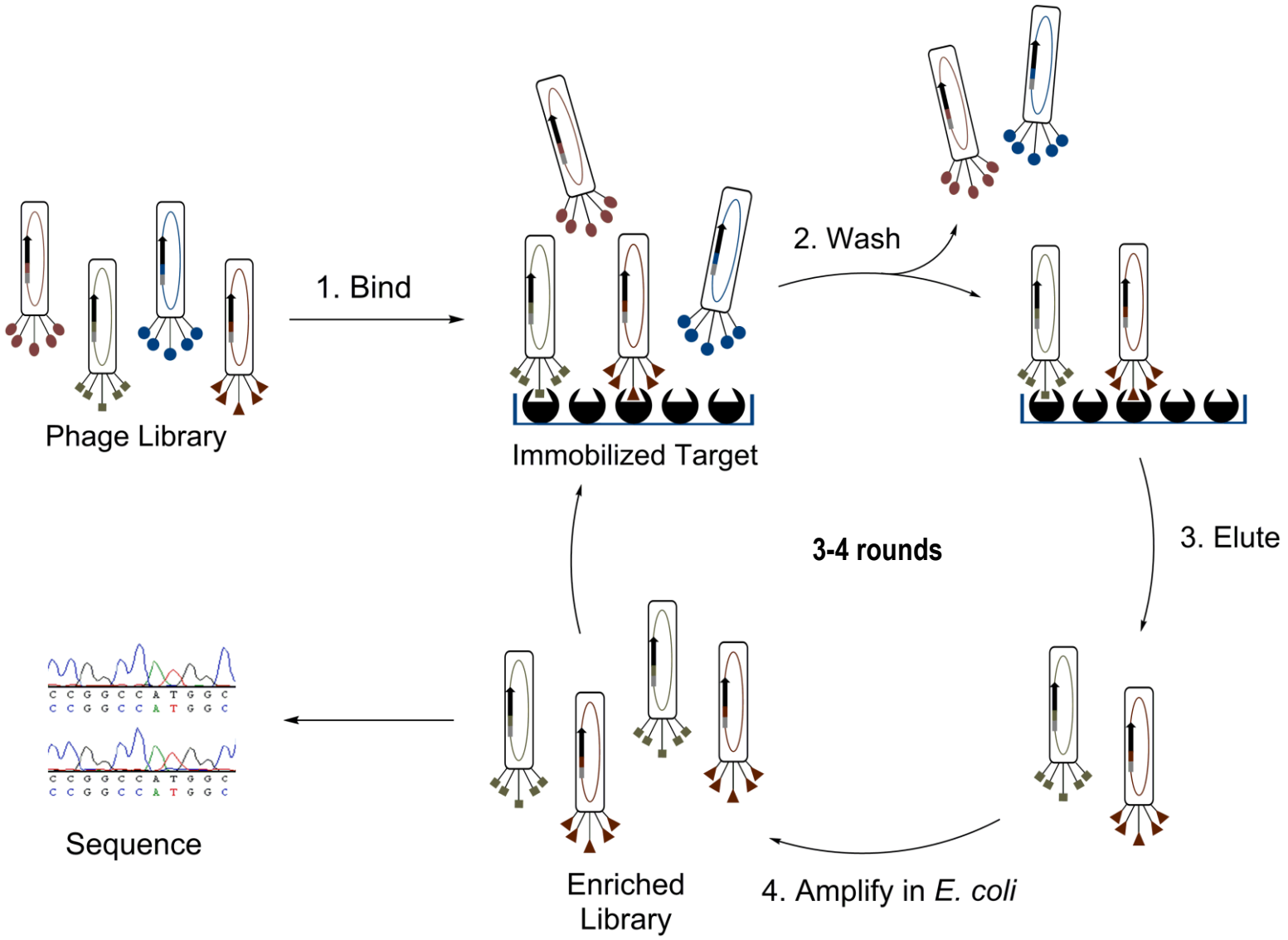
Peptides from no starting points – display techniques

DNA
RNA
Peptide

Peptide

Phage display, mRNA display, yeast display, DNA display, etc.

Directed evolution of phage-displayed peptides

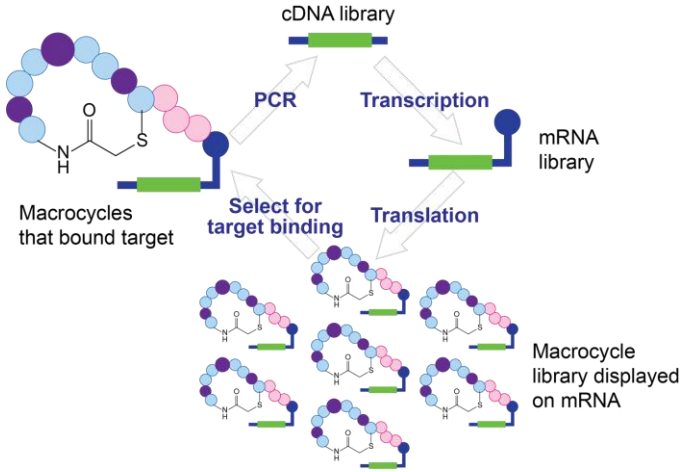


Smith and Petrenko, *Chem. Rev.* **1997**, *97*, 391-410.

