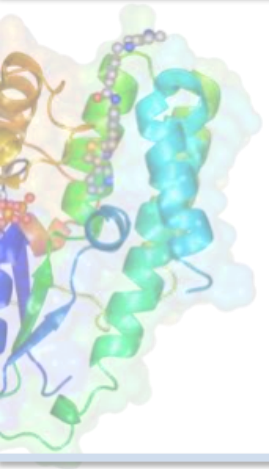
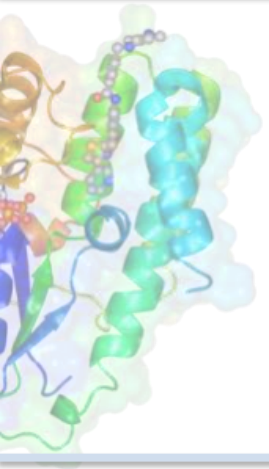


From a Drug Repositioning to a Fragment-Based Drug Design Approaches to Tackle Acute Lymphoblastic Leukemia (T-ALL)

Thomas Miller, PhD

Cancer Research Center of Marseille (CRCM) & Institut Paoli-Calmettes



From a Drug Repositioning to a Fragment-Based Drug Design Approaches to Tackle Acute Lymphoblastic Leukemia (T-ALL)

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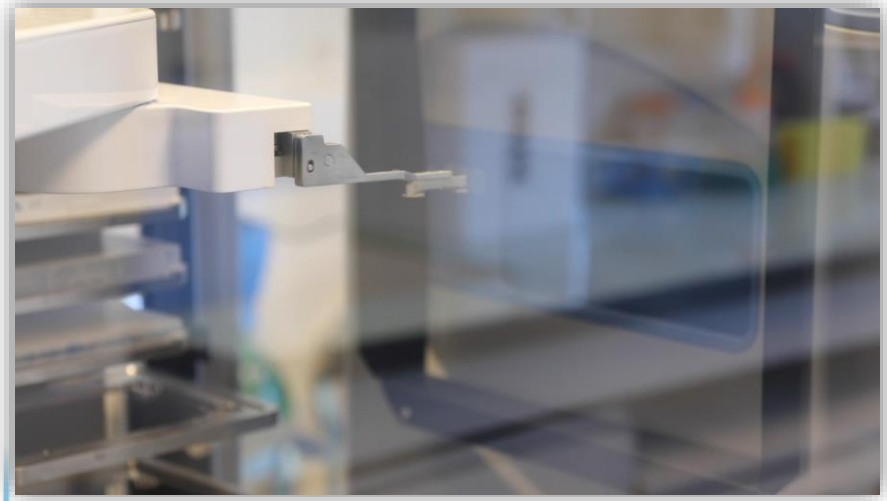
Introductions

Current : iSCB team/HiTS platform Marseille

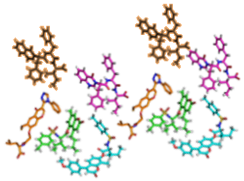
Former: ADST team, NCATS, NIH Bethesda MD USA



Strategies in Drug Discovery

Hit Identification

Chemical Library Design

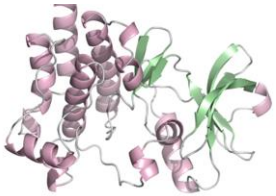


Oriented Libraries

- Machine Learning
- SVM/RF

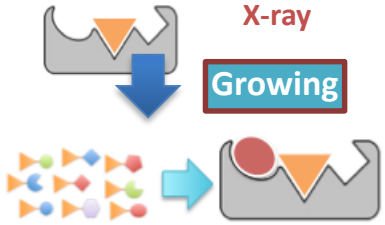
H2L Optimization – FBDD/LBDD

Virtual Screening



- Molecular Dynamics
- Docking
- Pharmacophore filtering

Fragment Screening



- Diverse Focused Database
- Conformational Sampling

❶ **"Fr-PPIChem" *:**
A PPI-Oriented Chemical library

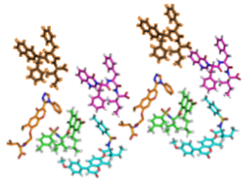
❷ **"DOTS" *:**
An integrated strategy To accelerate the Hit-to-Lead Process

*Open Science Programs

Strategies in Drug Discovery

Hit Identification

Chemical Library Design

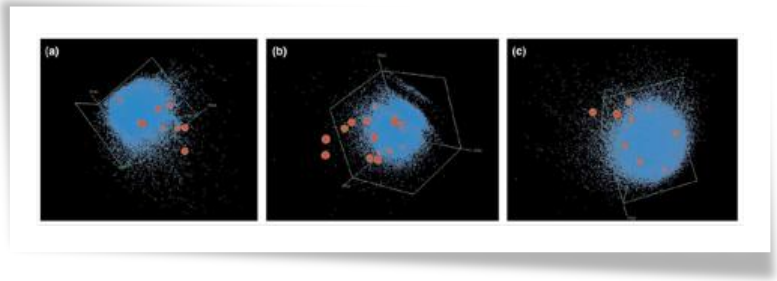


PPI-oriented Libraries

- Machine Learning
- SVM/Random Forest

First Step

Learn from
known inhibitors
to identify
new inhibitors



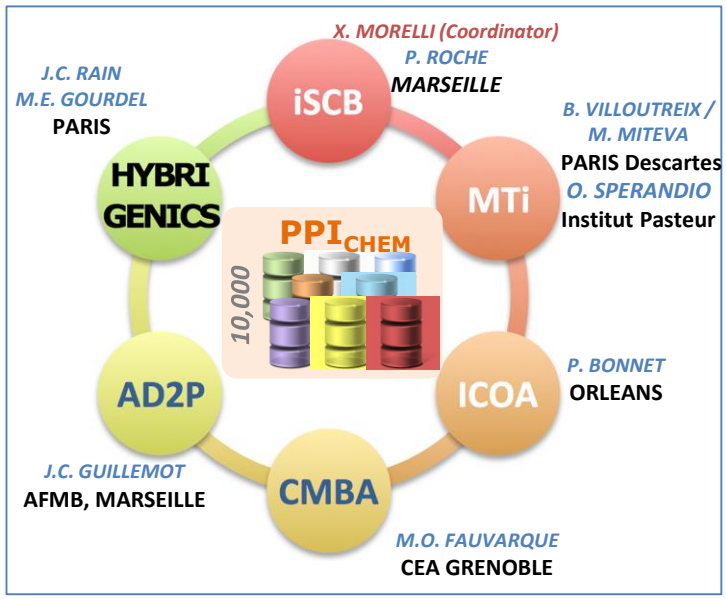
"Fr-PPIChem" : a French National Initiative

PPI CHEM
ANR
10,000 cpds

New Larger Version of 2P2I 3D

v2.0 (MedChem) using updated algorithms, **ADMET Filters**

➢ *National Program (ANR)* => **10.314 Compounds PPI-Library**



AMBINTER MolPort

Task 1
In silico Compounds Selection

Task 2
Acquisition / Plating / Distribution

Task 3
Cytotoxicity Evaluation

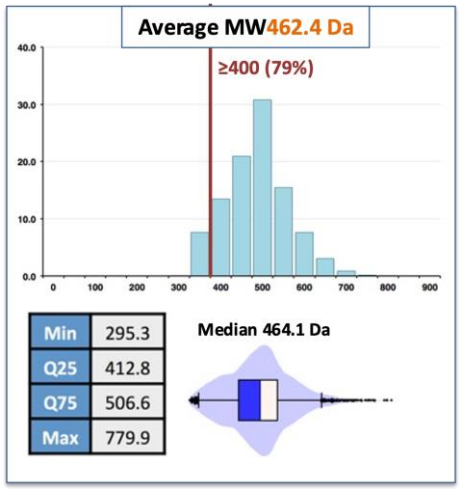
Task 4
Evaluation on selected PPIs

Made available to academic laboratories (MTA)

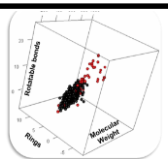
ChemBioScreen
Des petites molécules pour explorer le Vivant
GDR CNRS 3735

Bosc et al ACS Chem Biol. 2020 15(6): 1566–1574

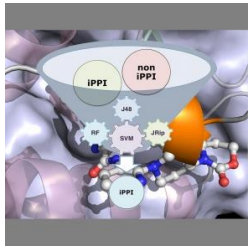
"Fr-PPIChem" : a French National Initiative



Machine Learning



PPI-focused Chemical Libraries



Fr-PPI Chem
10,314 cpds

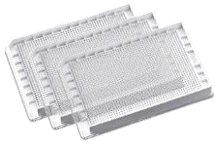
No IP

Requests

ChemBioFrance

Xavier Morelli (xavier.morelli@inserm.fr)
Philippe Roche (philippe.roche@inserm.fr)
Caroline Barette(caroline.barette@cea.fr)

https://chembiofrance.cn.cnrs.fr/fr/composante/chimiotheque#prestation-0-5



33 Plates (3 µl @10mM per well)

>40 sets of plates distributed (4 USA)

>20 Targets screened @ iSCB

Bosc et al ACS Chem Biol. 2020 15(6): 1566–1574

Strategies in Drug Discovery

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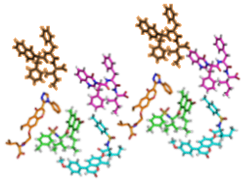

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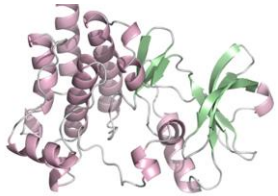

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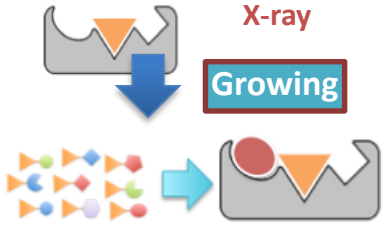
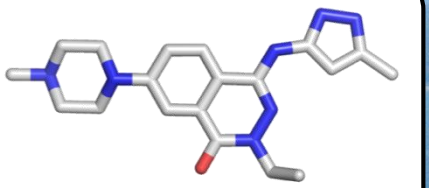


- Diverse Focused Database
- Conformational Sampling

❶ "Fr-PPIChem": PPI-Oriented Chemical library

❷ "DOTS": An integrated strategy To accelerate the Hit-to-Lead Process

Drug Chemical Space is nearly infinite…



MW < 500 Da

C H O N S

Commercial Compounds

Number of compounds that could be synthesized ?

$\approx 10^{63}$

Dune du Pilat (sand grains)

$\approx 10^{20}$

30.10^{6}

0.00000000000000000000000000000000000000000000003% of possible synthetic compounds

Finding a new drug is an almost unattainable challenge…

"The best way to discover a new drug is to start with an old one…"

Sir James W. Black (Prix Nobel 1988)

"Off-target concept":
The drug acts on new targets, out of the original scope, with a new therapeutic indication

Both the targets & the indications are new.

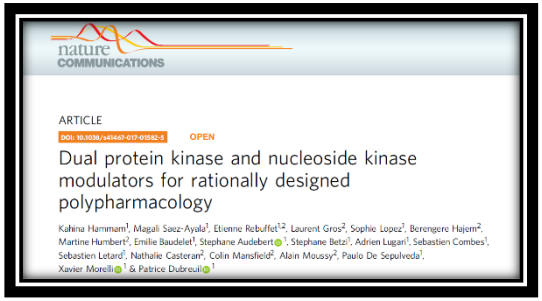
Drug	Original Indication	New Indication
Aspirin	Inflammation, pain	Antiplatelet, Cancer
Bupropion	Depression	Smoking cessation
Disulfiram	Alcoholism	Melanoma
Doxepin	Depressive disorder	Antipruritic
Gemcitabine	Antiviral	Cancer
Methotrexate	Cancer	Psoriasis, rheumatoid arthritis
Minoxidil	Hypertension	Hair loss
Sildenafil	Angina	Erectile dysfunction (Viagra®)
Thalidomide	Morning sickness	Leprosy
Tretinoin	Acne	Leukemia

Hopkins, The druggable genome, Nat Rev Drug Discov, 2002

Drug Repurposing in Cancer: Turning Liabilities into Opportunities…

Masitinib (Masivet®)

Patrice Dubreuil & AB Science


nature COMMUNICATIONS

ARTICLE

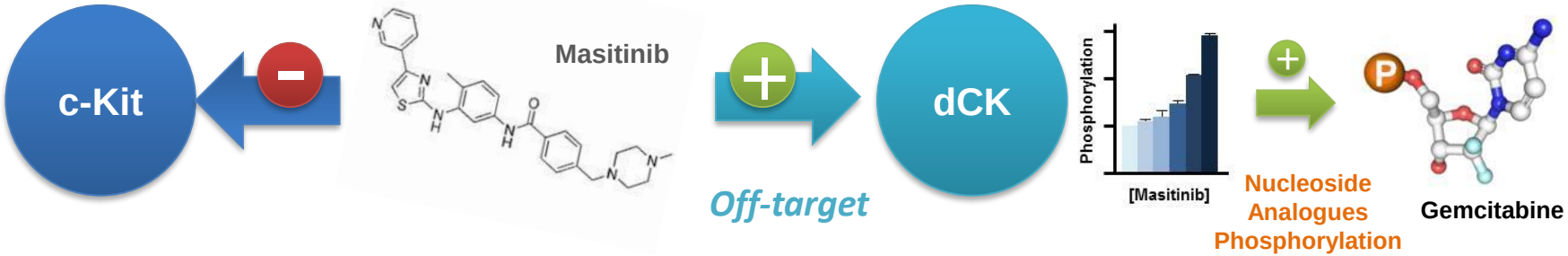
DOI: 10.1038/s41467-017-01582-5 OPEN

Dual protein kinase and nucleoside kinase modulators for rationally designed polypharmacology

Kahina Hammam[1], Magali Saez-Ayala[1], Etienne Rebuffet[1,2], Laurent Gros[2], Sophie Lopez[1], Berengere Hajem[2], Martine Humbert[2], Emilie Baudelet[1], Stephane Audebert[1], Stephane Betzi[1], Adrien Lugari[1], Sebastien Combes[1], Sebastien Letard[3], Nathalie Casteran[3], Colin Mansfield[3], Alain Moussy[2], Paulo De Sepulveda[1], Xavier Morelli[1] & Patrice Dubreuil[1]

Fondation pour la recherche sur le cancer

Nucleoside Kinase Activator



Oral Targeted Therapy (TKI)	Mastocytosis
Neurological diseases	Gastrointestinal stromal tumors (GIST)

Therapeutic benefit: Sensitize patients to chemotherapy	Reducing toxicity of therapeutic agents
Maintaining effectiveness at lower doses	Counteracting drug resistance initiated *via* down modulation

