Etude préclinique d’un nouveau médicament : lignées cellulaires, modèles animaux

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- « Laboratory of Integrative Structural & Chemical Biology (iSCB) »
- « TrGET, preclinical platform »
Drug discovery is an inherently inefficient process, particularly in oncology.

- >800 M€
- 1 out of 50

* Source: Hutchinson & Kirk, NRCO 2011
The drug development pipeline
The drug discovery workflow

Target selection
- Studies of Disease Mechanisms
  - Molecular Studies
- Animal Studies
  - relevant species
  - transgenic KO mice
  - conditional KOs
  - agonists/antagonists
  - antibodies
  - antisense
  - RNAi

Discovery
- Target
  - receptor
  - ion channel
  - transporter
  - enzyme
  - signalling molecule
- Lead Search
  - Develop assays (use of automation)
  - Chemical diversity
  - Highly iterative process
- Lead optimization
  - selectivity
  - efficacy in animal models
  - tolerability: AEs mechanism-based or structure-based?
  - pharmacokinetics
  - Highly iterative process

Development
- Drug Candidate safety testing
- Human Studies
  - Phases I, II, III
- Drug Approval and Registration

CRCM
Centre de Recherche en Cancérologie de Marseille
What are Pharmacology Studies for Anti-Cancer Biologics?

- Evaluation of ability of a new agent to induce the desired therapeutic effect
  - *in vitro* studies of product binding, tumor cell killing, and other effects
  - *in vivo* studies of anti-tumor activity
    *e.g.*, human tumor xenograft models

- Demonstration of pharmacologic and/or biologic activity is the first step in the development of ANY new drug or biologic
Goals of Non-Clinical Testing of Small Molecule Drugs and Biologicals

- To characterize potential adverse drug effects
  - Define end organ toxicities
  - Define reversibility of toxicity

- To characterize pharmacokinetic profile

- To characterize beneficial pharmacodynamic effects
  - Proof of principle

- To guide safe use in human clinical studies
  - To determine a safe & reasonable starting dose
  - Provide monitoring guidelines for the clinical study

- Provide sufficient data to conclude that patients are not exposed to unreasonable risks
  - Potential for benefit must also exist
What Are These Small Molecules we Test?

Practical Definition:

- An **organic molecule** of less than 1000 Daltons
  Typically in the range of 300-700 Daltons

- Small organic molecules made by living organisms
  (e.g., natural products)

- Small organic molecules made by chemists
  (e.g., organic compounds, RNAi)

- In all cases one is looking for a small ‘drug-like’ organic molecule that displays a biological activity
  (e.g., agonist, antagonist) with the target of interest.

- **Protein** (recombinant, Ab)
How Does One Select a Library to screen?

- **Random Selection**
  Random high throughput screening
  Little is known about the target
  Few or no active compounds as guides

- **Computational Chemistry/Virtual Screening**
  Creation of ‘Focused Libraries’
  Requires prior knowledge about target
  Active compounds, 3D-Structure
  Sequence homology

- **Prior Experience**
  Library successfully used for similar
  or related targets
High Throughput Screening (HTS)

- HTS enables the testing of large numbers of chemical substances for activity in diverse areas of biology in a relatively short time.

- The entire chemical space of small organic molecules is estimated to be $> 10^{60}$. Of those, $\sim 2.7 \times 10^7$ compounds have been registered and made. (Nature Insight, 2004)

- Responses studied can range from biochemical systems of purified proteins or enzymes to signal transduction pathways to complex cellular networks (Systems Biology).
History of cancer chemotherapy

**Figure 1. Key advances in the history of cancer chemotherapy**

- Advances in drug screening
- Events with national impact
- Advances in cancer therapeutics

- Arsenicals (1, 2)
  - Animal models (1–4)
  - 1900

- Transplantable tumors (5–11)
  - 1912

- Nitrogen mustard in lymphomas (15–18)
  - 1943

- Model development (7)
  - 1935

- Thiopurines (24, 25)
  - 1949

- Antifolates (22)
  - 1948

- 5-Fluorouracil (26)
  - 1951

- Antitumor antibiotics (23)
  - 1959

- Methotrexate in choriocarcinoma
  - 1955

- Cancer Chemotherapy National Service Center
  - L1210 as primary screen (27–30)

- Concept of cure
  - 1960s

**AACR Centennial Series**
In vitro Drug testing: The NCI60 experience
In vitro Drug testing: The NCI60 experience

Wainberg
The TrGET preclinical platform

ETOH

Physicians
- Access to innovative compounds
- Preclinical validation

Searchers
- Research tools
- Valorisation

Drug company
- Partnership
- Biological valorisation of Clinical trials
The TrGET initiative

A screening strategy to search for in vivo efficient (subtype-specific?) anti-AML drug combinations

1. Drug set design:
   - Diverse set
   - Specific set (Kl, epidrugs,...)

2. Target cell design:
   - Diverse cell lines
   - Primary AML

3. Cell test design:
   - Cytotoxicity
   - Caspase
   - ...

Drugs

Drug A

Drugs

21 different drug compounds + X

Diverse
Kinase inhibitors
Epidrugs

+ Cells

AML cell lines
Primary AMLs

24-120h

Cell test

Dr Vey & Prébet
Hematology Dpt
IPC

TrGET
The TrGET initiative

4. Identification of AML subtype(s) with specific sensitivity towards drug subset(s):
- Clinical-biology
- Cytogenetics
- Transcriptome
- Methylome
- Mutations
- ...
Expanding on the NCI60 experience

> 500 cell lines!