A macrocyclic peptide inhibitor of PCSK9

Hit-finding platform

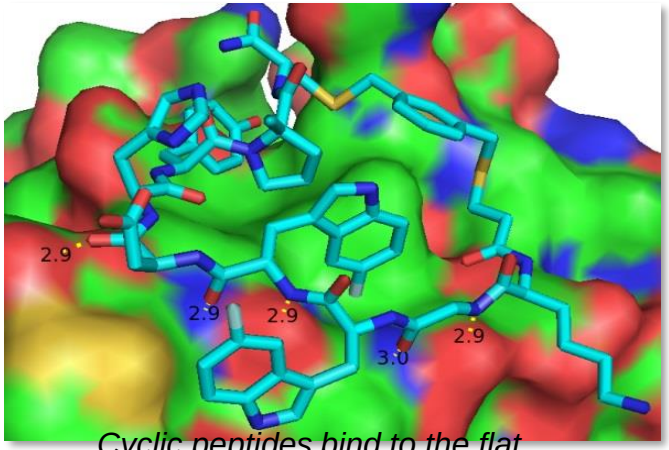
mRNA display



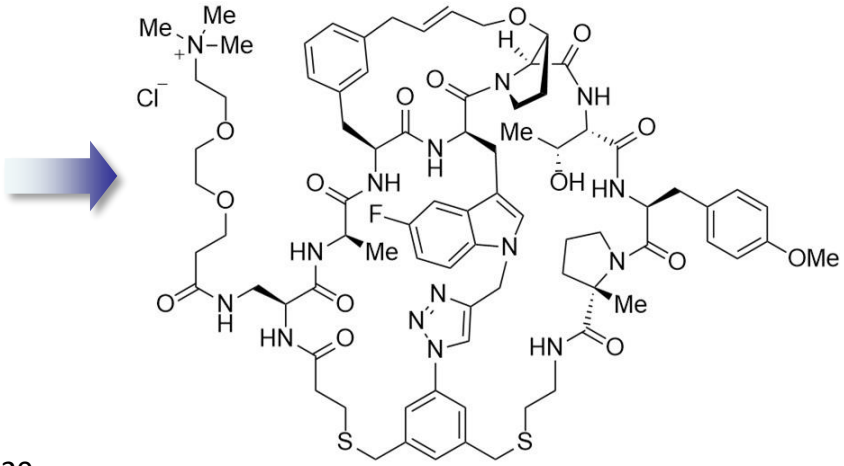
Identifies macrocyclic peptides as inhibitors of protein-protein interactions (PPI)