Hamman et al, Nat Commun, 2017

dCK *vs*. Cancer …

dCK is a key enzyme in the Nucleoside Salvage Pathway (SP)…

dCK *vs*. Cancer …

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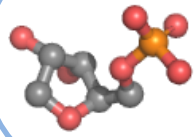
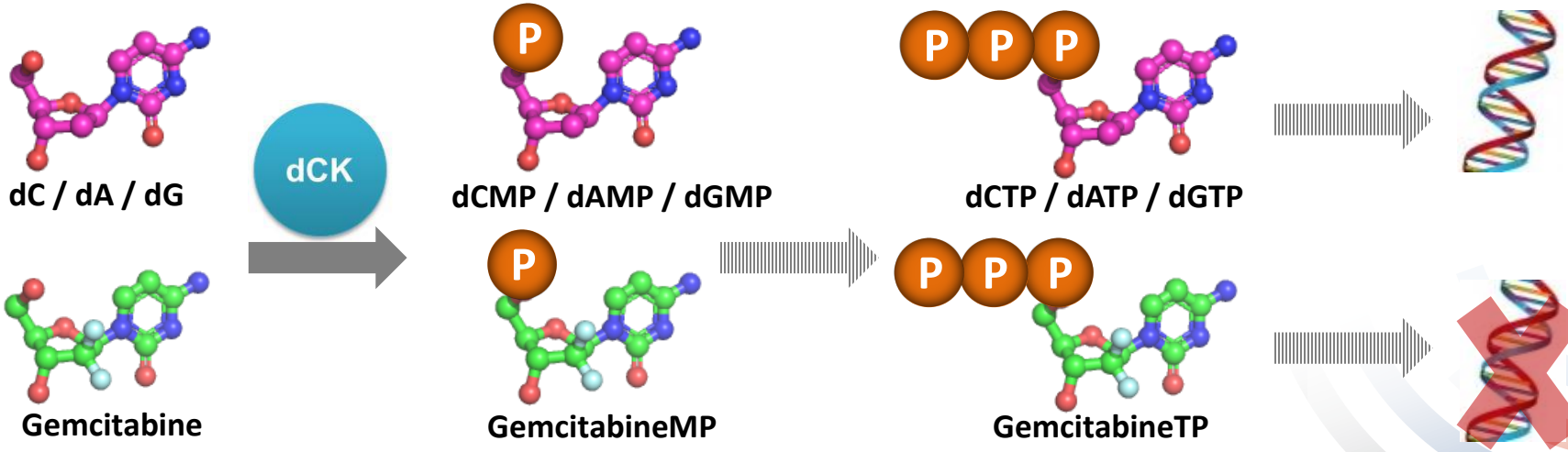


… wich catalyzes the phosphorylation of physiologic & nucleoside analogue drugs (but dT)

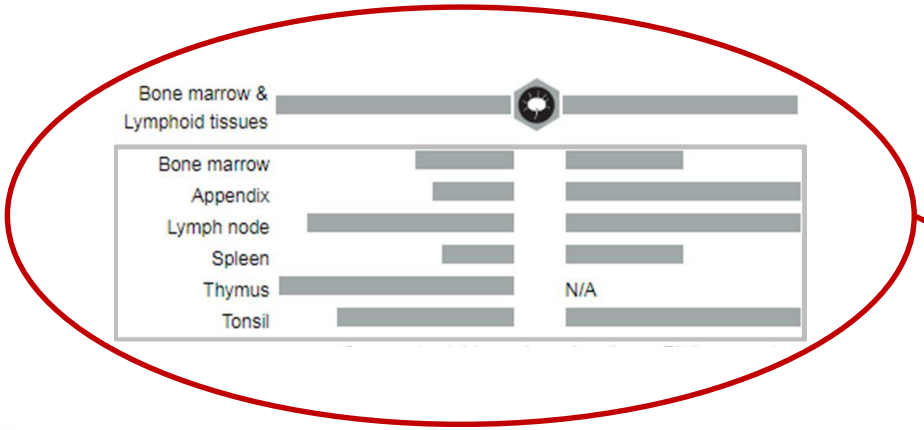
…. which recycles bases and nucleosides originating from the degradation of RNA and DNA to achieve the biosynthesis of deoxyribonucleotides, required for DNA replication and repair

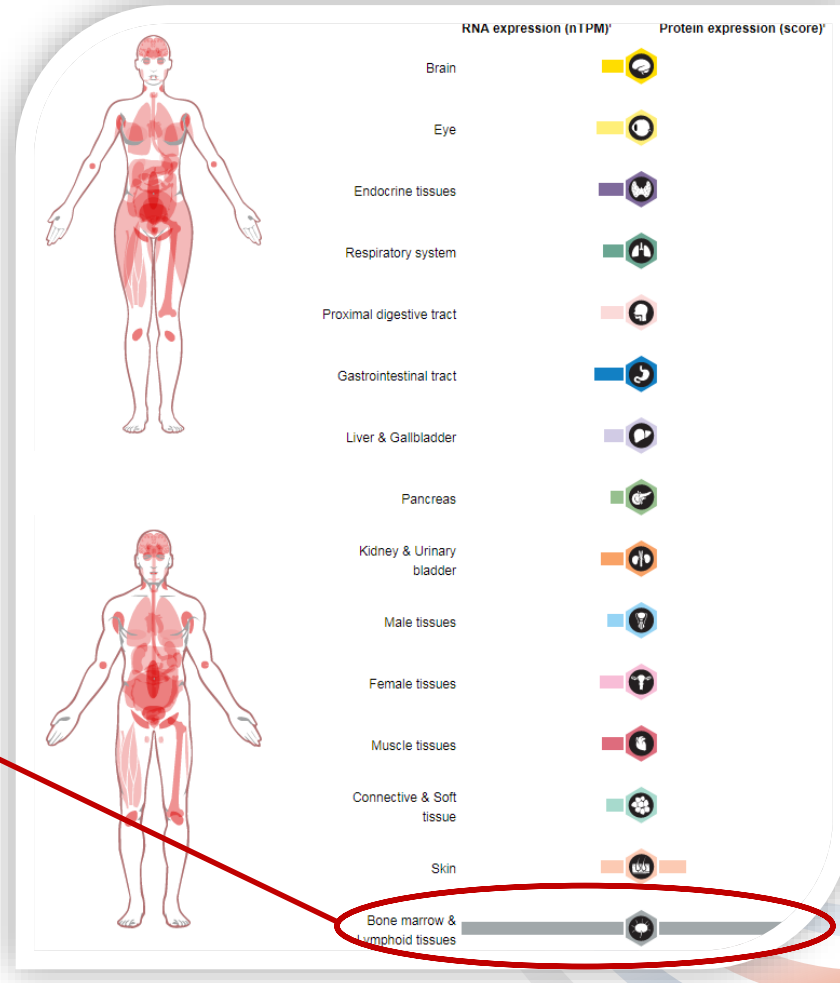


+ numerous anticancer and antiviral nucleoside analogues

dCK *vs*. Cancer …

Highly expressed in bone marrow and lymphoid tissues

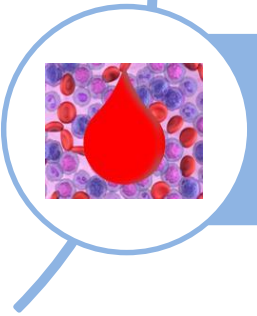




Bone marrow & lymphoid tissues

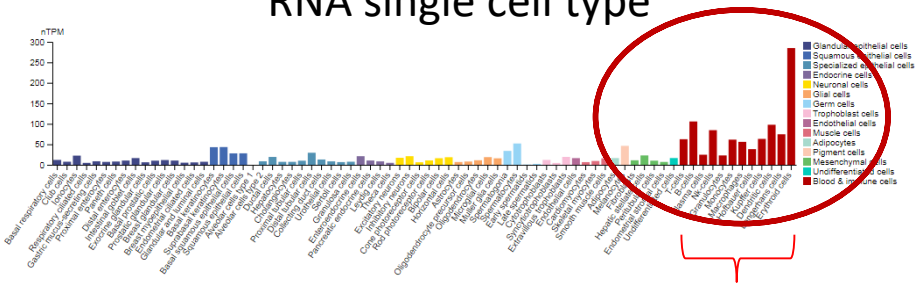
dCK *vs*. Cancer …

Highly expressed in bone marrow and lymphoid tissues



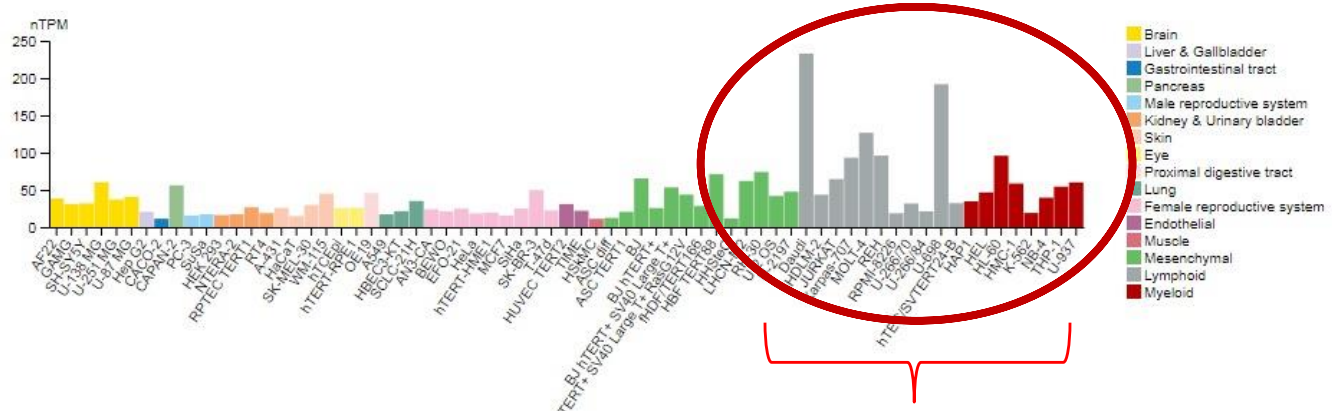
Highly expressed in lymphoid and myeloid cancer cell lines