McDermott et al PNAS 2007

AZ628 selective
Sorafenib poor selectivity / BRAF

1/3 V600E are insensitive!
Breaking resistance?

Chou & Talalay: \[ CI = \frac{(D_1)}{(D_x)_1} + \frac{(D_2)}{(D_x)_2} \]

\((D_1)\) et \((D_2)\) : doses de la drogue 1 et de la drogue 2 en combinaison

\((D_x)_1\) et \((D_x)_2\) : doses de la drogue 1 et de la drogue 2 utilisées seules inhibant x% de la viabilité.
Breaking resistance?

Challenges for application to clinical trial

- Link of observed synergism with underlying biochemical/physiological mechanism difficult to discover
- In vitro drug ratio vs in vivo/MTD
- As compared to standard treatment
- Impact on tumour heterogeneity not assessed in vitro
- Lack of correlation between in vitro cytotoxicity and patient benefit
Established cell lines

- Difficulties to outgrow many cancer cells
  in vitro
- Nbr models < cancer subtypes
- In vitro adaptation
- Insufficient molecular characterization
- Tumour initiating cells
- Lack of key features (ex: stromal-tumour interactions)

Primary cell cultures...

Drug testing

48h
The TrGET initiative

- Expansion
- CGH array
- Mutational status
- Transcriptomic profile

Death ↔ Cytotoxicity (EC50 value) ↔ Viability
Seulement 5% des molécules passent le stade de la recherche et seules 2% passent les essais précliniques. Parmi celles-ci 20% passent avec succès les essais cliniques et parviennent sur le marché. On voit que l’expérimentation animale joue le rôle d’un filtre très efficace à la fois en recherche et en toxicologie.

Figure 2 | Preclinical models to evaluate anticancer drugs. Selected steps in anticancer drug validation. Each step adds specific information that will be used in the drug validation process before beginning a clinical trial.

In vivo drug testing, validation
Animal Models in Cancer

- Spontaneous tumors
  - Idiopathic
  - Carcinogen-induced
  - Transgenic/gene knockout animals: p53, RB, etc

- Transplanted tumors
  - Animal tumors: Lewis lung, S180 sarcoma, etc
  - Human tumor xenografts: human tumor lines implanted in immunodeficient mice (current NCI standard in vivo efficacy testing system)
  - Human tumors growing in vivo in implantable hollow fibers
Immunocompromized mice

- Athymic “nude” mice developed in 1960’s
- Mutation in nu gene on chromosome 11
- Phenotype: retarded growth, low fertility, no fur, immunocompromised, Lack thymus gland, T-cell immunity
- First human tumor xenograft of colon adenocarcinoma by Rygaard & Poulson, 1969

- irradiation
- low relevance to evaluate host (immune system)/ tumor interactions
Some of the tumor cell lines already xenografted at the TrGET plateform.

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Xenograft study end points

- control n=7
- AraC 10mg/kg x 1x n=8
- AraC 10mg/kg x 10x n=8
- P<0.001
- MS=25
- MS=30,5
Xenograft Biomaging

Luciférase

Luciférase + Mg²⁺ + O₂ + ATP → Oxyluciférase + CO₂ + ADP

Monitoring

\[
\begin{array}{cc}
\text{Normalized Values} & 10,000,000% \\
1,000,000% & 100,000% \\
100,000% & 10,000% \\
10,000% & 100% \\
\end{array}
\]

Time (days after implantation)

\[R^2 = 0.96\]

\[R^2 = 0.98\]

Ilar J. et al; PMC 2009.

Sensitivity

Rabinovich B. et al, PNAS 2008

TryGET
Xenograft Biomaging
Xenograft Biomaging
Xenograft Biomaging

**Primary AML**

[Diagram showing fold changes over time for different groups labeled with dates and drug treatments.]

- DUC
- 2ry xenograft
- J70
- Fold
- 1G1 (+Arac)
- 2D1
- R1 (+Arac)
- 1D2
- 1G2 (+AraC)
- AraC

Dates:
- d25
- d54
- d67
Xenograft Biomaging

4 views

Non Inj Control

KG1 Lluc

MOLM14 Lluc

organs

Luminescent signal repartition among organs ("cells preferential locations")

% of total luminescent signal

prostate Liver kidneys heart spleen lungs femur

MOLM14 Lluc n=3 KG1 Lluc n=3
Xenograft Biomaging

Human Vγ9Vδ2 T Cells Specifically Recognize and Kill Acute Myeloid Leukemic Blasts

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