Merck Global Chemistry

Structure-Based Drug Discovery



Cyclic peptides bind to the flat PCSK9:LDL-R interface with mAb-like affinity



Cyclic Peptide

redecessor to MK-0616

MW = ~1500 g/mol
Ki = 2-5 pM

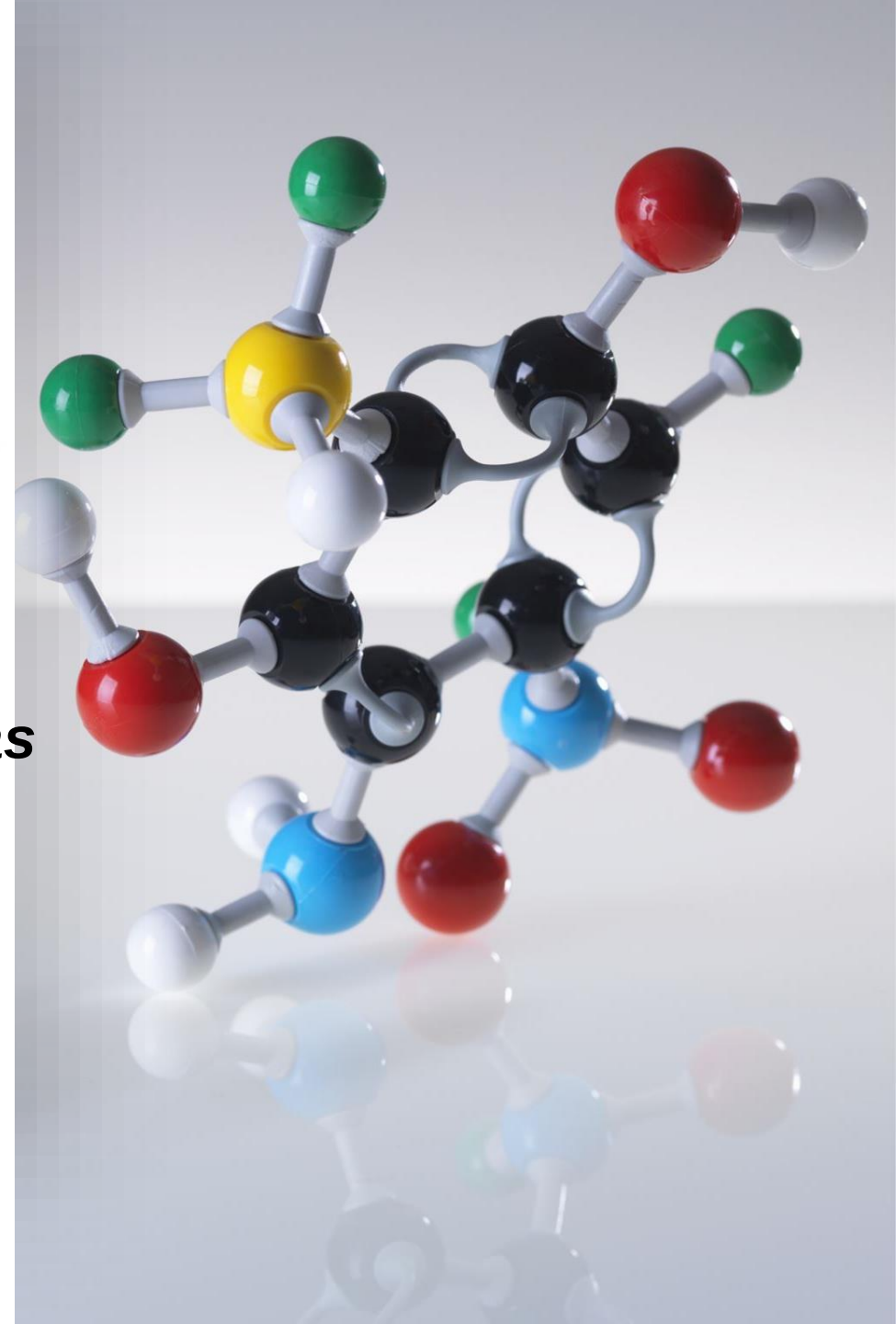
J Med Chem 63(22):13796-13824, 2020

Small Molecule Inhibitors

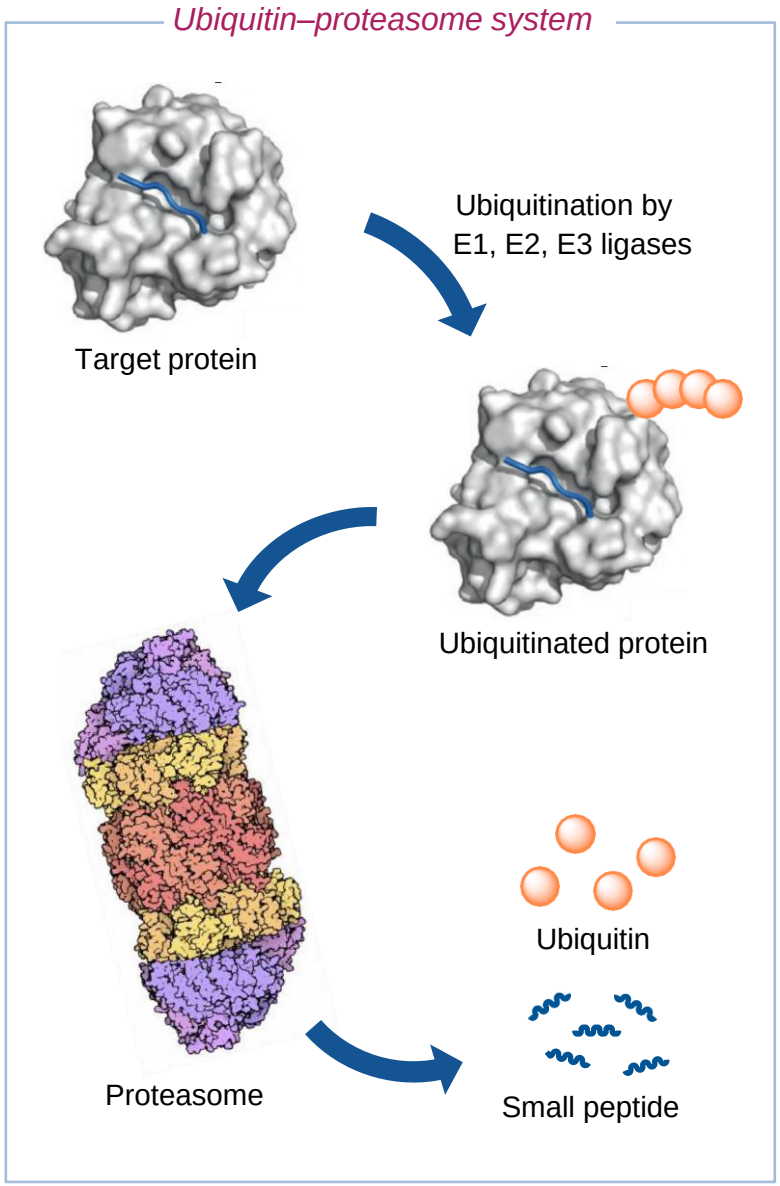
- Stoichiometric (occupancy-based)
- Target active or allosteric sites (undruggable targets)
- Selectivity and toxicity concerns
- Difficult to target protein-protein interactions (PPIs)

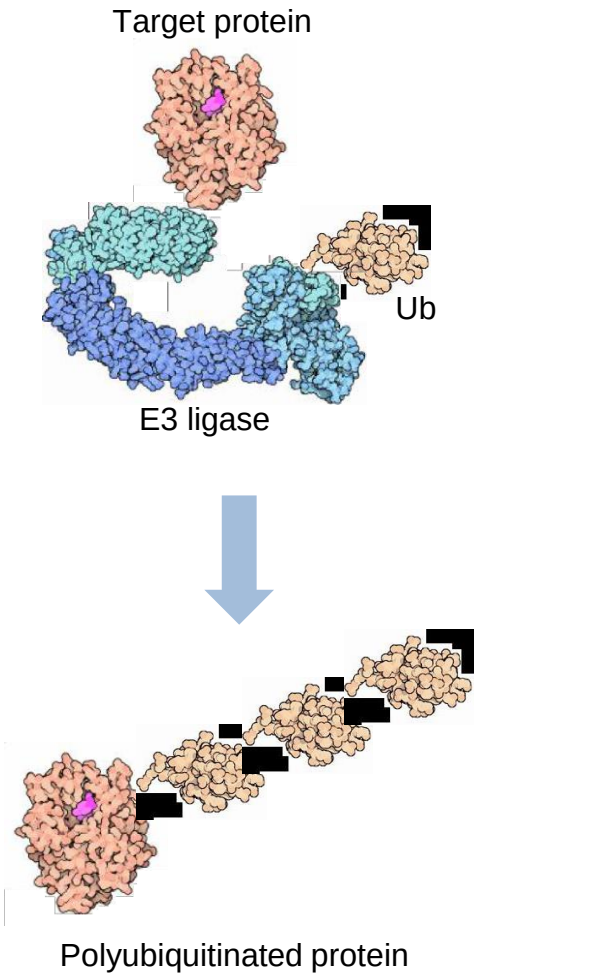
Proteolysis Targeting Chimeras (PROTACs)

- Small molecule triggered protein degradation by proteosome
- Target undruggable targets
- Catalytic (sub-stoichiometric)
- Less toxicity (sub-stoichiometric)
- Improved selectivity in comparison to occupancy-based inhibitors