RNA single cell type



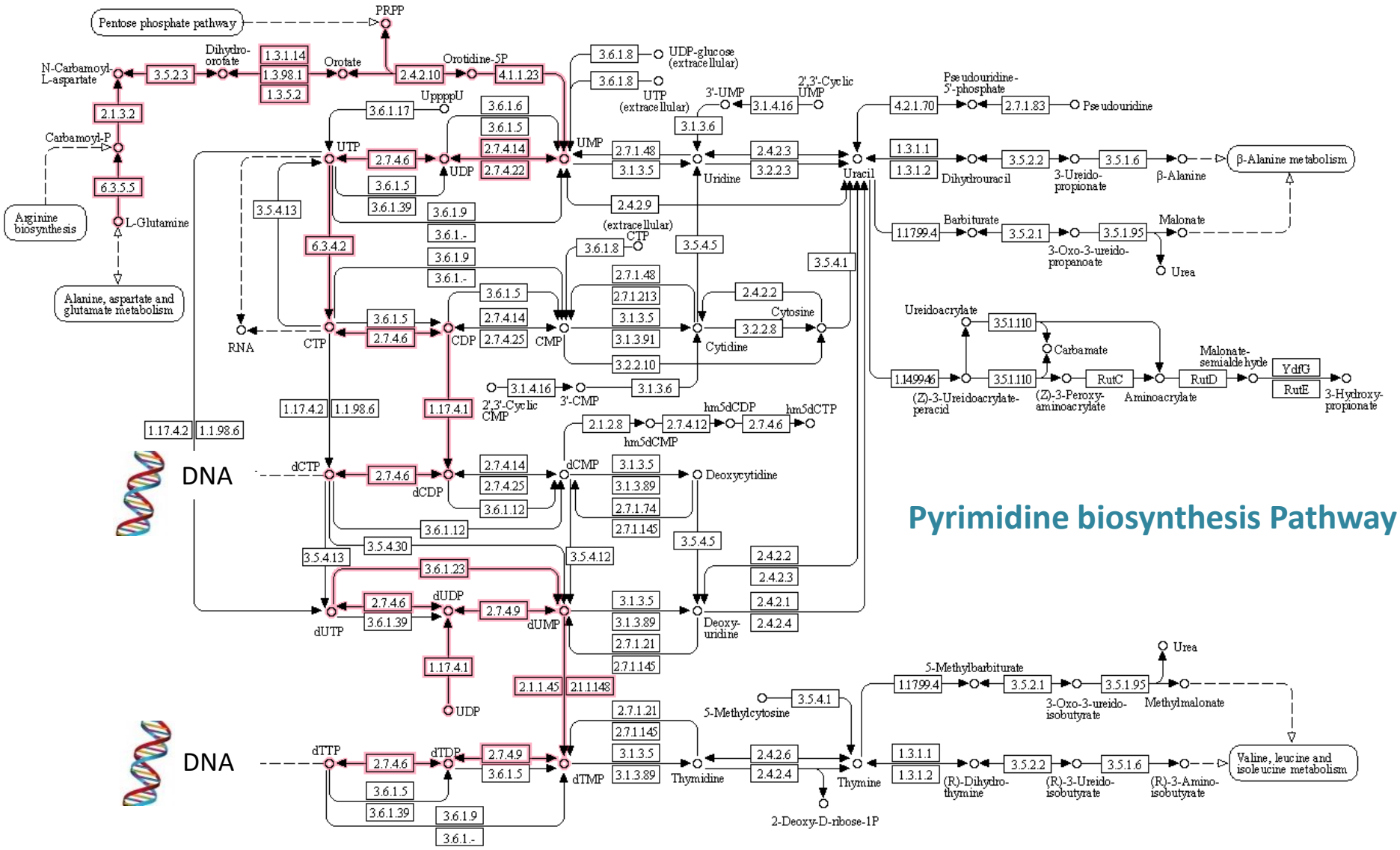
Blood & Immune cells

Cancer Cell lines



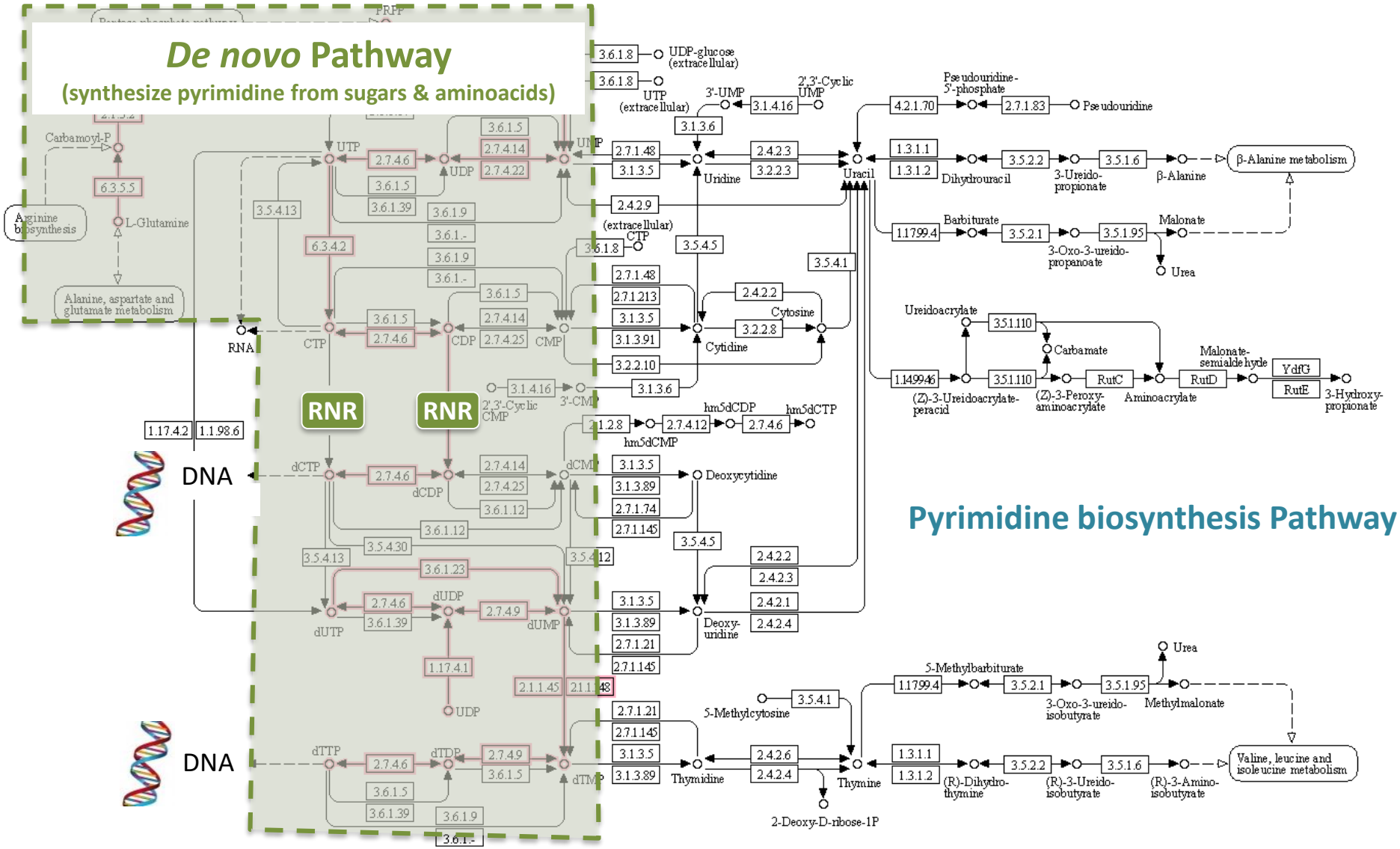
Lymphoid & Myeloid cell lines

dCK *vs.* Deoxyribonucleotides biosynthesis Pathways…

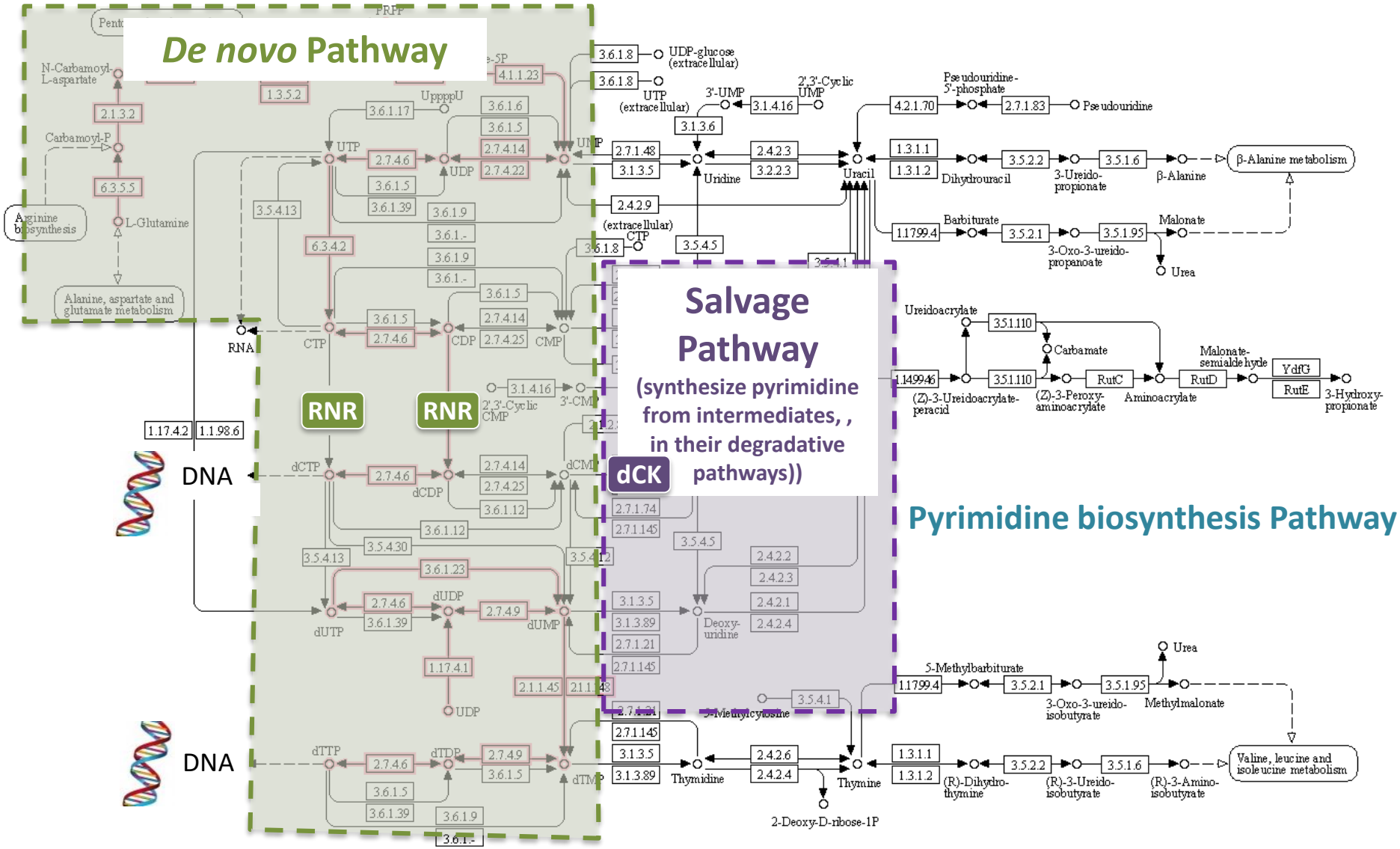


Pyrimidine biosynthesis Pathway

dCK *vs. De novo* & Salvage Pathways…



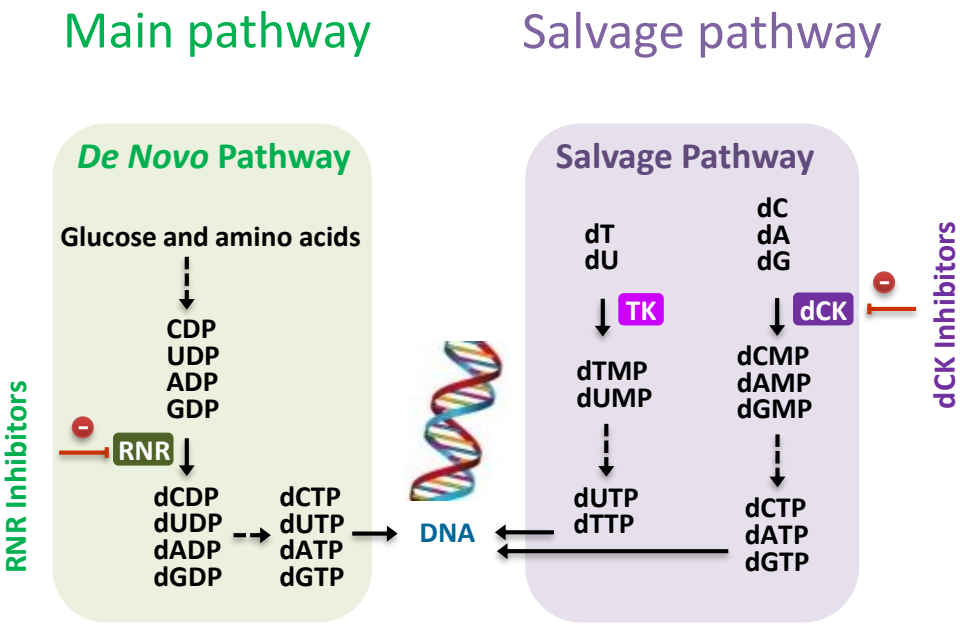
dCK *vs. De novo* & Salvage Pathways…



KEGG: Kyoto Encyclopedia of Genes and Genomes

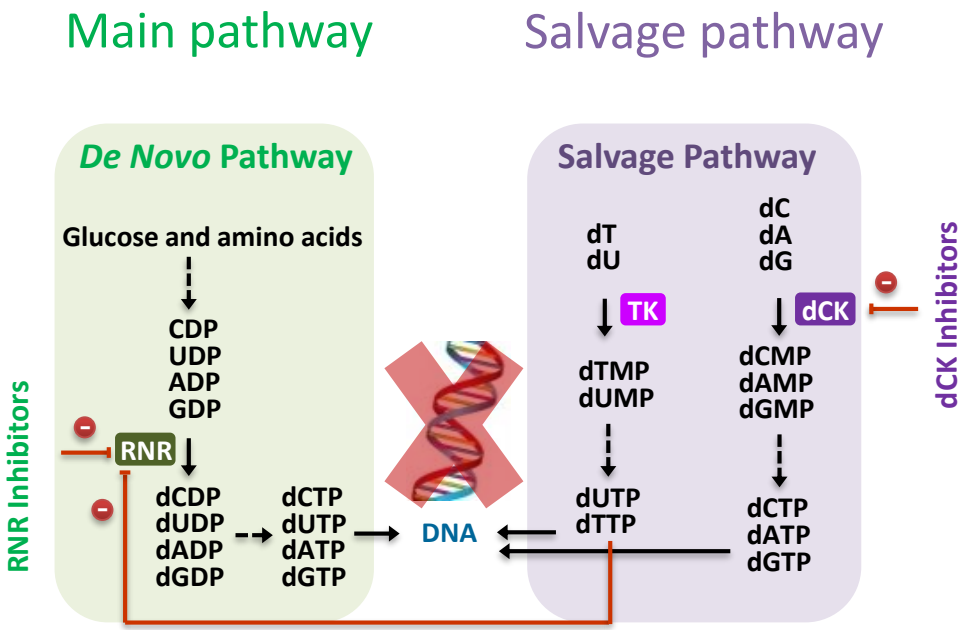
dCK *vs. De novo* & Salvage Pathways…

Inhibition of one pathway alone do not stop DNA synthesis



dCK *vs. De novo* & Salvage Pathways…

Inhibition of both pathways stops DNA synthesis

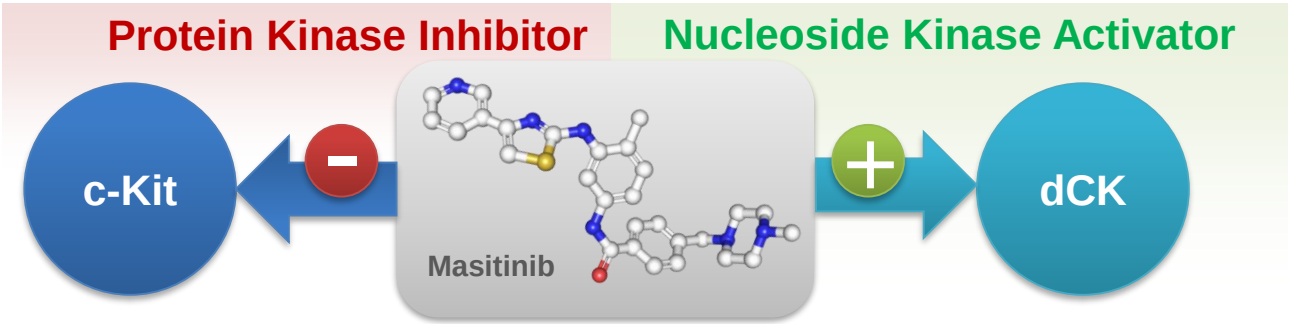


Concept of Synthetic lethality (DNP + SP)

Nathanson et al, J Exp Med, 2014

dCK *vs. De novo* & Salvage Pathways…

Hypothesis: Repositioning of masitinib to develop an inhibitor of dCK ?
Increase affinity to become an inhibitor ?



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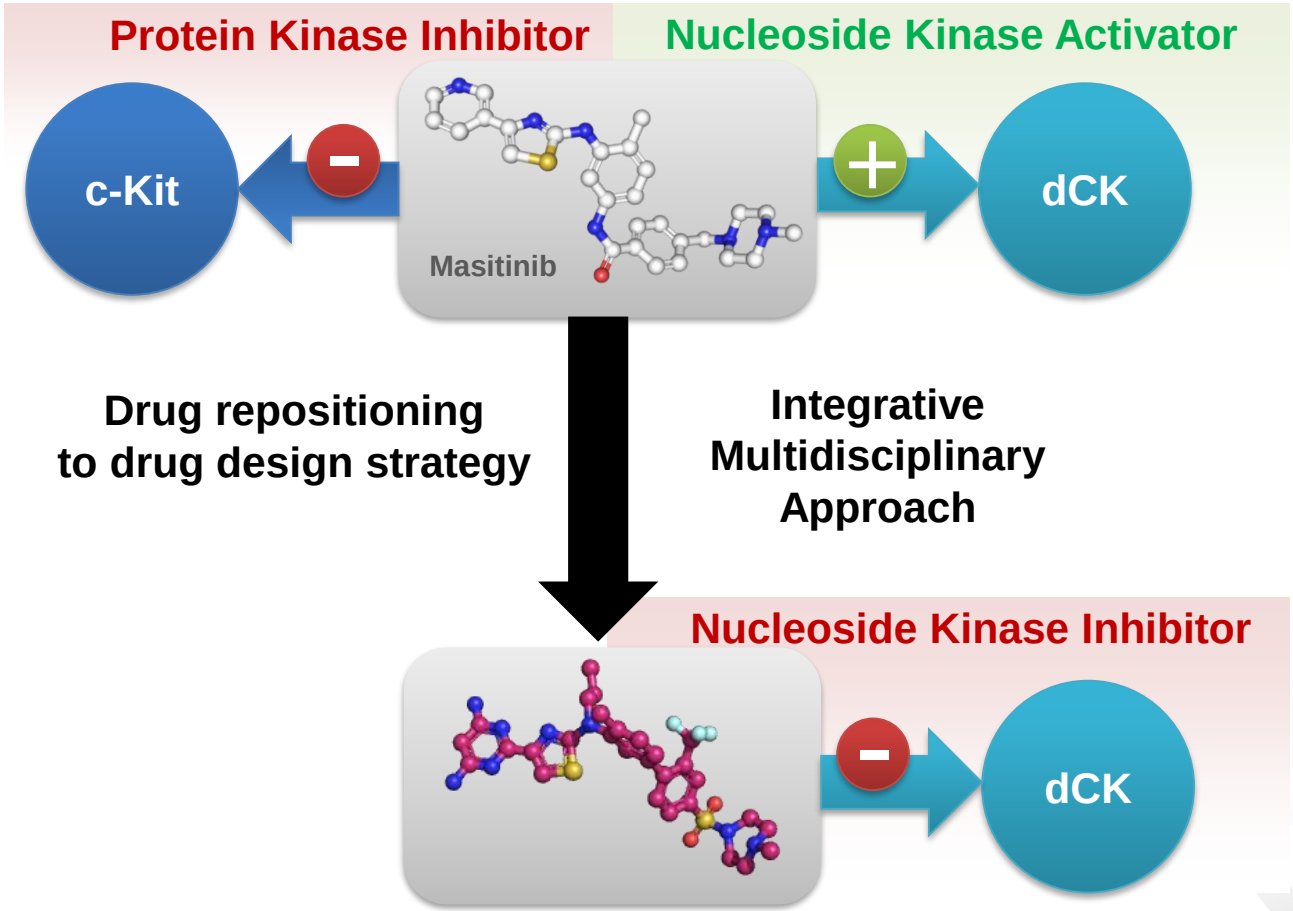
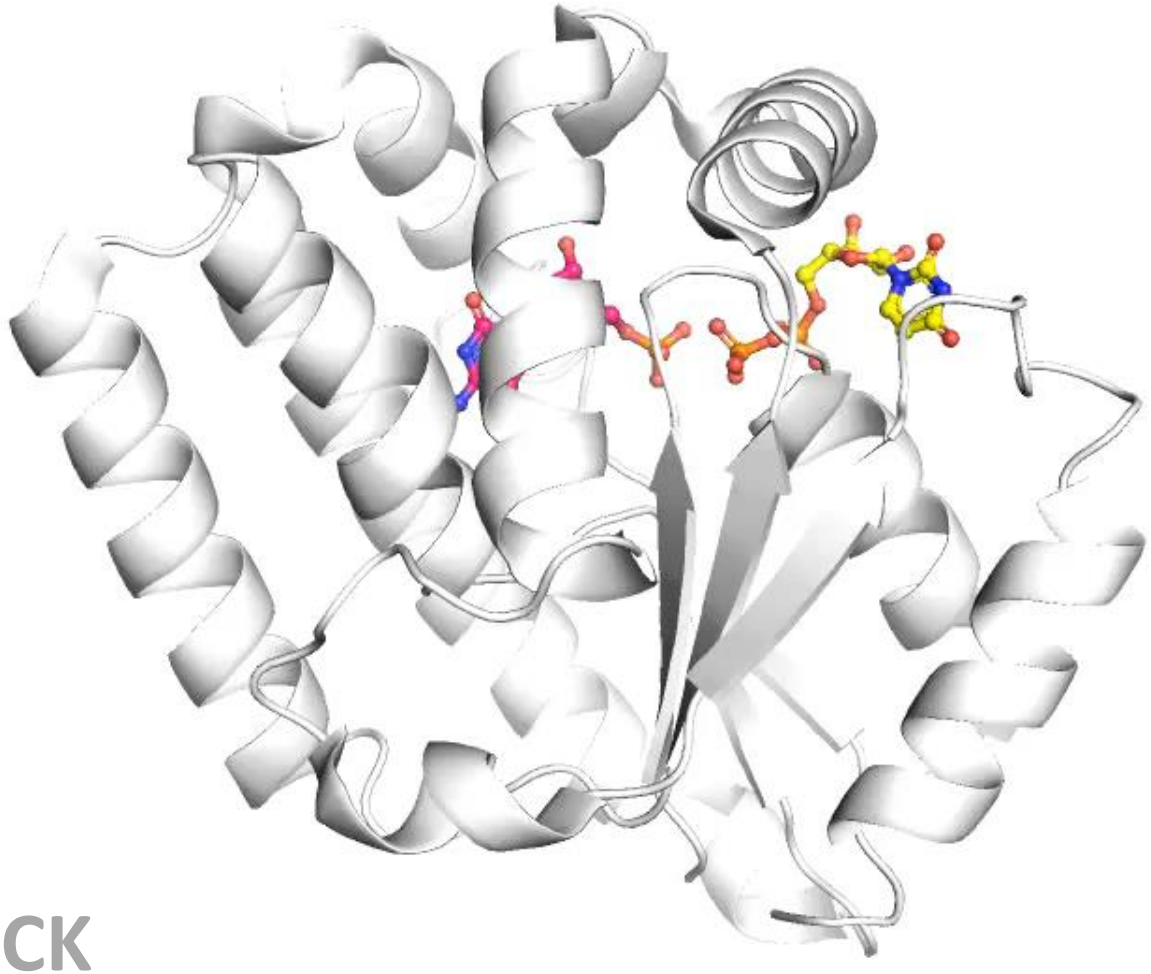


