Drug discovery based on innovative academic science

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VIB drug discovery course
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The Centre for Drug Design and Discovery (CD3)

- Established in 2006 by KULeuven R&D and European Investment Fund (EIF)
- Mission: closing the innovation gap by translating early-stage academic insights into new valuable assets
- Focus: Innovative small molecule drug discovery
- Hand-in-hand partnerships with university research groups and biotechs
- CD3 III : EUR 60 mil fund – started Sept 2016
- Access to an interdisciplinary expert team with strong industry pedigree
Transforming early-stage projects into valuable assets

Drug Discovery & Development Engine
Integrated core competencies & expertises in small molecule drug discovery

- Academic centers
- Spin-offs

- Small molecules (related)
- Starting points:
  - Targets
  - Hit compounds
  - Lead compounds
  - Preclinical candidates
- Develop until (pre)clinical candidate & to phase I studies
Transforming early-stage projects into valuable assets

CD3 Drug Discovery & Development Engine
Integrated core competencies & expertise in small molecule drug discovery

- Academic centers
- Spin-offs

In continued collaboration

As Investment

- Biotechs
- Pharma
CD3 collaboration model

Shared risk – reward model
Investment model

CD3 Drug Discovery Engine
Integrated core competencies & expertises in small molecule drug discovery

- Team: > 30 drug discovery experts (bio & chem)
- Assay development, HTS, H2L, ADMET, PK, IP, project management, BD

Scientific Advisory Board
Investment Committee
Consultants

- Cistim
- HDC

30-40 collaborators on a constant basis

Pharma
Research Group X
Research Group Y
Spin-off Company Z
Biotech
Spin-off
Pharma

CROs
CD3 is open for projects WW in all disease indications

- Project proposals
  - from European and other research groups / companies
  - in any disease indication
  - in different stages: target, assay, hits, leads

Project proposals by disease indication

Oncology 37%
CNS 14%
Infection 14%
Immunology 6%
Ocular 1%
Musculo-skeletal 2%
Metabolic 8%
CVD 5%
Airway 2%
Urogenital 2%
Pain 2%
Platform 2%
Aging 1%
Other 4%
## Example partnered CD3 projects

<table>
<thead>
<tr>
<th>Target</th>
<th>Disease</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEDGF – Integrase inhibitors</td>
<td>HIV infections</td>
<td>Pfizer, Viiv Healthcare</td>
</tr>
<tr>
<td>Tau induced toxicity inhibitors</td>
<td>Alzheimer’s disease</td>
<td>reMYND</td>
</tr>
<tr>
<td>NS4B inhibitors</td>
<td>Dengue virus infections</td>
<td>Janssen</td>
</tr>
<tr>
<td>Malt 1 inhibitors</td>
<td>Auto-immune, cancer</td>
<td>Galápagos</td>
</tr>
<tr>
<td>HRV inhibitors</td>
<td>COPD / Asthma</td>
<td>Novartis</td>
</tr>
<tr>
<td>Target X</td>
<td>Remyelination / MS</td>
<td>REWIND Therapeutics</td>
</tr>
</tbody>
</table>
Drug Development: Timelines, cost and attrition

Target ID & Validation

Hit

Lead Generation

Lead Optimisation

Preclinical development

Target ID & Validation

Hit Generation

Lead Generation

Lead Optimisation

Preclinical development

Target Validation & Lead Selection

Pre-Clinical (Lead)

Phase I

Phase II

Phase III

Submission

IND

NDA

674

4.5

49%

24.3

150

15

36%

12.4

319

2.5

56%

8.6

314

2.5

30%

4.6

48

1.5

9%

1.6

1.1

1

Capitalized Cost ($ mn)

Duration (years)

Probability of Attrition

Programmes

Translation Gap
‘The Valley of Death’
Drug Discovery: 5 steps from idea to first-in-human

1. Target Identification & Validation
2. Hit Identification & Validation
3. Hit-to-Lead
4. Lead Optimization
5. Selection of Preclinical Candidate
Step 1: target identification & validation

- Novelty
- Medical need – linkage to human disease (market)
- Biological rationale and experimental validation of target (efficacy)
- Safety of target modulation
- Druggability/ availability of tools / assays
- Clear clinical development path (biomarkers, patient selection and recruitment)
- IP / FTO

✔ Define a **Target Compound Profile** at onset
✔ Not all criteria will be fully met at onset of project: derisking strategy!
  - Risk mitigation within portfolio
  - Avoid double/triple risks within a project
Step 1: target identification & validation

http://www.smd.qmul.ac.uk/bioinf/drugdiscovery.html
**Step 1: target identification & validation**