The Ubiquitin Proteasome System

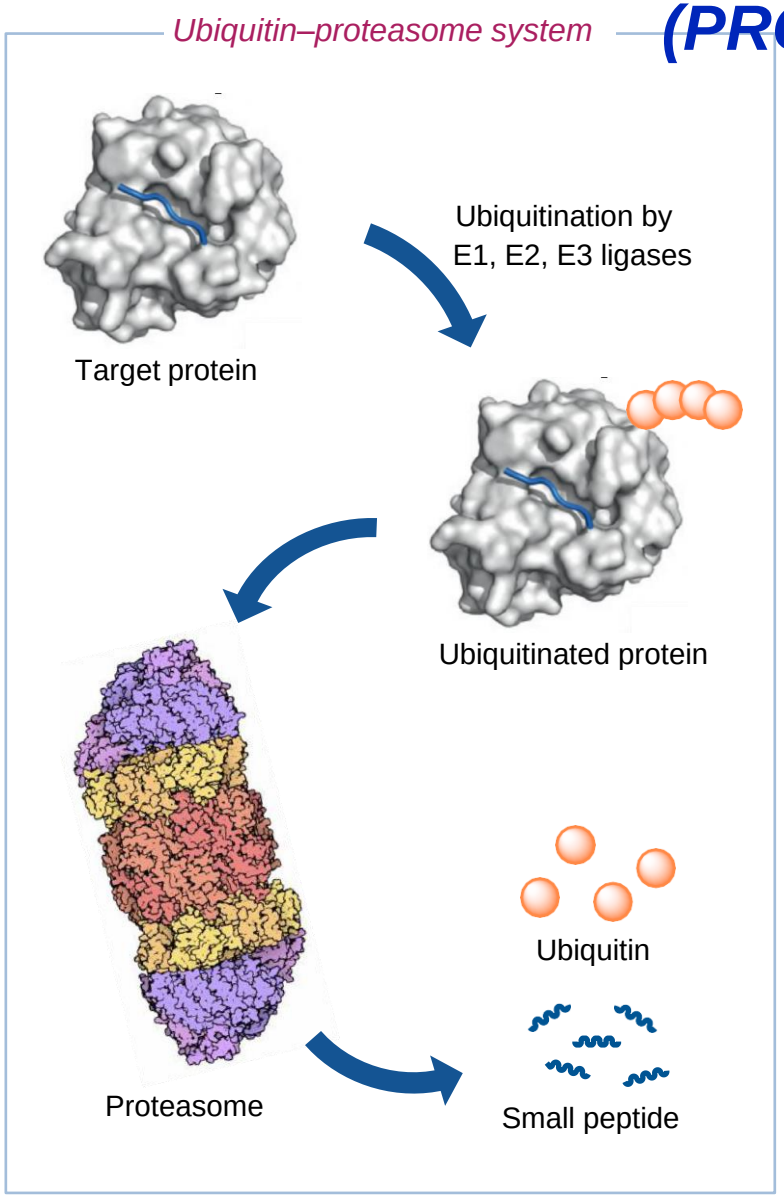


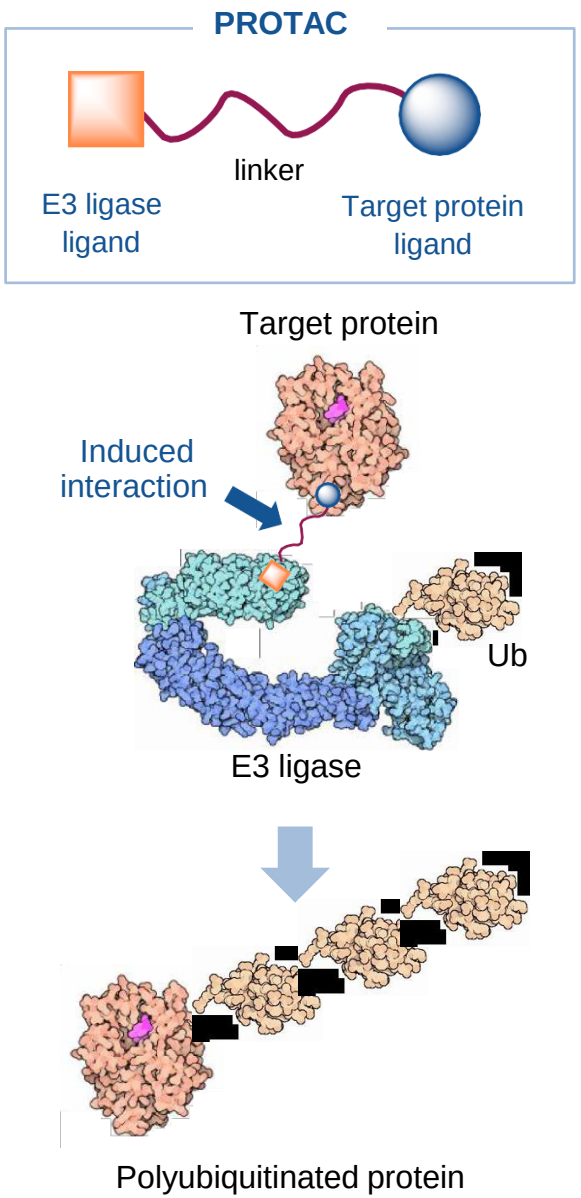


Can we induce artificial interaction between E3 ligase and POI?

Slides were adapted from a literature presentation by Junyong Kim at

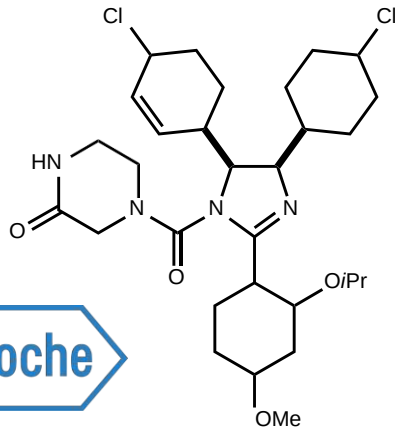
Proteolysis Targeting Chimera (PROTAC)





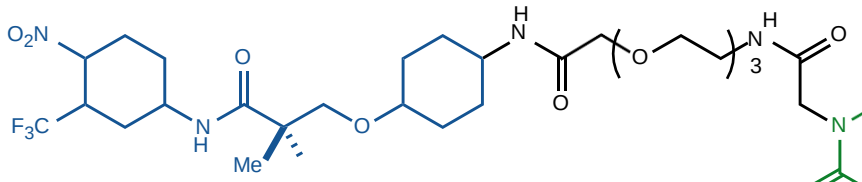
Slides were adapted from a literature presentation by Junyong Kim at

Small Molecule PROTACs



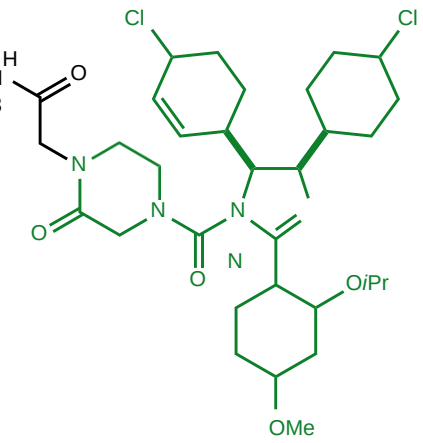
Roche

Nutlin-3 (MDM2 E3 ligase inhibitor)
Science **2004**, 844.

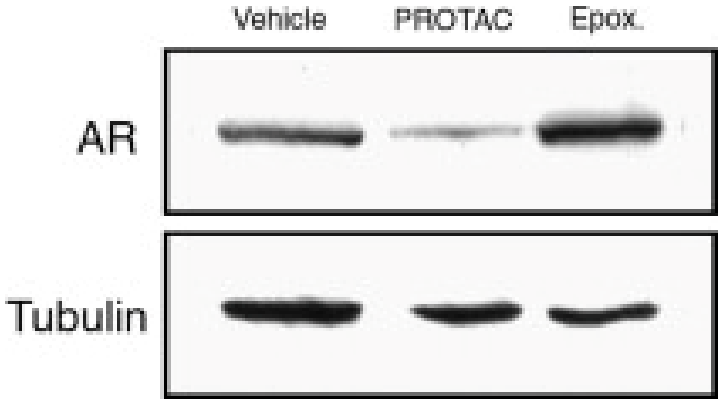


SARM (Androgen receptor ligand)

First cell permeable PROTAC demonstrating pharmaceutical utility



Nutlin-3 (MDM2 ligand)