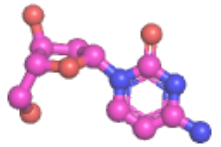
Turning an Activator into Inhibitor

Masitinib interacts with dCK and links to a pocket that overlaps with the active site

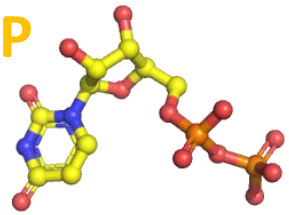


dCK

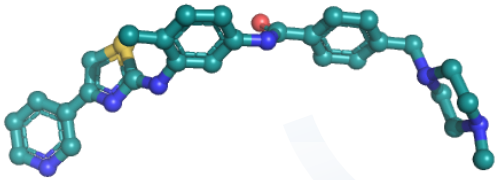
dC



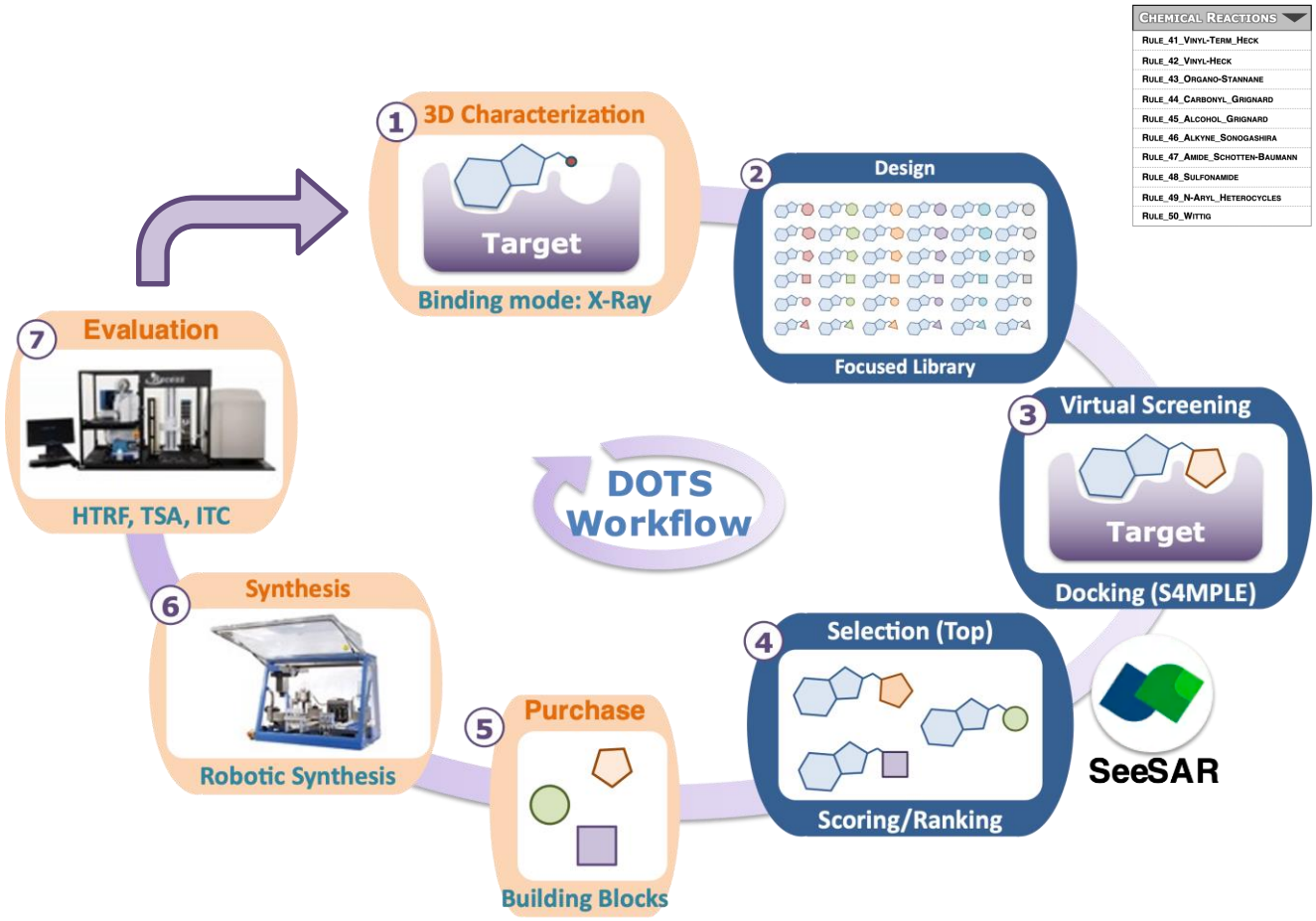
UDP



Masitinib



Diversity-Oriented Targeted Synthesis: DOTS



Hoffer *et al* (**2018**) *J Med Chem* **61** 5719-5732; Hoffer et al (2024) Nucleic Acids Res. Apr 30:gkae326.

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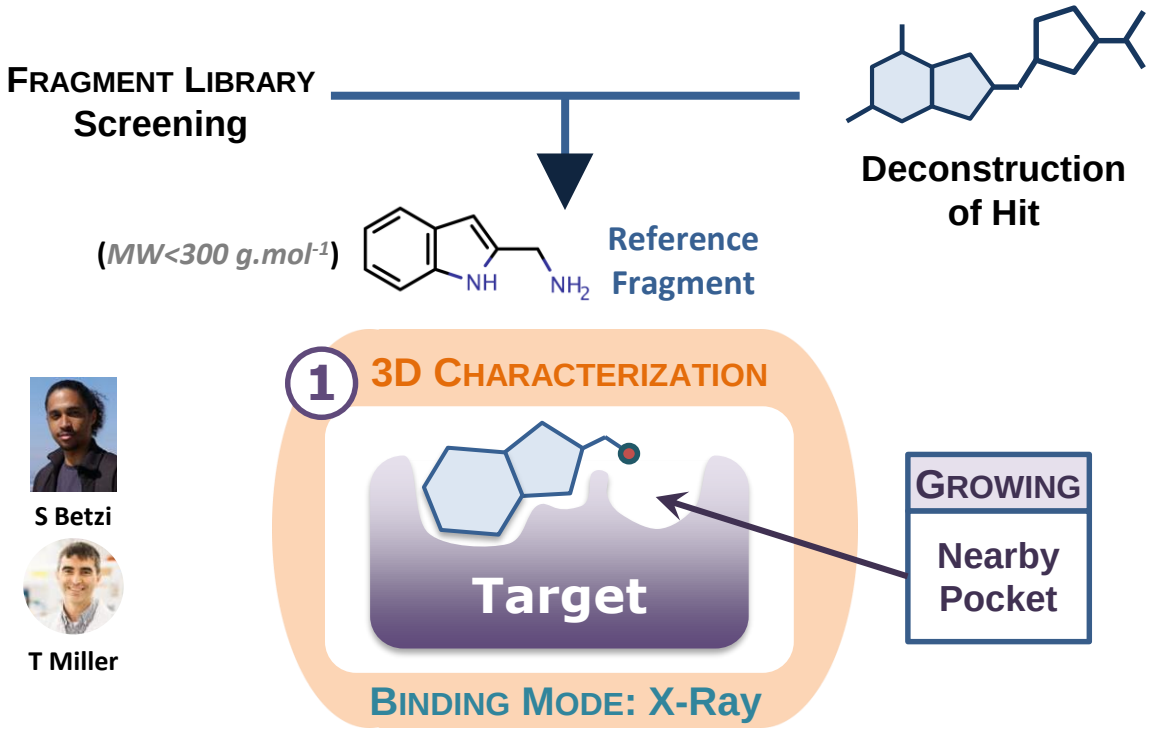

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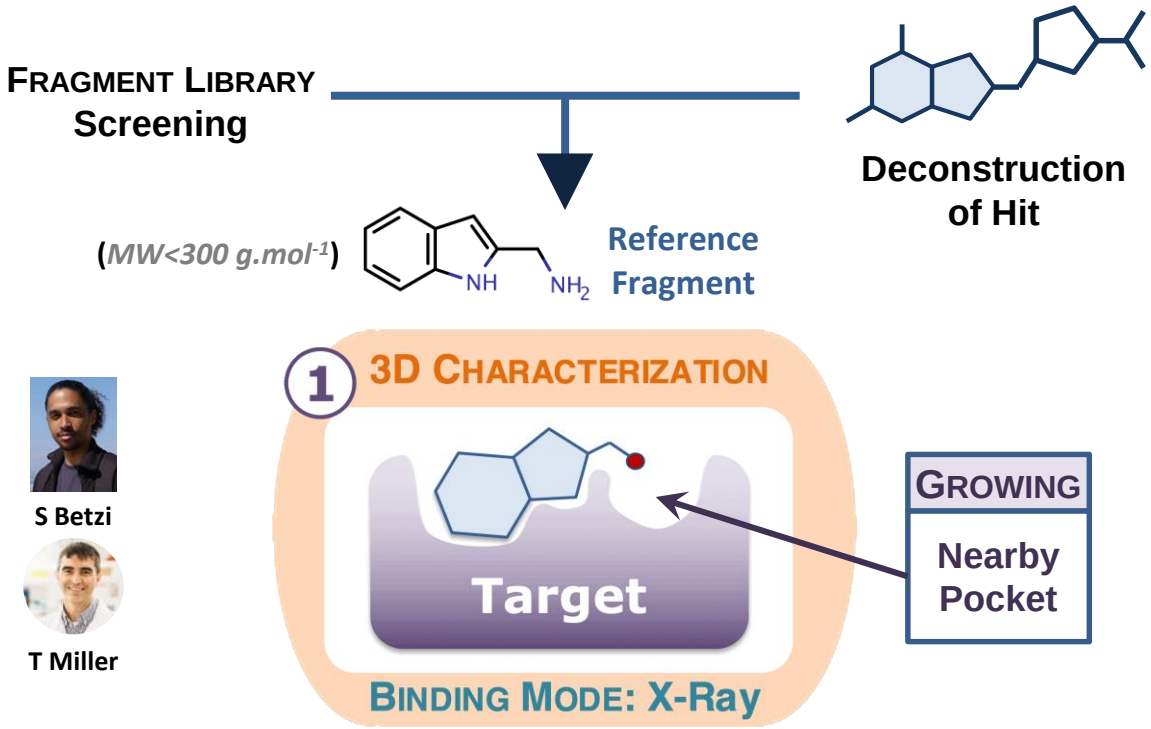

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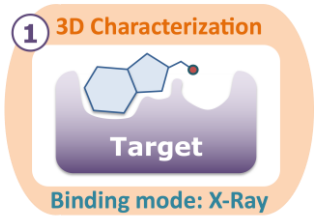
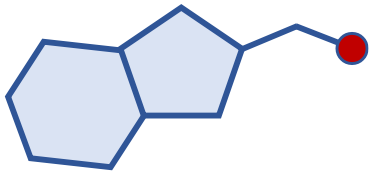
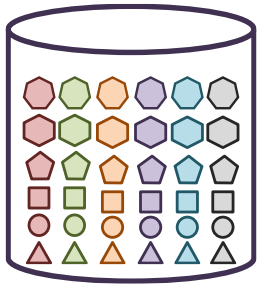


Virtual Chemical Library Design

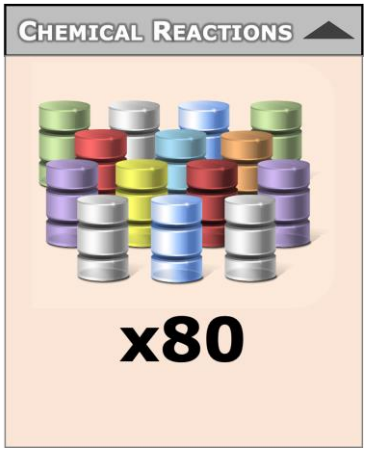
L Hoffer



Reference Fragment

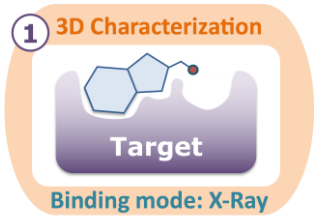


Building Block Library (>6M)