<table>
<thead>
<tr>
<th>Target Compounds Profile</th>
<th>Kinase inhibition (IC(_{50}))</th>
<th>&lt;10 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinase selectivity</td>
<td>&lt;10% kinome at 100x Target Kd</td>
<td></td>
</tr>
<tr>
<td>Cellular inhibition of substrate phosphorylation</td>
<td>&lt;100nM (SAR consistent with kinase inhibition)</td>
<td></td>
</tr>
<tr>
<td>Target-mutant lymphoma proliferation (IC(_{50}))</td>
<td>&lt;100nM (SAR consistent with kinase inhibition)</td>
<td></td>
</tr>
<tr>
<td>Selectivity window over WT lymphoma cell proliferation</td>
<td>&gt;100-fold</td>
<td></td>
</tr>
<tr>
<td>Single dose PK/PD (mouse)</td>
<td>Inhibition of substrate phosphorylation and sustained repression of downstream transcriptional program with single oral dose &lt; 20mg/kg</td>
<td></td>
</tr>
<tr>
<td>Efficacy/tolerability (mouse)</td>
<td>Complete tumor growth inhibition in target mutant xenograft models using doses &lt;20 mg/kg. Maximal Tolerated Dose &gt;100m/kg</td>
<td></td>
</tr>
<tr>
<td>Polypharmacology, CVS, genotox</td>
<td>No alerts</td>
<td></td>
</tr>
<tr>
<td>Predicted human daily dose</td>
<td>&lt; 1g, oral route, formulation identified</td>
<td></td>
</tr>
<tr>
<td>Patent application</td>
<td>Compound matter</td>
<td></td>
</tr>
</tbody>
</table>

- Target
- Cellular phenotype
- In vivo target modulation
- In vivo efficacy & tolerability
- Safety
- Developability
- IP
Step 1: target identification & validation

- Pharmacologic target validation ≠ full loss-of-function (knock-out, knock-down)
- Developmental vs adult setting

Case example: MALT1
Step 1: target identification & validation

Case example: MALT1
Step 2: Hit identification

- Target-based:
  - High-throughput-Screening (HTS)
  - Rational design
  - Fragment-based drug discovery
  - DNA-encoded libraries

- Phenotypic screening:
  - Disease-relevant context
  - Target identification usually essential to move forward
Step 2: Hit identification
Assay Development and HTS

Technical requirements:

- Compound library (100-500K, <500 Mw)
- 384 or 1536 well format
- Z’ (S/N and %CV)
- Homogenous assay (no wash steps)
- Assay time/stability

Assay relevance:

- Sensitivity to pharmacologic modulation
- Physiologic relevance Validation using tool compounds/peptides, mutagenesis
Step 2: Hit identification
Rational design

Technical requirements:

• Crystal structure available
• ‘druggable’ pocket (~size, shape and hydrophobicity of the pocket)
• Based on known small-molecule or peptide binders
• Selectivity (primary sequence divergence across family members)

Relevance:

• Binding → functional impact
• Validation using tool compounds/peptides, mutagenesis
2003 LEDGF/p75 is a co-factor of HIV replication (Cherepanov et al., J. Biol. Chem.)

2006 LEDGF/p75 tethers IN to the chromatin (Llano et al., Science)

2006 Overexpression of the LEDGF/p75 integrase binding domain (IBD) inhibits HIV replication (De Rijck et al., J. Virol.)

2007 Start investment in drug discovery project in a collaboration with Prof. Z. Debyser (KULeuven)
# Case example: Novel HIV inhibitors – LEDGF-Integrase

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>LEDGF/p75-integrase IC₅₀ᵃ</th>
<th>EC₅₀ᵇ</th>
<th>EC₉₀ᶜ</th>
<th>CC₅₀ᵈ</th>
<th>SIᵉ</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX0516</td>
<td><img src="structure1.png" alt="Structure" /></td>
<td>1.37 ± 0.36</td>
<td>2.35 ± 0.28</td>
<td>5.36 ± 1.48</td>
<td>59.8 ± 0.50</td>
<td>25</td>
</tr>
<tr>
<td>CX05045</td>
<td><img src="structure2.png" alt="Structure" /></td>
<td>0.58 ± 0.30</td>
<td>0.76 ± 0.08</td>
<td>1.86 ± 0.521</td>
<td>72.2 ± 5.15</td>
<td>95</td>
</tr>
<tr>
<td>CX14442</td>
<td><img src="structure3.png" alt="Structure" /></td>
<td>0.046 ± 0.012</td>
<td>0.069 ± 0.003</td>
<td>0.114 ± 0.052</td>
<td>96.0 ± 16.0</td>
<td>1,391</td>
</tr>
</tbody>
</table>

ᵃ Concentration (μM) required to inhibit *in vitro* protein-protein interaction by 50%.
b Effective concentration (μM) required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
c Effective concentration (μM) required to reduce HIV-1-induced cytopathic effect by 90% in MT-4 cells.
d Cytotoxic concentration (μM) reducing MT-4 cell viability by 50%.
e Selectivity index: ratio, CC₅₀/EC₅₀. The averages and standard deviations for at least 3 independent experiments are shown.
f Identical with compound 6 in reference 23.
g Identical with compound 7 in reference 23.
2003  LEDGF/p75 is a co-factor of HIV replication  
(Cherepanov et al., J. Biol. Chem.)