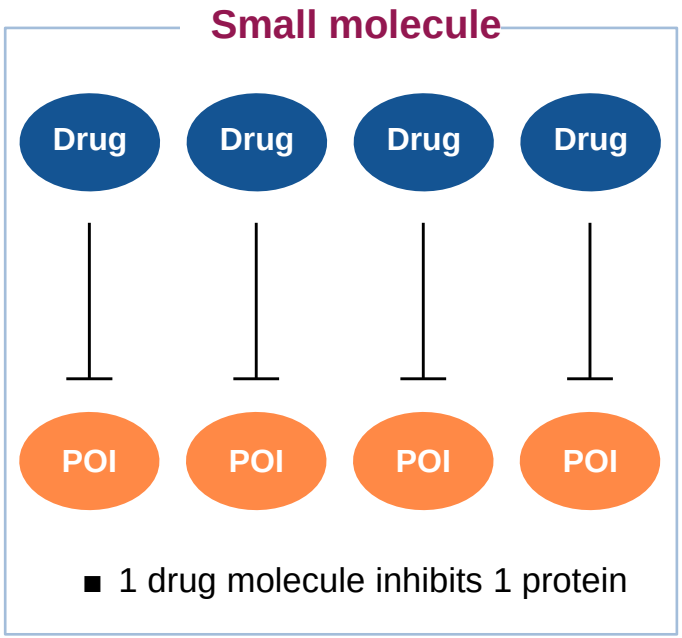
expoxomicin: a proteasome inhibitor

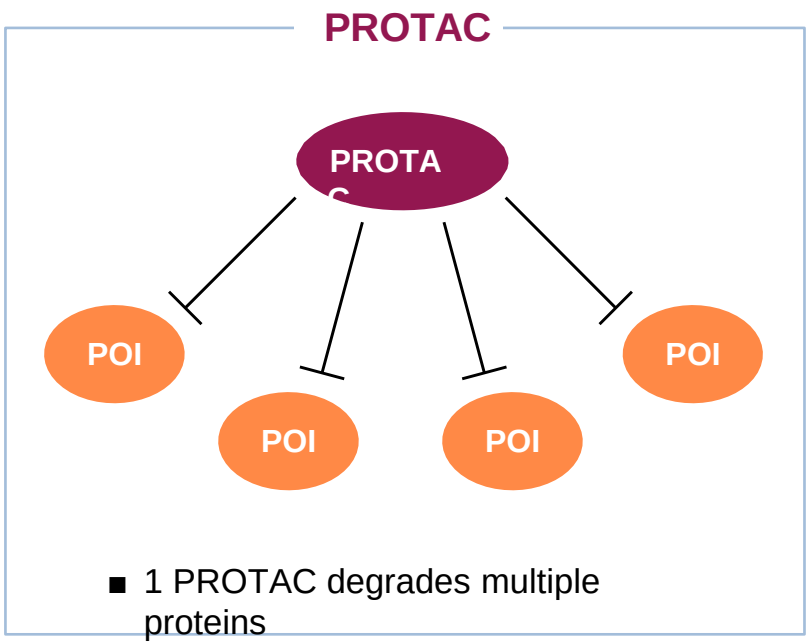
10 uM treatment for 7h

Schneekloth, A.R.; Pucheault, M.; Tae, H.S.; Crews, C.M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5904–5908.

Slides were adapted from a literature presentation by Junyong Kim at

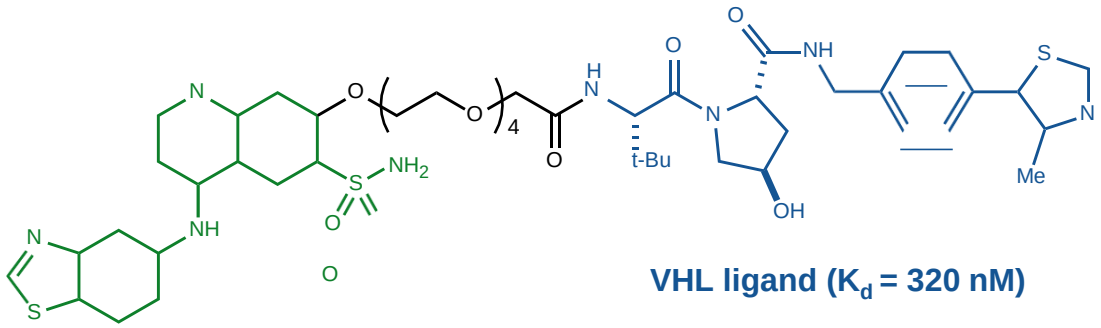
Small Molecule Drugs v.s. PROTACs





Slides were adapted from a literature presentation by Junyong Kim at

Small Molecule Drugs v.s. PROTACs

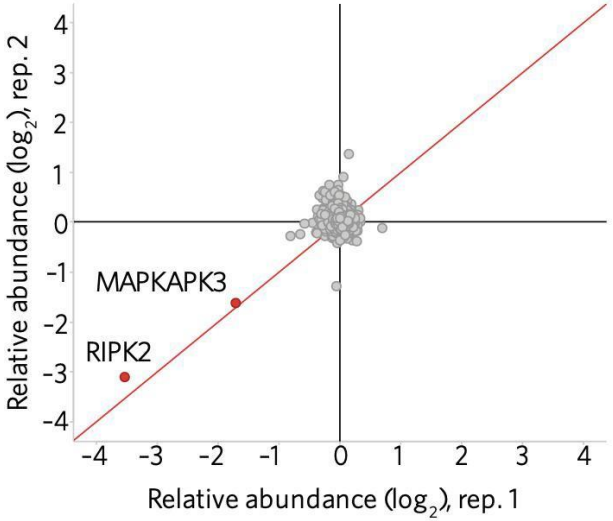


RIPK2 ligand (IC_{50} = 660 nM)

VHL ligand (K_d = 320 nM)

DC_{50} = 1.4 nM
DC_{max} = 10 nM

DC_{50}: 50% degrading conc.