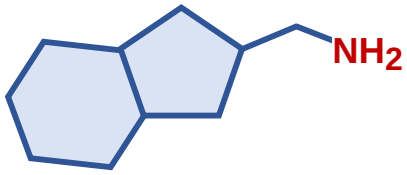
MedChem Chemical Reactions

Diversity-Oriented Targeted Synthesis: DOTS

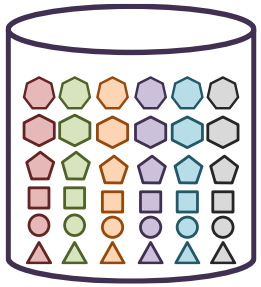


Virtual Chemical Library Design

L Hoffer



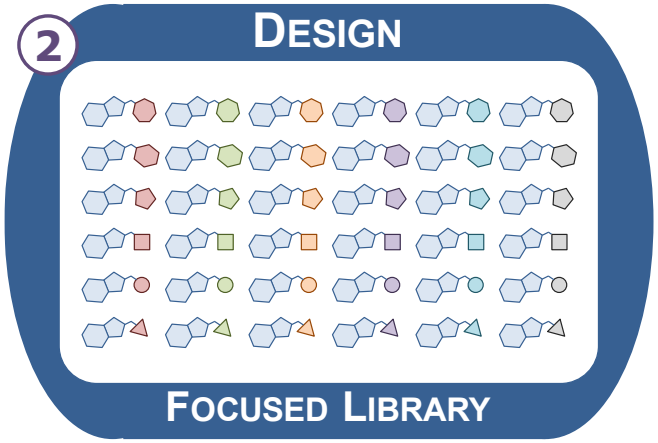
Reference Fragment



Building Block Library (>6M)

Chemical Reactions
Rule_41_Vinyl-Term_Heck
Rule_42_Vinyl-Heck
Rule_43_Organo-Stannane
Rule_44_Carbonyl_Grignard
Rule_45_Alcohol_Grignard
Rule_46_Alkyne_Sonogashira
Rule_47_Amide_Schotten-Baumann
Rule_48_Sulfonamide
Rule_49_N-Aryl_Heterocycles
Rule_50_Wittig

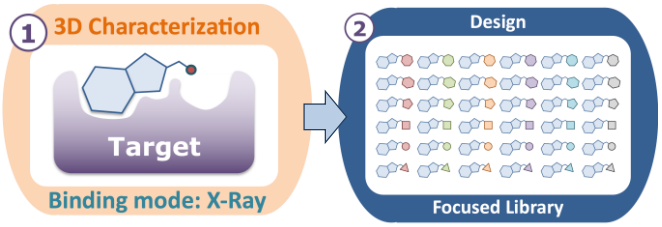
MedChem Chemical Reactions

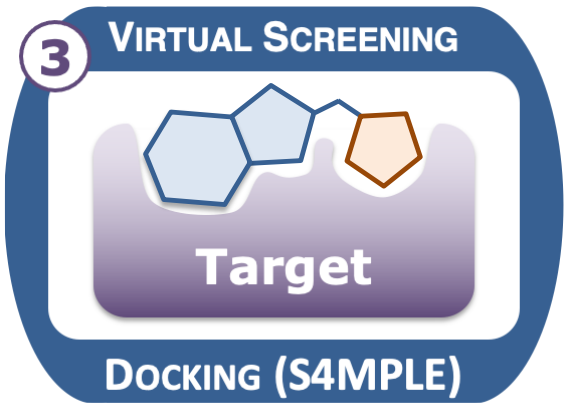


All compounds in the virtual library are amenable to organic synthesis

Hoffer *et al* (**2018**) *J Med Chem* **61** 5719-5732; Hoffer et al (2024) Nucleic Acids Res. Apr 30:gkae326.

Diversity-Oriented Targeted Synthesis: DOTS

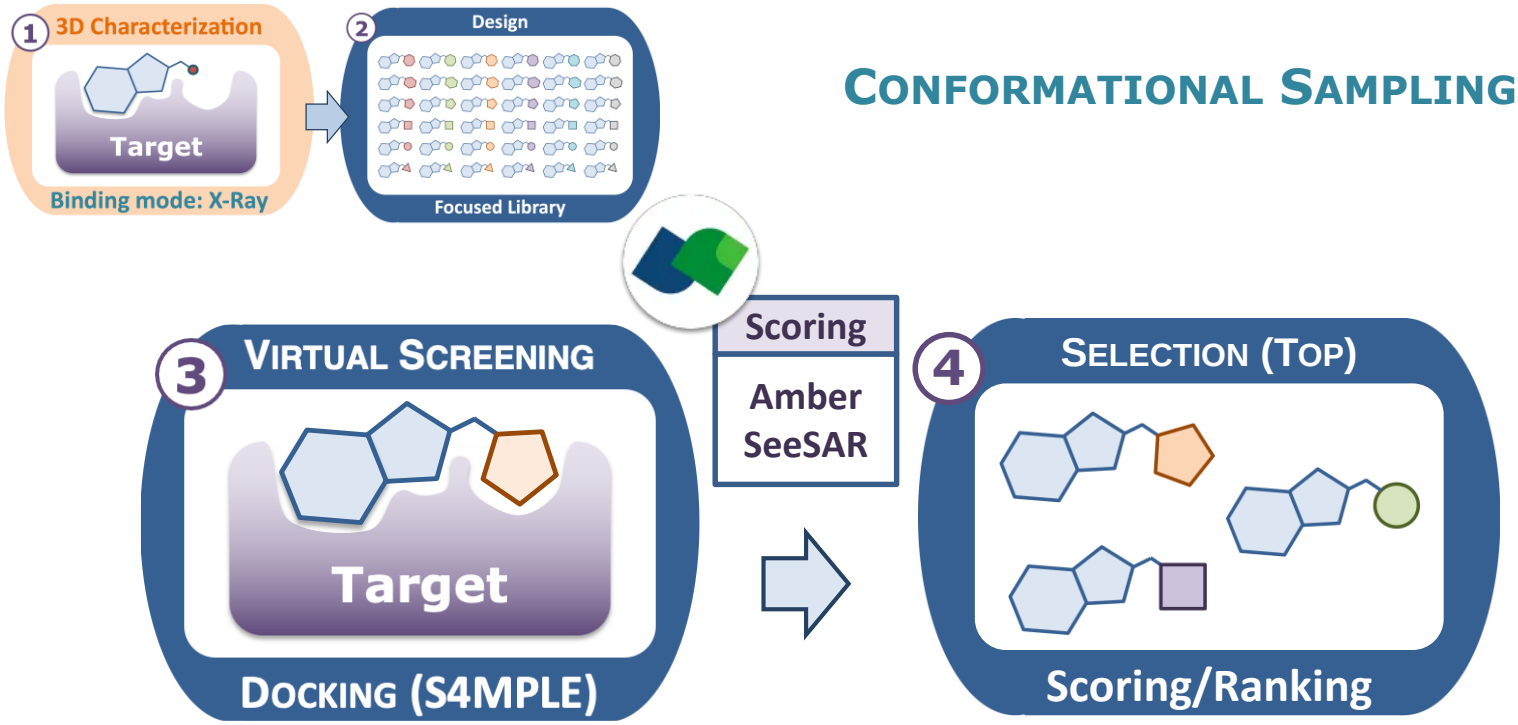




Conformational Sampling

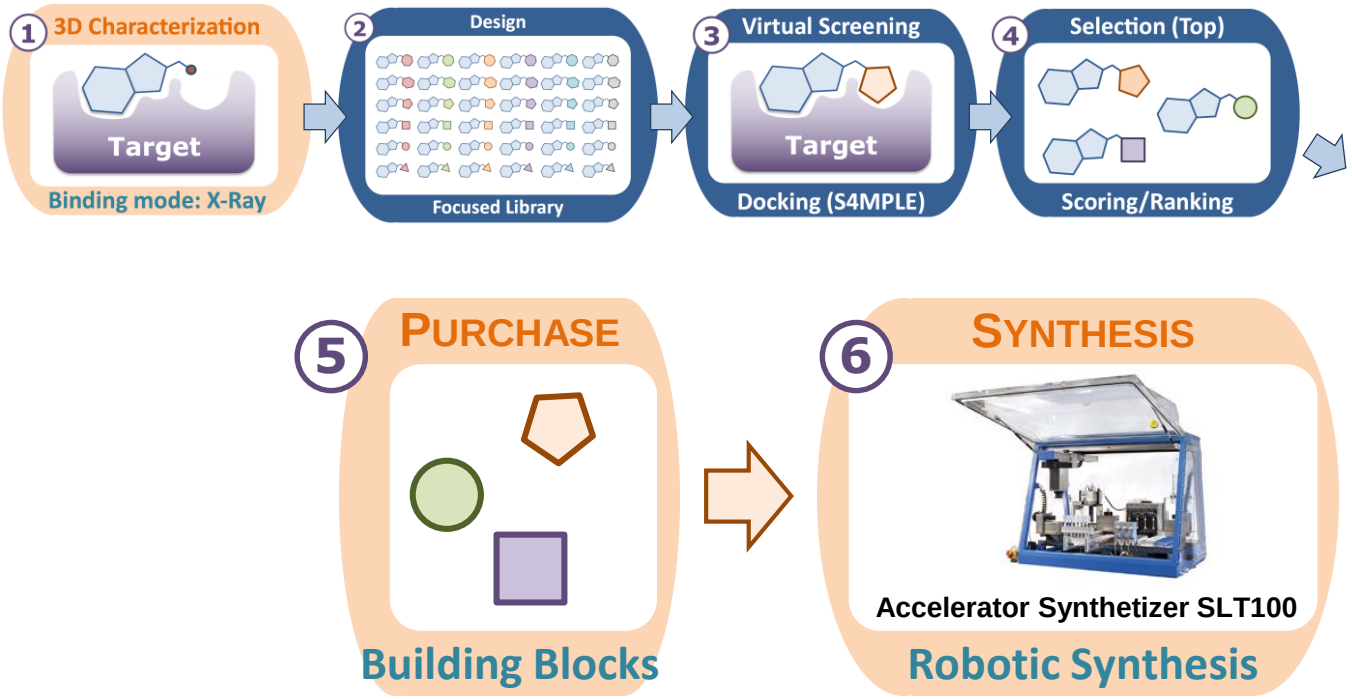
L Hoffer

Diversity-Oriented Targeted Synthesis: DOTS



L Hoffer

Diversity-Oriented Targeted Synthesis: DOTS



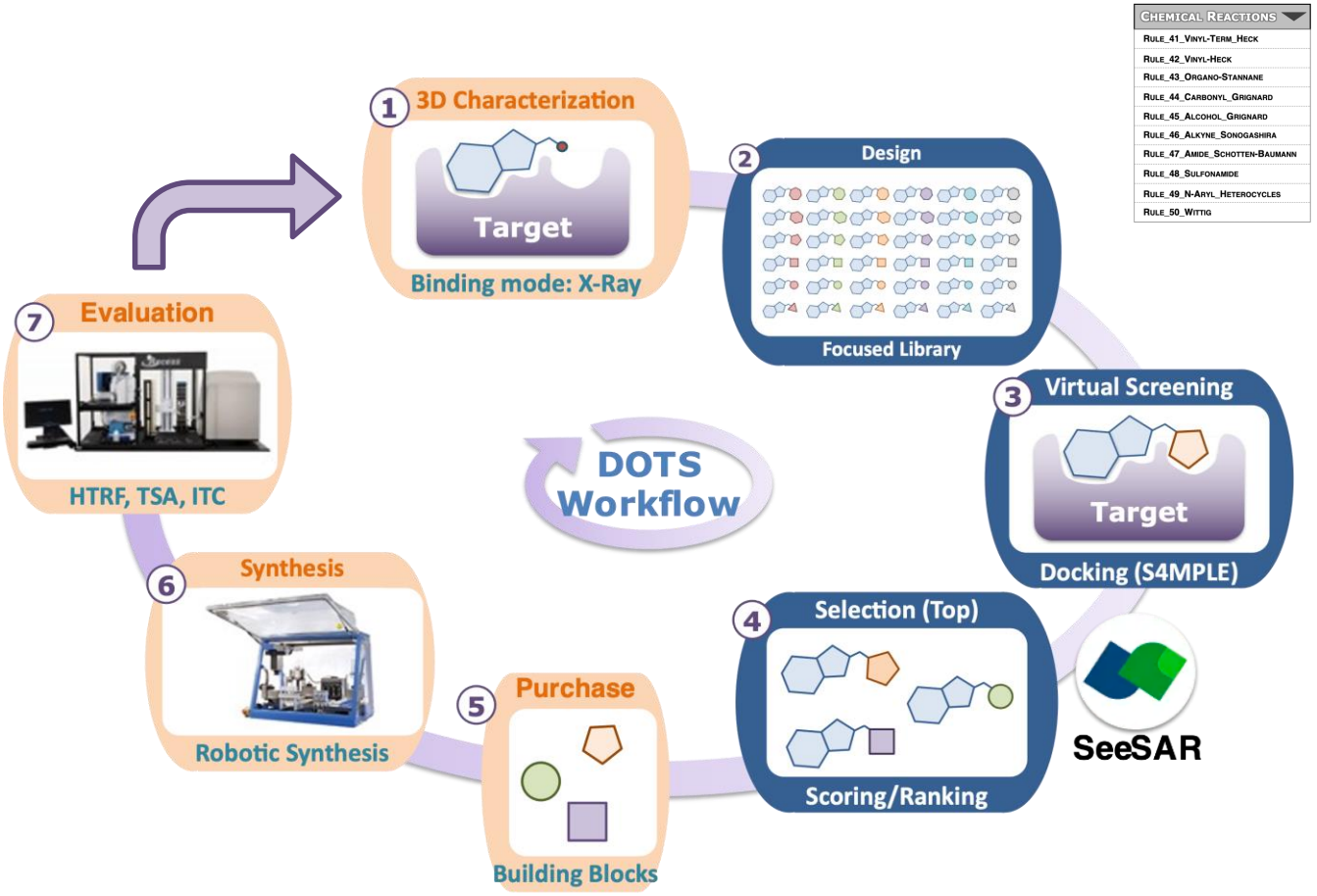

P Bremond S Combes

Chemical Synthesis

Hoffer *et al* (**2018**) *J Med Chem* **61** 5719-5732; Hoffer et al (2024) Nucleic Acids Res. Apr 30:gkae326.

Diversity-Oriented Targeted Synthesis: DOTS

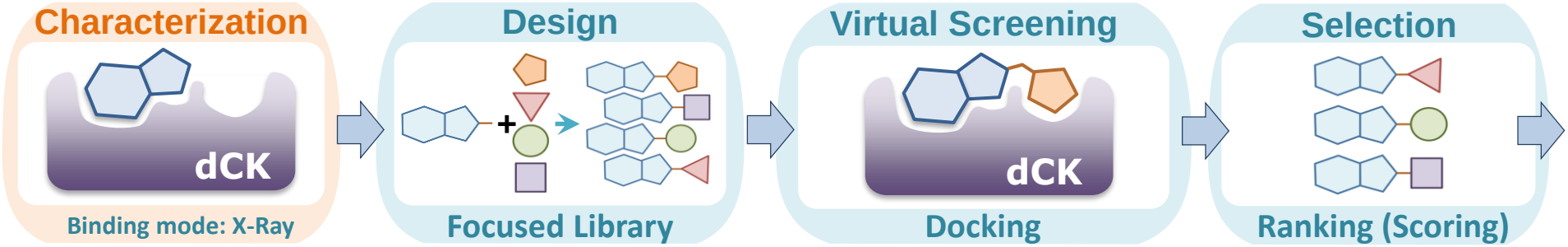
Diversity-Oriented Targeted Synthesis: DOTS



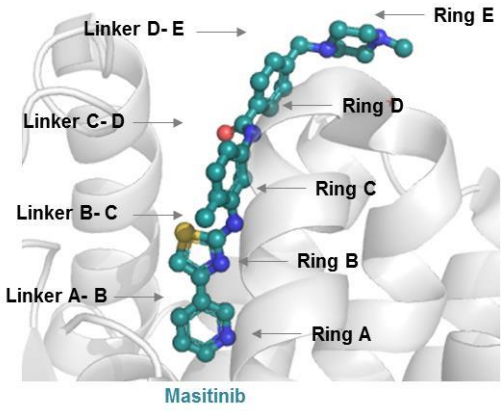
The ChemoDOTS (web server generating the virtual librairies) will be available Q3 2023 / Q1 2024

Hoffer *et al* (**2018**) *J Med Chem* **61** 5719-5732; Hoffer et al (2024) Nucleic Acids Res. Apr 30:gkae326.

