Développement d'un médicament : de la biologie à la recherche clinique

Modèles *in vitro*

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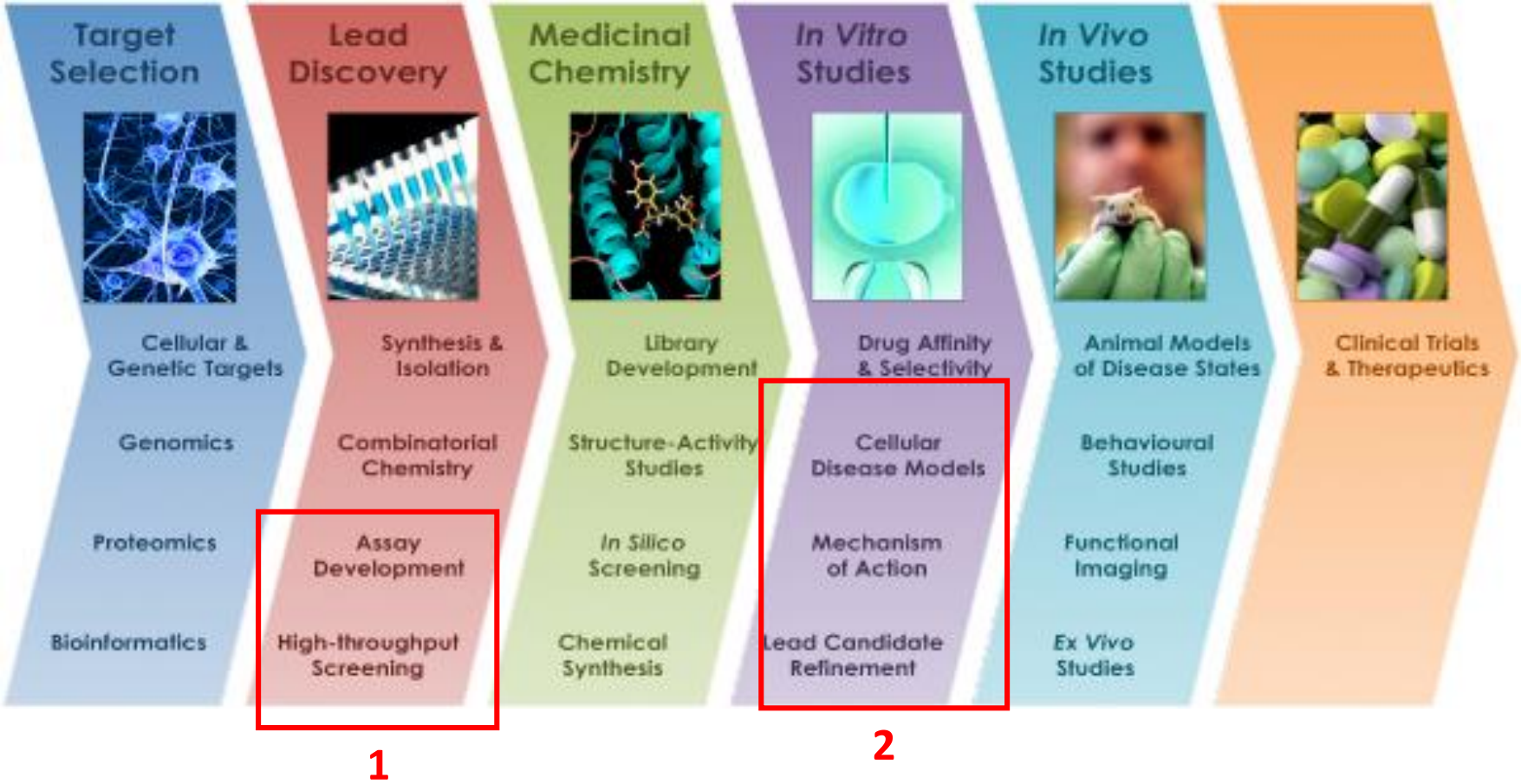
Positionnement des modèles *in vitro* dans le pipeline du développement des anticancéreux



Distinction entre:

1- Modèle *in vitro* stricto sensu (modèles acellulaires)