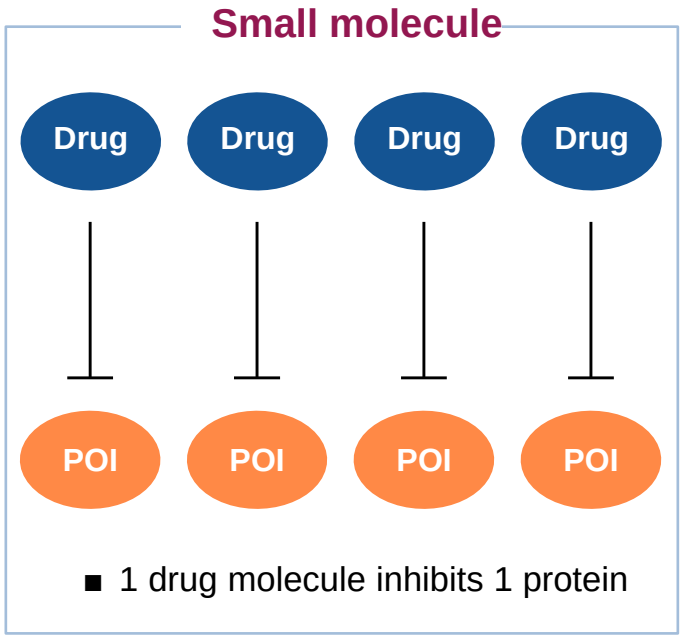


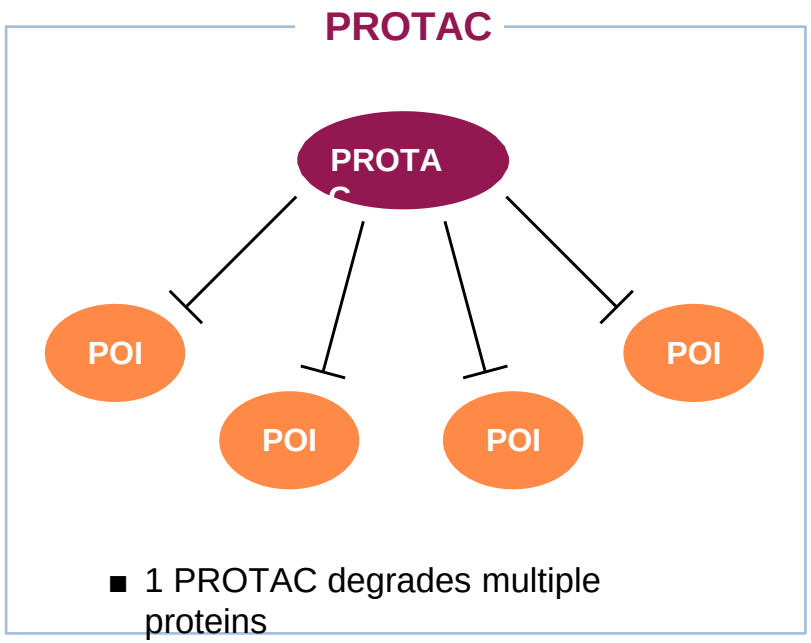
Only RIPK2 and MAPKAPK3 were degraded among 7640 proteins

Bondeson, D.P.; Mares, A. *et al*. *Nat. Chem. Biol.* **2015**, *11*, 611–617.

Adapted from a literature presentation by Junyong Kim at

Small Molecule Drugs v.s. PROTACs





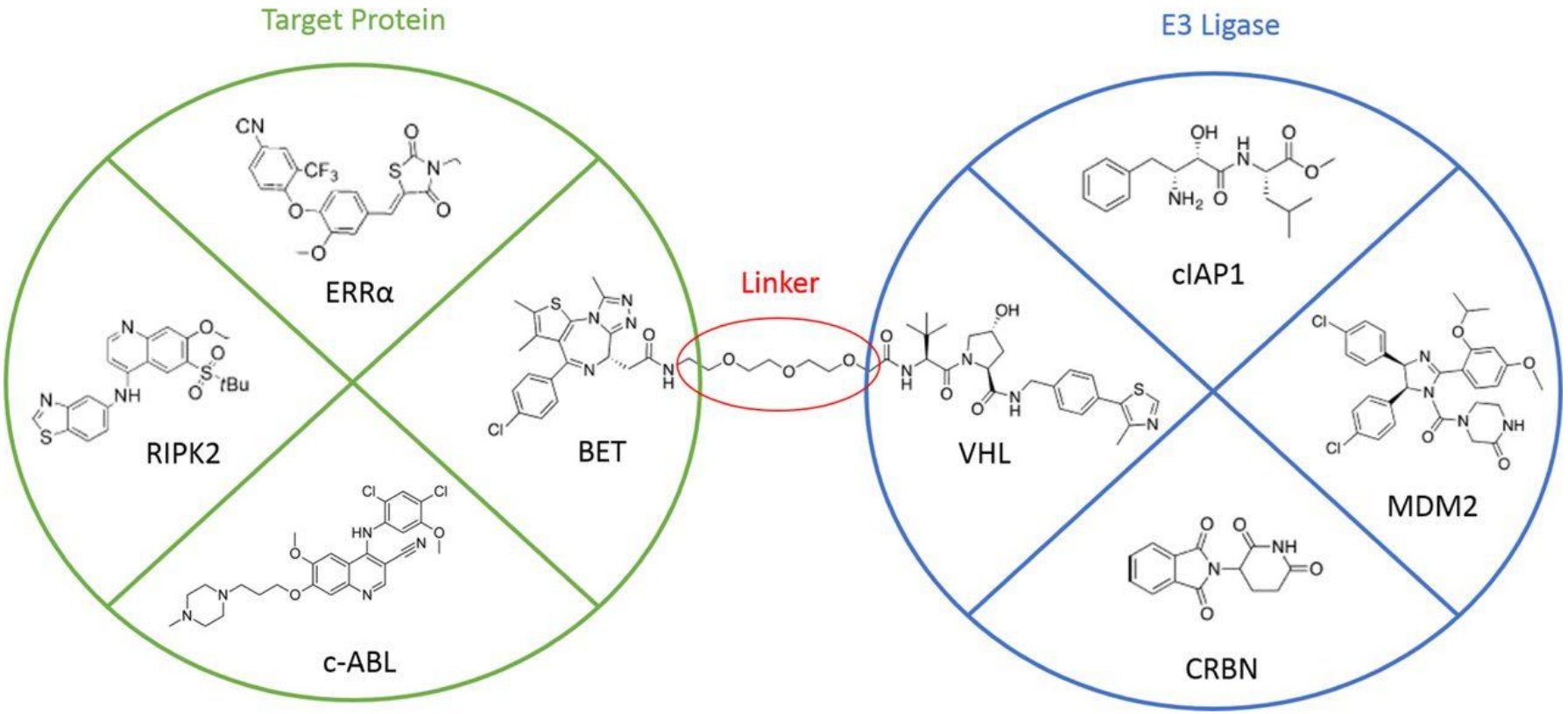
Catalytic mode of action can provide high potency and selectivity

Only affinity probes are required – no need to be inhibitors

Removal of a protein instead of inhibition can provide additional therapeutic effect