Turning an Activator into Inhibitor

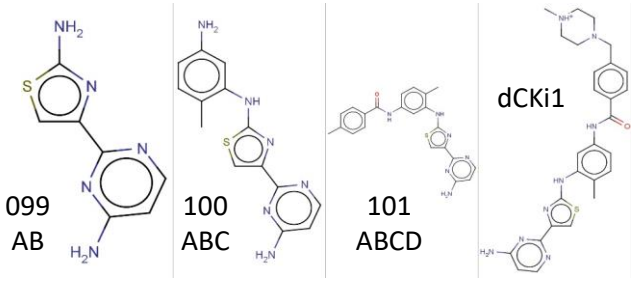


Structure modifications



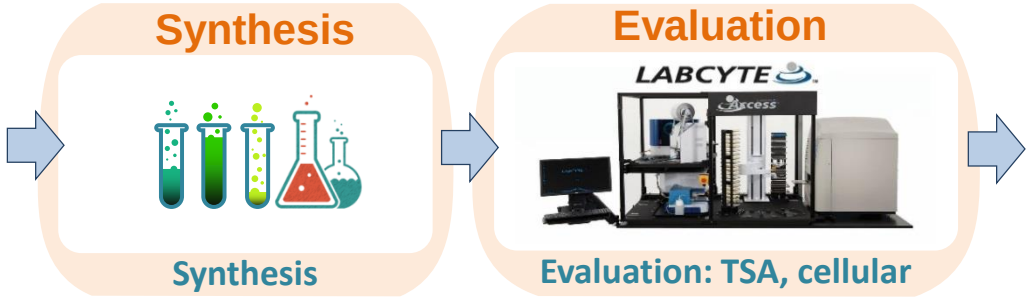
>500
Designed compounds

Scoring
Amber
SeeSAR

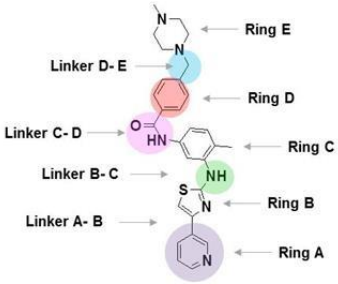


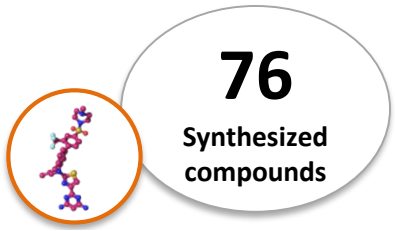
Name	Src	Estimated affinity	LLE	LE	Tor.	Intra	Inter	MW	LogP
099								193.23	0.76
100								298.37	2.82
101								416.51	4.80
0_dcki_1								515.66	2.82

Turning an Activator into Inhibitor

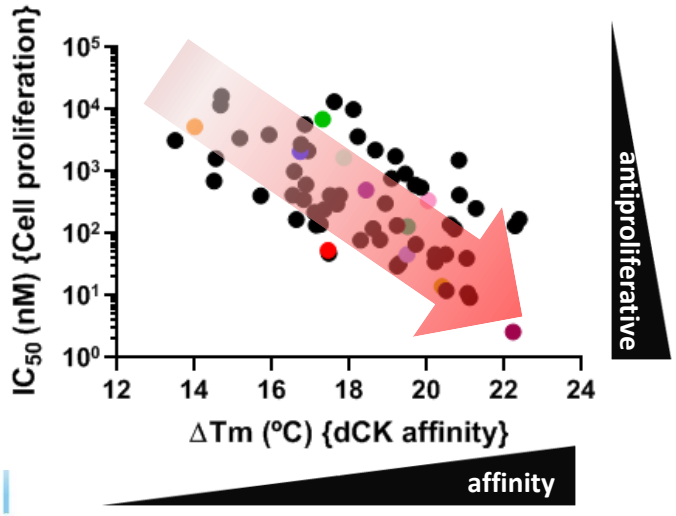


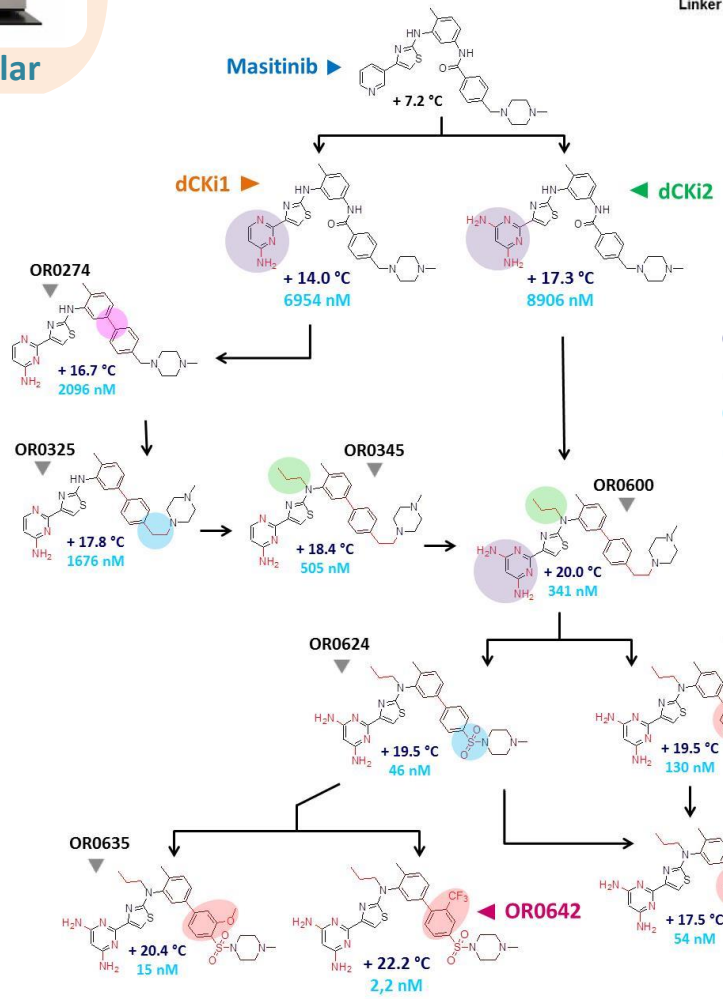
Milestone modifications





76
Synthesized compounds





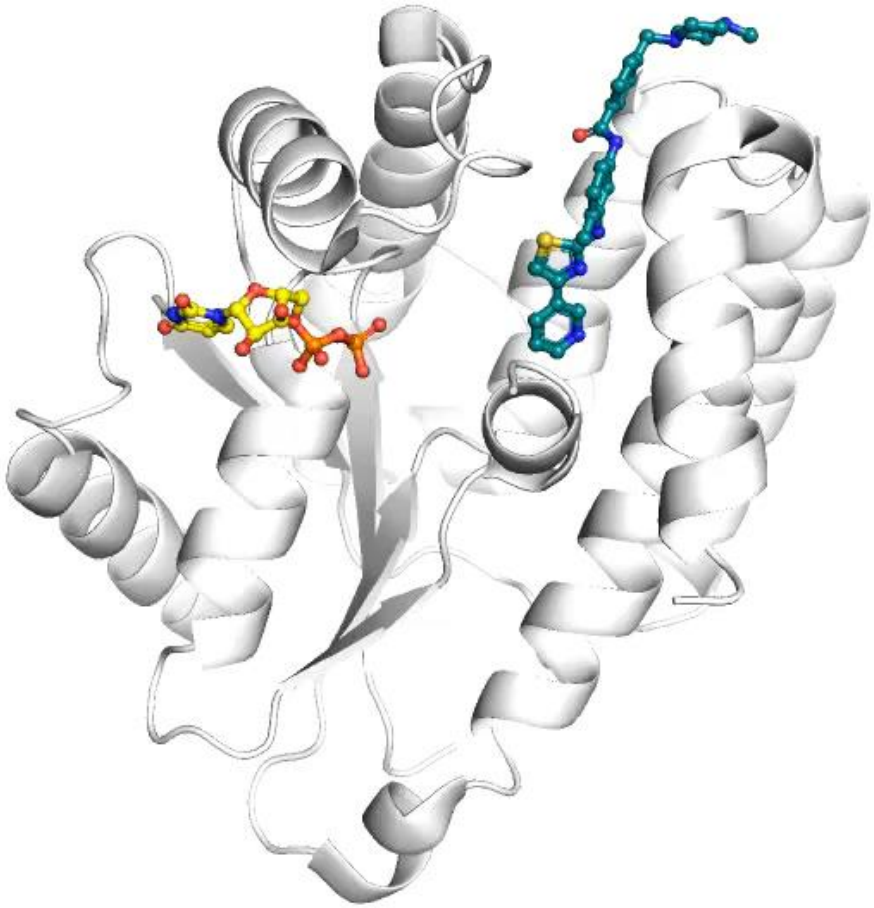
dCK stabilization
(+ ΔT_m, °C)
Cellular dCK inhibition
(IC_{50}, nM)

Turning an Activator into Inhibitor

Characterization

Binding mode: X-Ray

30
Crystal structures



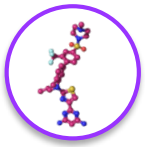
Modifications:

Cycle A	→ Di-amino
Linker B-C	→ *N*-propyl
Linker C-D	→ Direct
Linker D-E	→ Sulfonamide
Cycle D	→ CF_3

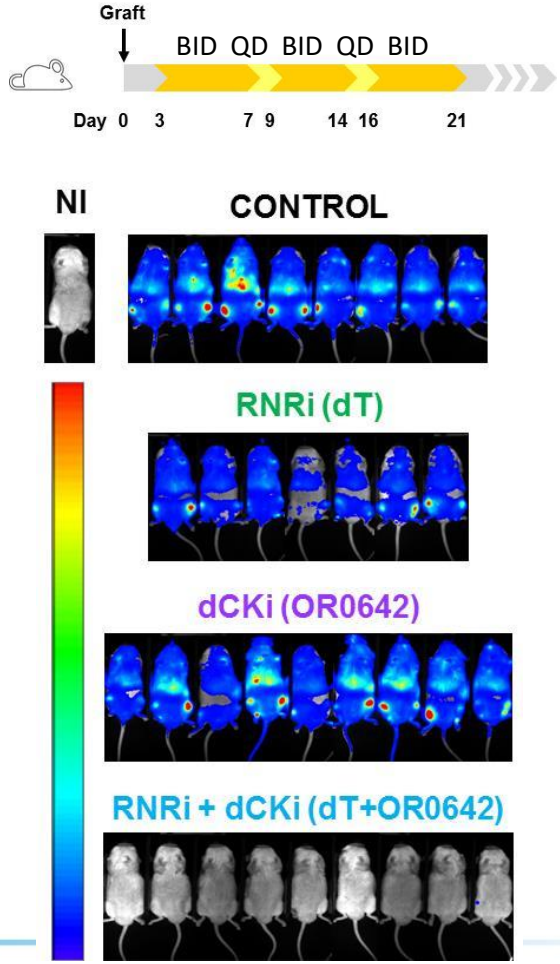
‘(pre)Lead’ Compound & *in vivo* validation (CCRF-CEM/T-ALL model)

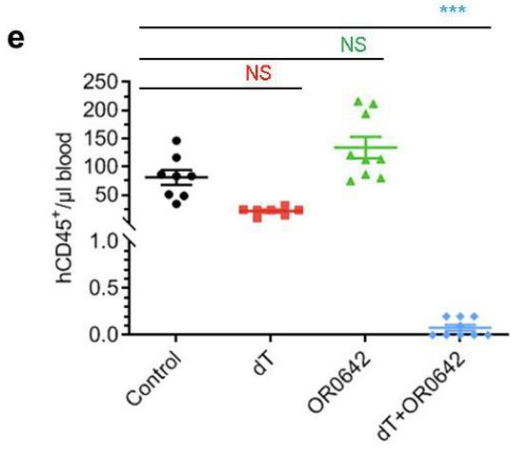
CDX

In vivo cell-line-derived xenograft

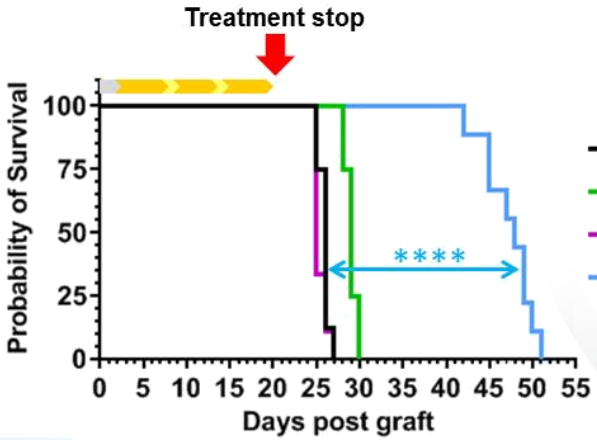


OR0642
CCRF-CEM
T-ALL
EC_{50} = 2.6nM





-- Control
-- dT
-- OR0642
-- dT+OR0642



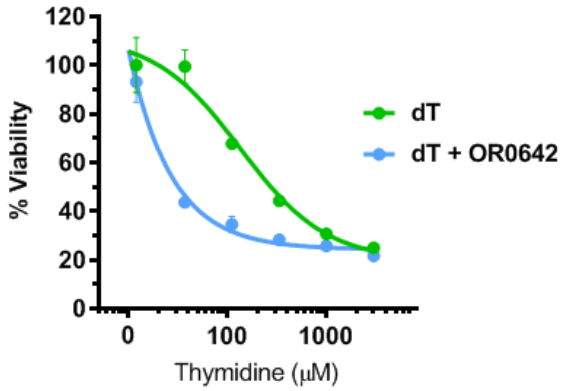
Median Survival
26
29
25
48

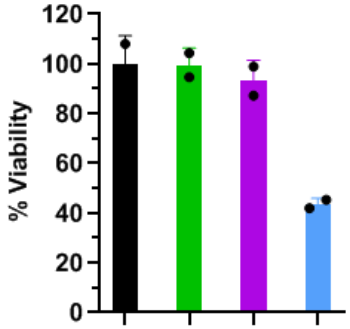
‘(pre)Lead’ Compound & *in vivo* validation (PDX / T-ALL)