2006  LEDGF/p75 tethers IN to the chromatin  
(Llano et al., Science)

2006  Overexpression of the LEDGF/p75 integrase binding domain (IBD) inhibits HIV replication  
(De Rijck et al., J. Virol.)

2007  Start investment in drug discovery project in a collaboration with Prof. Z. Debyser (KULeuven)

2009  New anti-HIV drugs inhibiting LEDGF-integrase interaction identified

2009  Multiple patent applications filed - Business Development initiated

2010  Highly active anti-HIV drugs identified ~existing drugs – kills all resistant viruses

2010  Publication Nature Chemical Biology

2010  Exclusive license established with Pfizer
Step 2: Hit identification
Fragment based drug discovery

- Fragment library (1-3K, <200 Mw)
- Binding assay: NMR, SPR, TSA, ...
- Crystal structure needed: grow/link fragments
Step 2: Hit identification
Phenotypic screening

- Drug discovery timelines longer
- More risk later on (target ID)
- Restrict to disease indication with lack of tractable drug targets?
Step 2: Hit identification and validation

WORST OFFENDERS

Pan-assay interference compounds (PAINS) fall into hundreds of chemical classes, but some groups occur much more frequently than others. Among the most insidious are the eight shown here (reactive portions shown in red and purple). These and related compounds should set off alarm bells if they show up as ‘hits’ in drug screens.

‘PAINS’ pan-assay interference structures:

- Unselective covalent modifiers
- Redox cyclers
- Aggregators
- Metal chelators, ...
Step 2: Hit identification and validation

- Counterscreens (technology hitters, specificity)
- Orthogonal assays (*eg* enzyme inhibition and enzyme binding)
- Chemical resynthesis, purification
- Analogs
**Step 2: Hit identification and validation**

Primary screening assay:
biotin-TAZ/GST-TEAD HTRF

Counter screen:
Biotin/GST HTRF

Selectivity assay:
biotin-VGLL/GST-TEAD HTRF

Orthogonal assays:
TEAD binding (TSA, NMR)

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![Graphs showing IC$_{50}$ values for TAZ peptide/TEAD, CS, and VGLL1/TEAD assays.]

**TAZpeptide/TEAD**: IC$_{50}$ 19 nM

**CS**: (Graph showing a trend line with IC$_{50}$ value)

**VGLL1/TEAD**: IC$_{50}$ 20 μM
Step 3: Hit-to-lead

- SAR: Structure-Activity-Relationships
- Activity correlations: biochemical to cell-based, and to phenotypes
- Early ADME-T
Step 3: Hit-to-lead

- SAR: Structure-Activity-Relationships
- Supported by crystallography/docking

~200 compounds synthesized

- Correlations: enzyme inhibition to target engagement in cells
Step 3: Hit-to-lead

• Early ADME-T (Absorption Distribution Metabolism Excretion – Toxicity):
  • Solubility
  • Cytotoxicity
  • Metabolic stability (clearance in liver microsomes)
  • Plasma protein binding (‘free fraction’)
  • Permeability (and PgP efflux)
  • hERG (and other) ion channel
Step 4: Lead optimization

• PK/PD relationships
  (pharmacokinetics/pharmacodynamics)

• Efficacy in animal models

• Cross-species pharmacology and ADME/PK

• Broad selectivity panels

• Tolerability in animal models
Step 5: Preclinical candidate selection
Regulated tox

- 1 rodent and 1 non-rodent tox species (rat and dog most common; but also: mouse, monkey (cyno), mini-pig)
- Single dose MTD
- Exposures achievable must exceed therapeutic exposure (in real terms and after plasma protein binding correction)
- Goal is to identify tox signals and define therapeutic index: therapeutic area matters (eg oncology), acute vs chronic use
- Clinical formulation --develop tox formulation or use alternative route of administration to boost exposure
- ~kg batch needed: GLP

- 9-12 months (~M €)
Conclusions

- Drug discovery is a long (4-5 year) and costly, but fun adventure
- Small molecules are usually unspecific - until proven otherwise
- Test, re-test, and test again
- Correlations and Structure-Activity-Relations are key
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