Slides were adapted from a literature presentation by Junyong Kim at

E3 Ligands

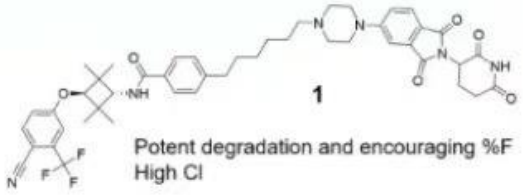

Target Protein
E3 Ligase
Linker
ERRα
RIPK2
BET
c-ABL
cIAP1
VHL
MDM2
CRBN

PROTACs on Clinical Trials (Arvinas)

Evolution of AR Degrading PROTACs Leading to ARV-110

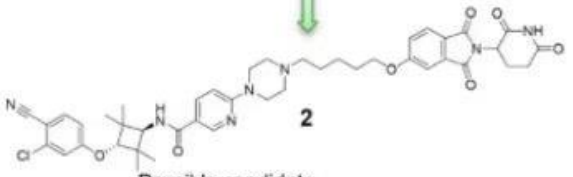
American Association for Cancer Research
FINDING CURES TOGETHER

Early Discovery Efforts
Multiple E3 recruiting ligands
Multiple AR binders



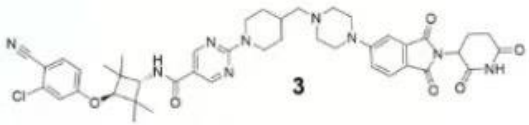
1

Potent degradation and encouraging %F
High Cl



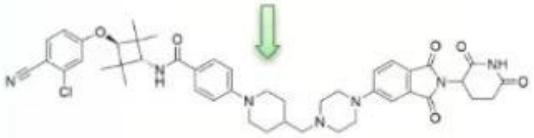
2

Possible candidate
In vivo potency suboptimal
Crystallized to high melting solid



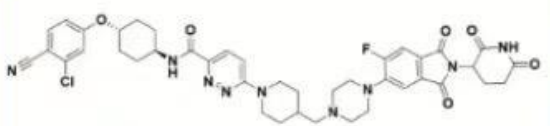
3

Good in vitro degradation potency
Possible autoinduction signal
AR ligand by itself agonist
In vivo potency superseded by **4**



4

Possible candidate
Dose escalation exposure suboptimal



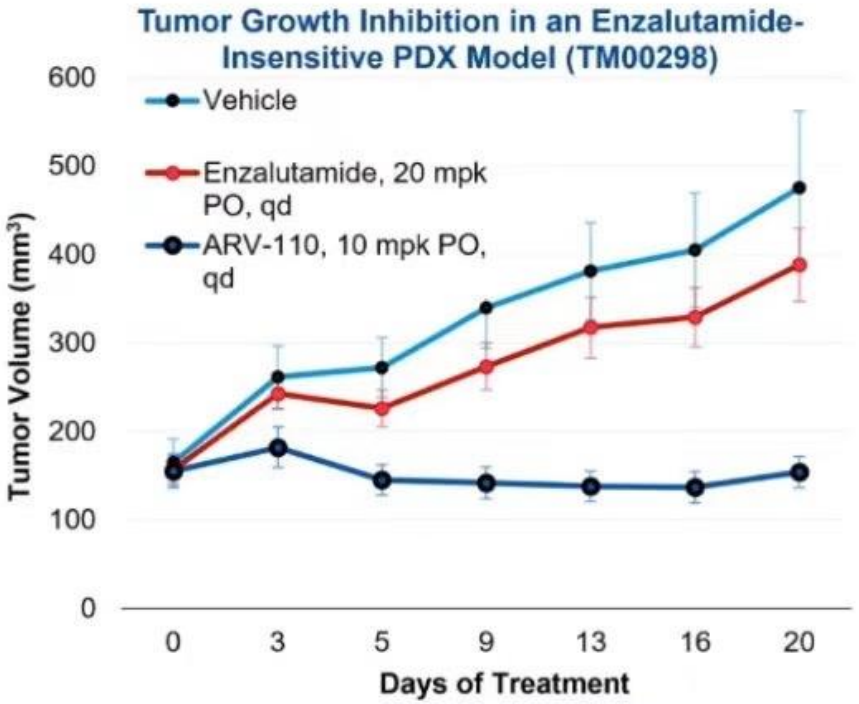
ARV-110

PROTACs on Clinical Trials

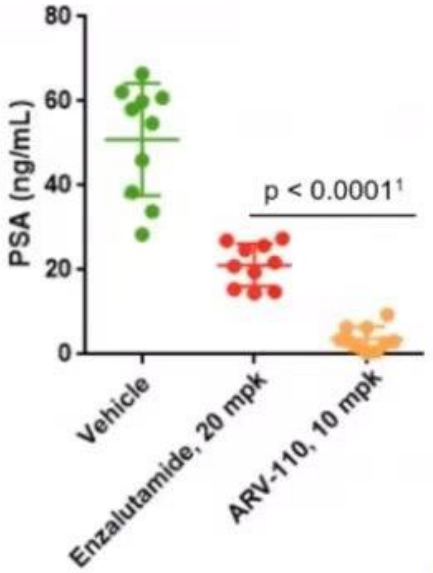
ARV-110 Demonstrates Efficacy and Plasma PSA Reduction in an Enzalutamide-Insensitive PDX Model

American Association for Cancer Research
FINDING CURES TOGETHER

- Orally delivered ARV-110 significantly inhibited tumor growth in these enza-insensitive tumors (TGI: 100%)



- Plasma PSA levels following ARV-110 treatment significantly decreased vs. mice treated with vehicle or enzalutamide