PDX

Ex vivo patient-derived xenograft

UPNT525
T-ALL

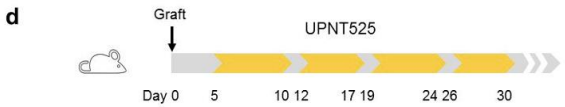


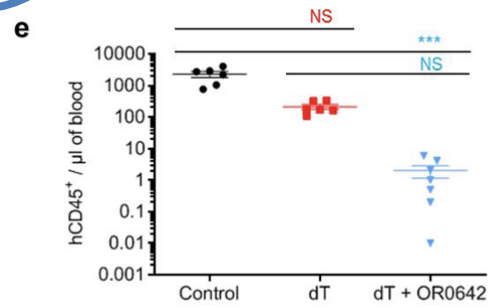


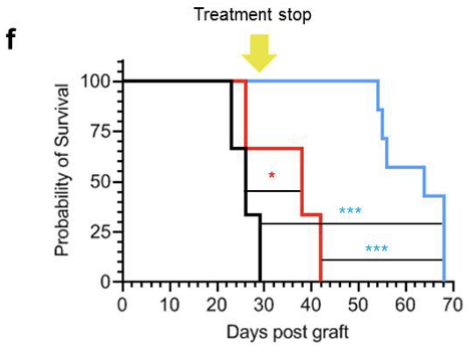
CONTROL
dT 37 µM
OR0642 250 nM
dT + OR0642

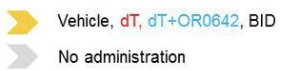
PDX

In vivo patient-derived xenograft









Median Survival

26
38
66

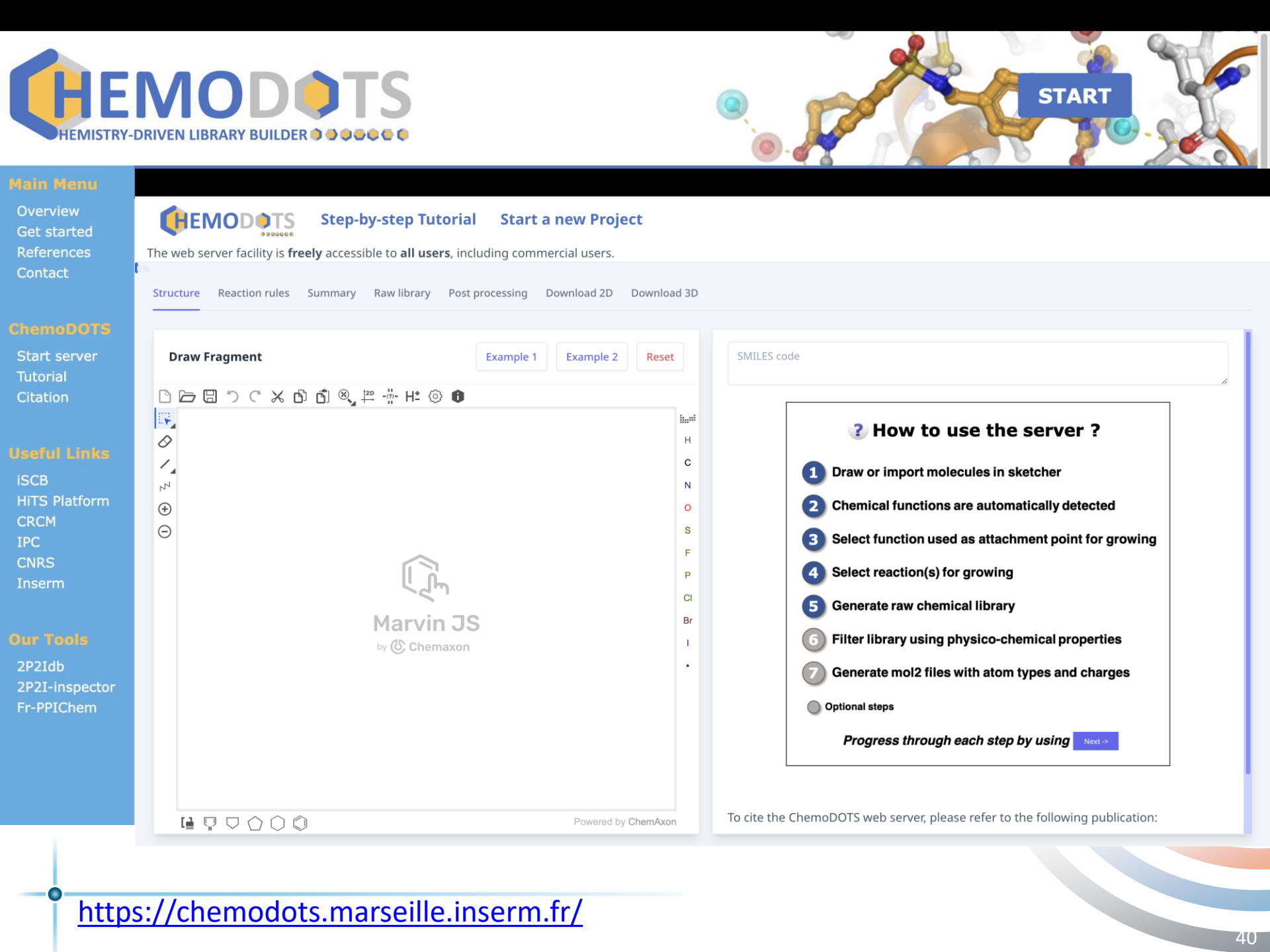
Conclusions & Perspectives

CONCLUSIONS

- From a Drug Repositioning to a Structure-Based Drug Design program
- **'chemoDOTS'** transformed a 'dCK' activator, Masitinib, into an inhibitor: **from *µM* to *sub-nM* IC_{50}'s**
- **OR0642** validation ***in vitro, ex vivo*** and ***in vivo*** (CDX & PDX)

PERSPECTIVES

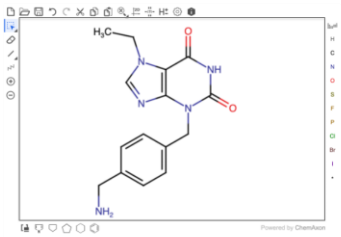
- Developing orally-compatible version (**on going synthesis)**
- PDX studies (on low & highly proliferative patient samples)
- Biomarkers correlation (w/ single seq RNA from patient samples)
- Preclinical regulatory studies in 2025
- Phase I in 2025-2026



https://chemodots.marseille.inserm.fr/

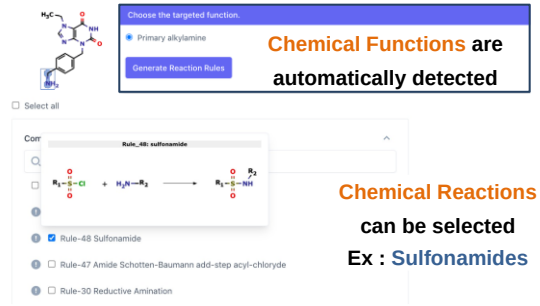
ChemoDOTS webserver functionality

Step 1 : Draw Fragment



The activated Fragment can be drawn or imported into the sketcher

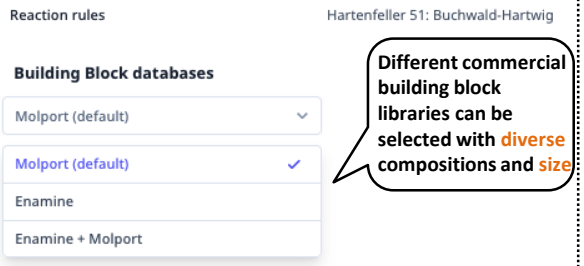
Step 2 : Chemical Reactions



Chemical Functions are automatically detected

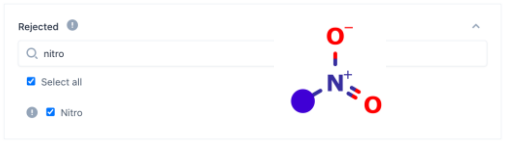
Chemical Reactions can be selected
Ex : Sulfonamides

Step 3 : commercial libraries



Step 4 : Undesired Structures

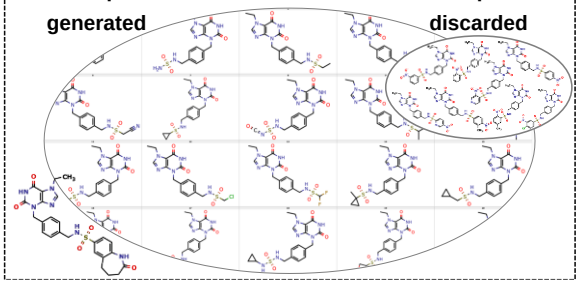
It is possible to select undesired sub-structures (ex Nitro groups). These sub-structures will be discarded from the final chemical library



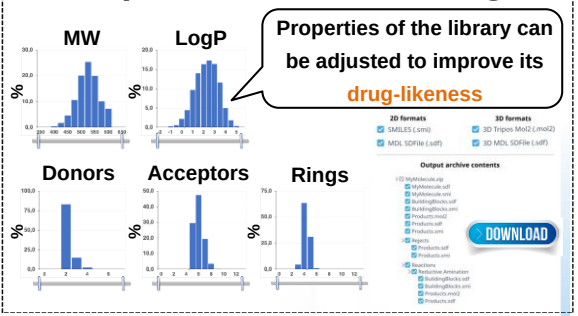
Step 5 : Library Design

1650 compounds generated

77 compounds discarded



Step 6 : Post-Processing



Rapid: generates ~1000 molecules per second
Practical: all building blocks commercially available
Integrated: compounds produced in “ready to dock” format

ChemoDOTS utility: retrospective hit to lead for approved drugs

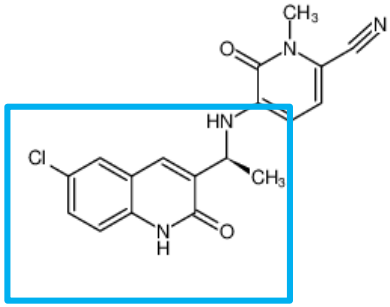
OLUTASIDENIB

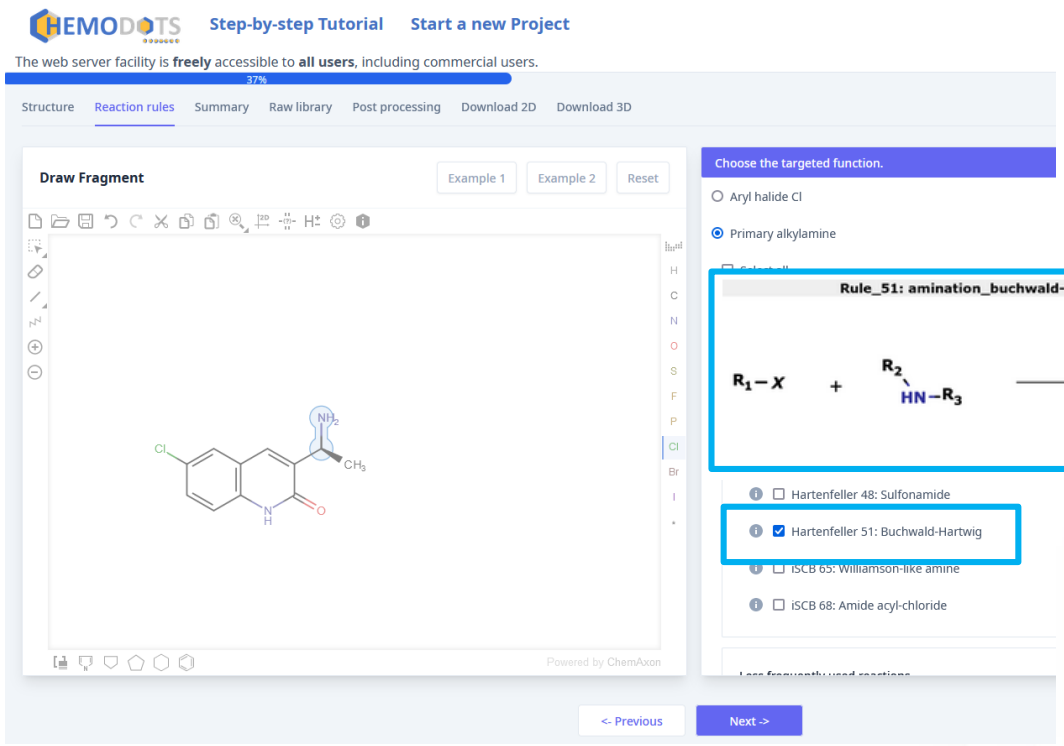
(approved in 2022 - RIGEL PHARMS INC)

ANTINEOPLASTIC

ISOCITRATE DEHYDROGENASE-1 (IDH1) INHIBITOR

r/rAML IDH1mut





Credit: Dominique Douguet

>80k synthetically accessible analogs generated in ~2 min

CHEMODOTS Step-by-step Tutorial Start a new Project

The web server facility is **freely** accessible to **all users**, including commercial users.

62%

Structure | Reaction rules | Summary | Raw library | Post processing | Download 2D | Download 3D

Raw library download

Here you can find some statistics about the raw library generation. The downloads are available **below**.
Duration of library generation: **00:02:05**

You can **bookmark** the following link to reload and **access the saved data**, which will be stored for **two weeks**:
https://chemodots.marseille.inserm.fr/growing?tab=4&experiment=a7c39758-27d1-4a05-8109-07cd2174b6ba

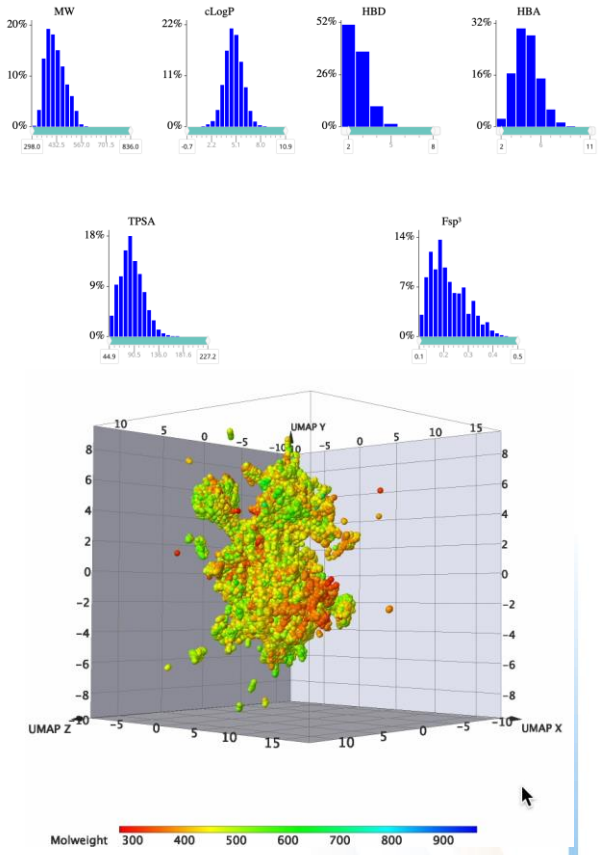
Overall

Total building blocks	501542
Reacted building blocks	16.2% (81340/501542)
Generated products	81340
Duplicate products	19.0% (15484/81340)
Final products	81.0% (65856/81340)
Overall contribution to chemical space	100.0% (65856/65856)