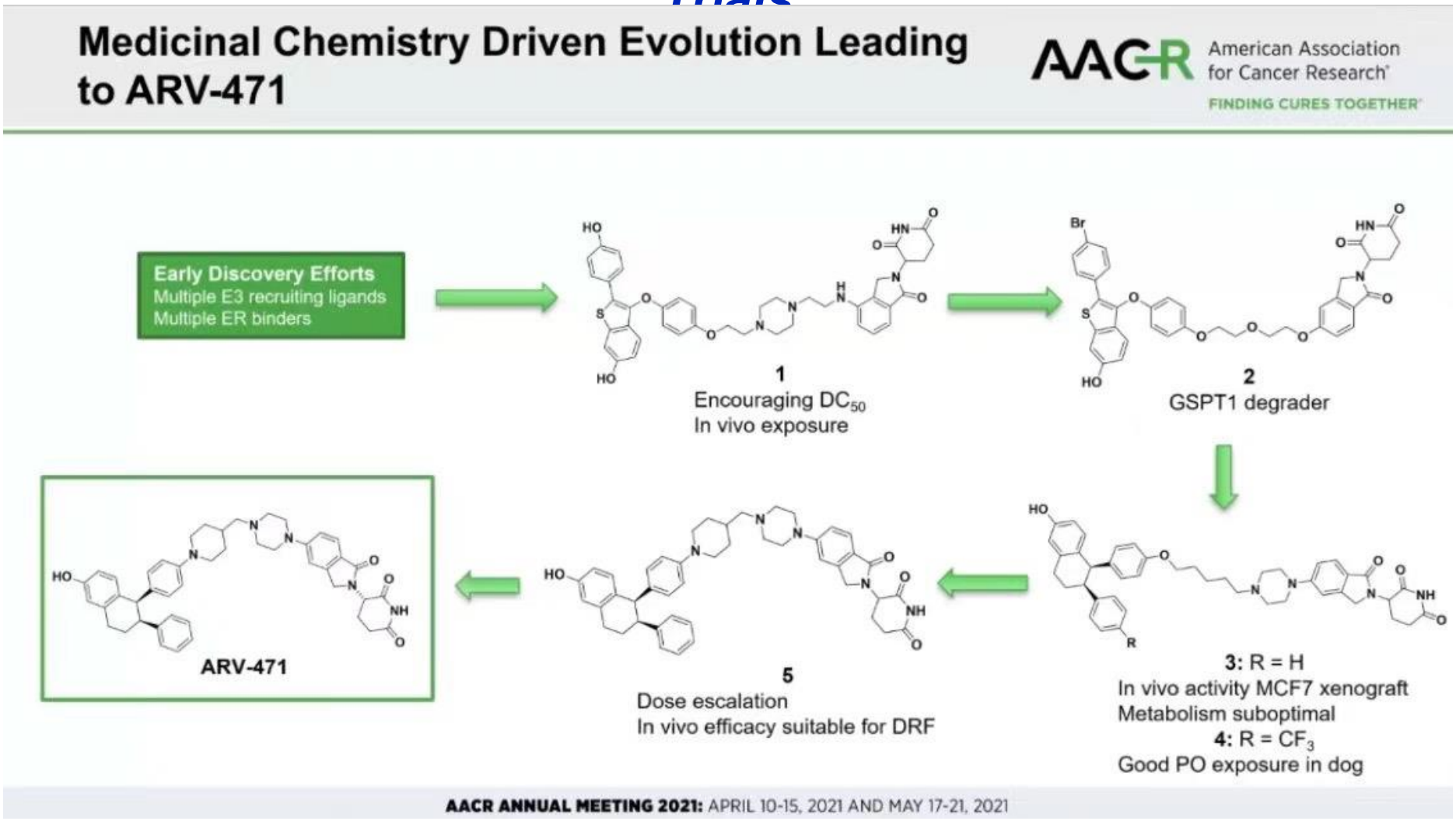
1 p value refers to ARV-110 vs. enzalutamide

ARVINAS

AACR ANNUAL MEETING 2021: APRIL 10-15, 2021 AND MAY 17-21, 2021

Slides were adapted from a literature presentation by Junyong Kim at

PROTACs on Clinical Trials



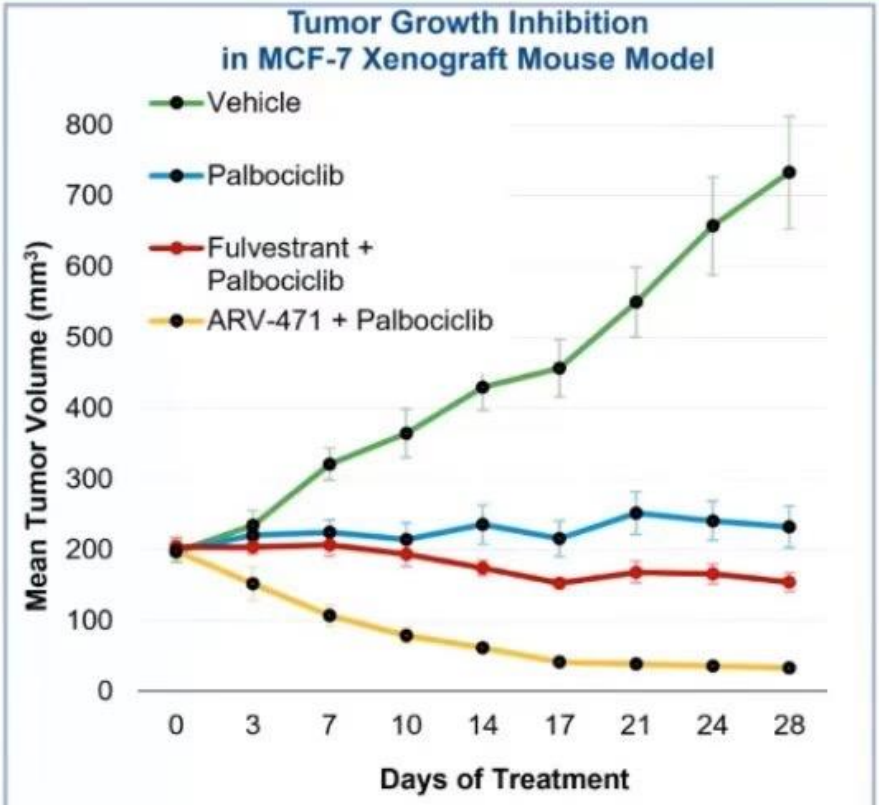
PROTACs on Clinical Trials

In Combination with Palbociclib, ARV-471 Exhibits Superior Tumor Shrinkage Versus Fulvestrant

AACR American Association for Cancer Research
FINDING CURES TOGETHER

ARV-471 *In Vivo* Preclinical Development

- Achieved significant tumor shrinkage in combination with palbociclib (131% TGI)
  - In all 10 mice in experiment, tumors reduced by >80%
- Superior tumor shrinkage (in combination with palbociclib) compared to fulvestrant (108% TGI)



-Palbociclib arm: 60 mpk po qd; 94% TGI.
-Fulvestrant + Palbociclib arm: Fulvestrant 200 mpk sc biwx 2, qwx 3 + palbociclib 60 mpk po qd; 108% TGI
-ARV-471 + Palbociclib arm: ARV-471 30 mpk po qd + palbociclib 60 mpk po qd; 131% TGI