Hartenfeller 51: Buchwald-Hartwig

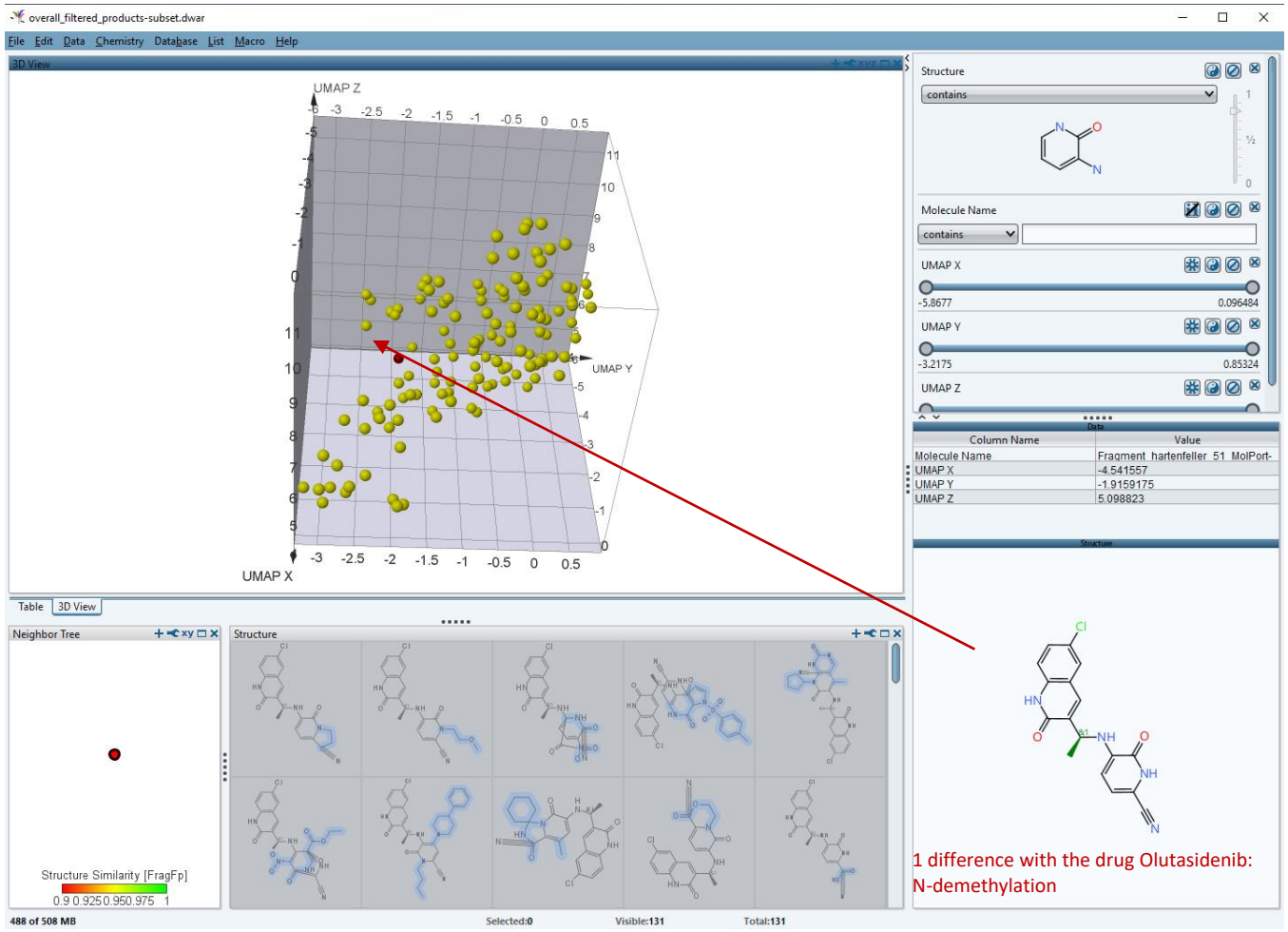
Generated products overview

<- Previous | Next ->

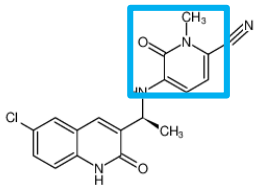


Credit: Dominique Douguet

131 analogs have a pyridinone group as in the drug Olutasidenib

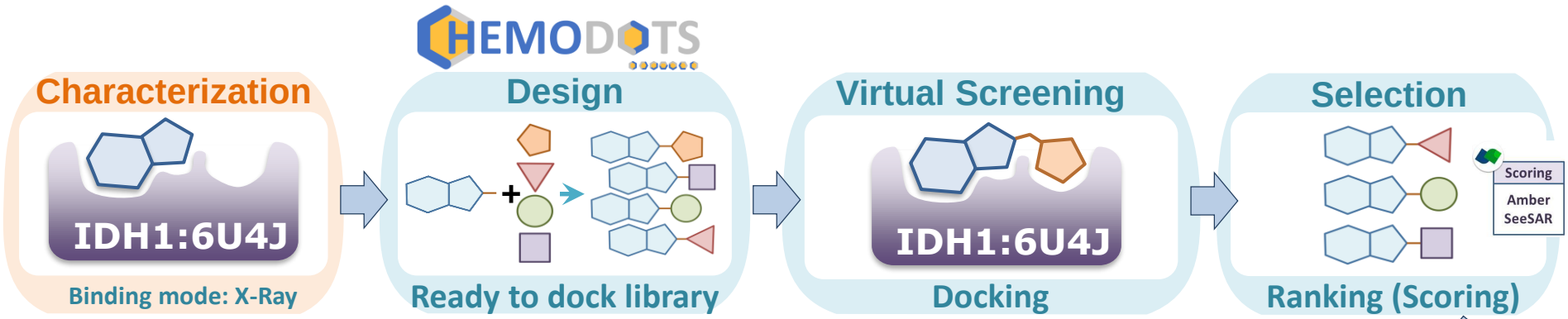


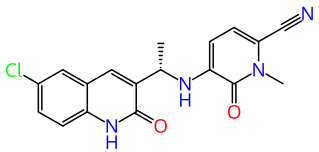
Olutasidenib



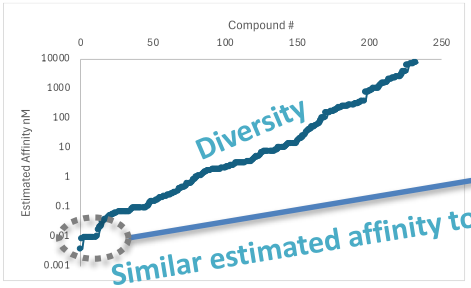
Credit: Dominique Douguet

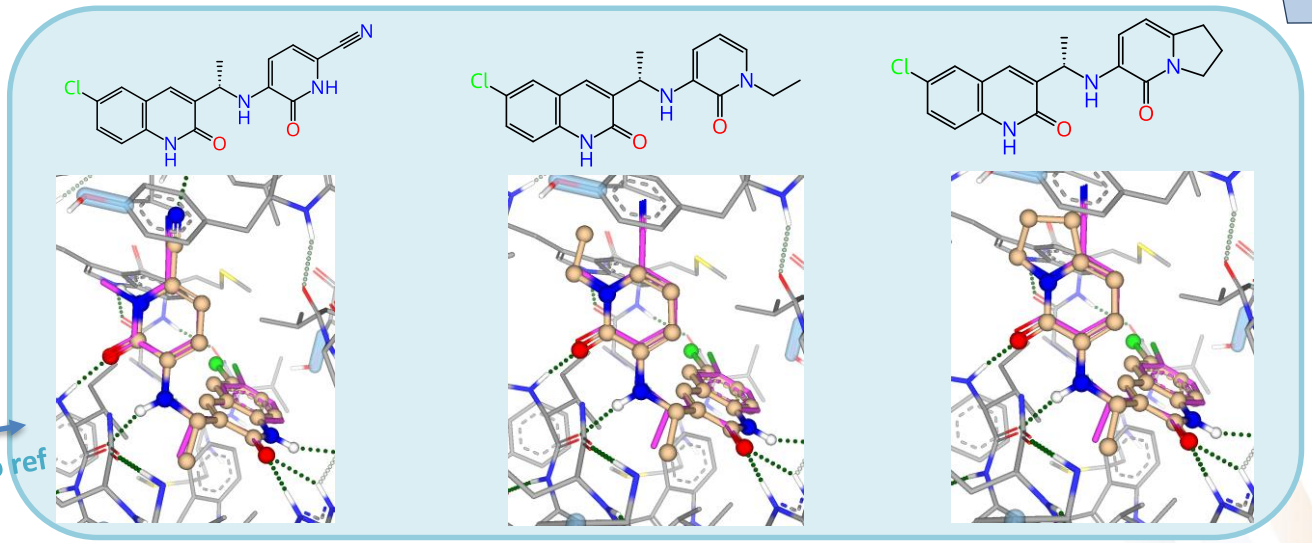
ChemoDOTS integration with virtual screening to select synthesis priorities





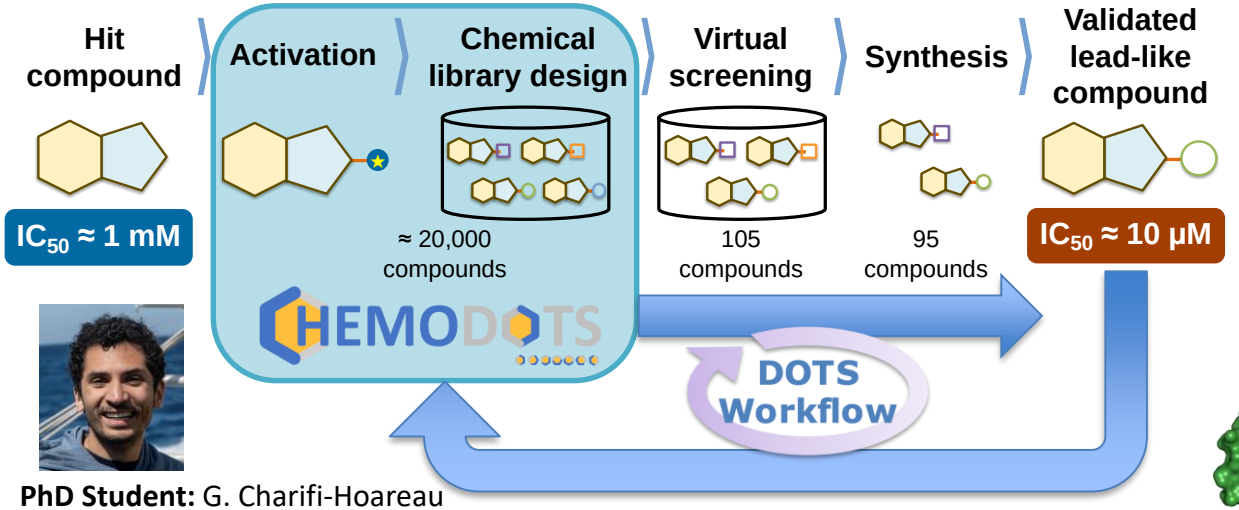
Olutasidenib





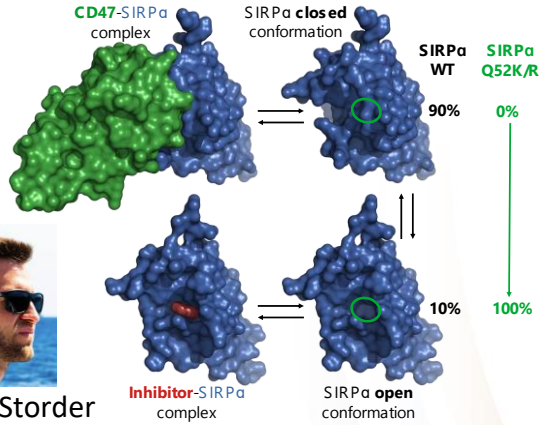
ChemoDOTS ongoing utilization: hit to lead optimization of novel CD47-SIRPα immune checkpoint inhibitors

A **low-affinity hit** identified from screening was refined into a **high-affinity lead-like** inhibitor of SIRPα-CD47 interaction



PhD Student: G. Charifi-Hoareau

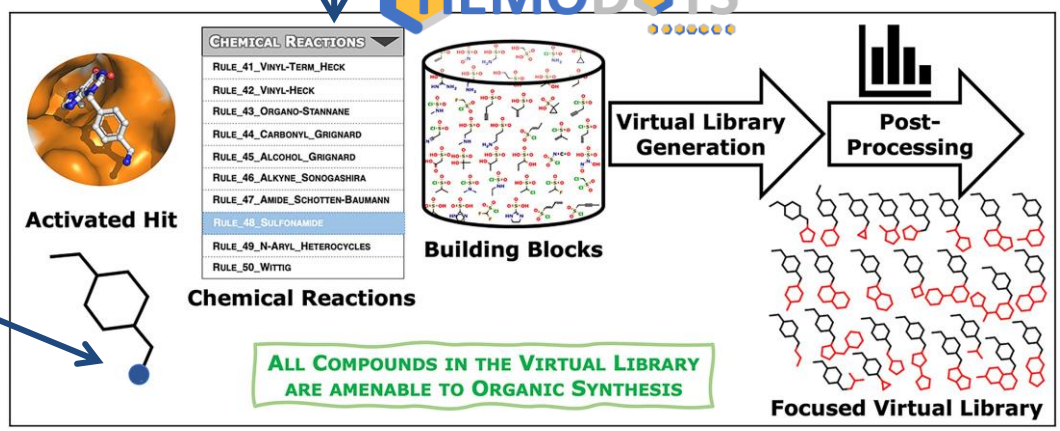
PhD Student : M. Storder



Continuous improvement and Perspectives

Update with latest robust chemical reactions (e.g. C-C coupling)

Automated proposition of activated fragment



Incorporate linking and covalent design strategies

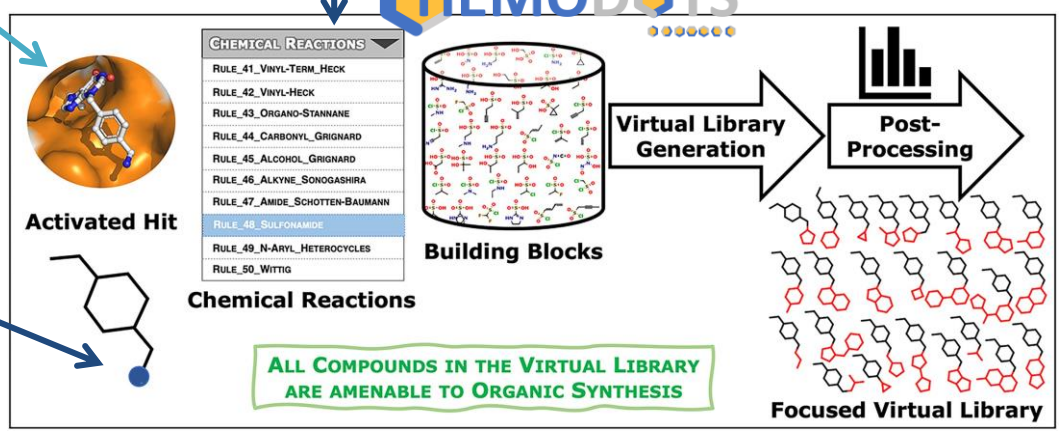
Direct integration in fragment screening libraries

Continuous improvement and Perspectives

Hit ID strategies
CryptoDOTS

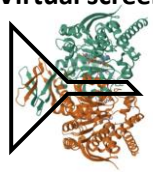
Update with latest robust chemical reactions (e.g. C-C coupling)

Automated proposition of activated fragment



Intelligent library refinement

Virtual screen



ChemoDOTS GOLD LIBRARY

Incorporate linking and covalent design strategies

Transform reversible ligands into covalent inhibitors

Direct integration in fragment screening libraries

Acknowledgements

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