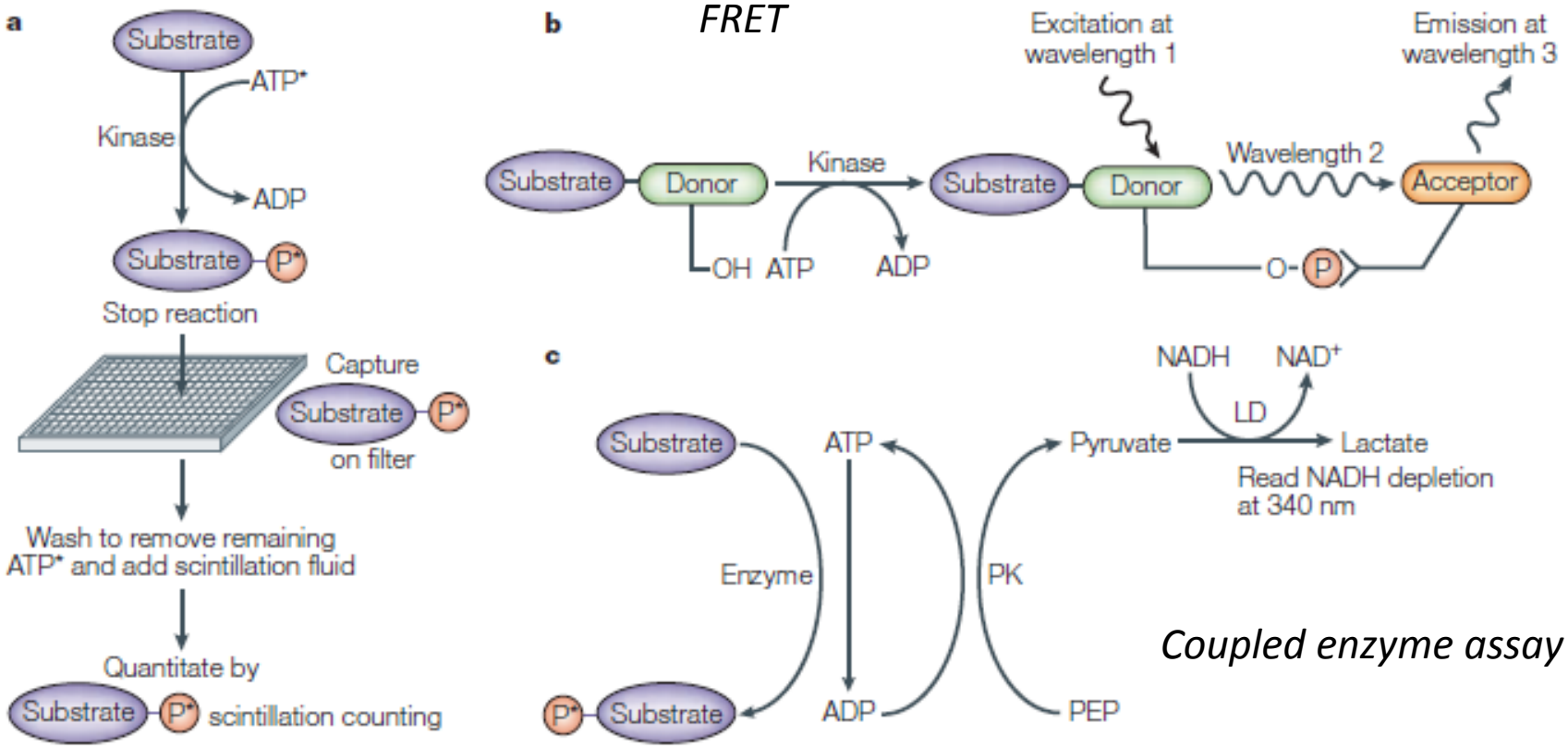
- développés spécifiquement pour l'évaluation de l'activité sur une cible particulière
- pour le screening à haut débit de nouveaux inhibiteurs

→ Nécessite l'obtention de la protéine cible purifiée

2- Modèles cellulaires (modèles *in cellulo*) utilisés pour:

- valider l'effet antiprolifératif de la molécule
- étudier son mécanisme d'action
- identifier les mécanismes de résistance
- tester des combinaisons
- etc…

Quelques exemples de tests in vitro adaptables au HTS



Filter binding assay

Coupled enzyme assay

Nat Rev Drug Discov 2003 2:259-66

L'utilisation des modèles *in cellulo*

Les différents types de cultures de cellules tumorales:

- ✓ **cultures primaires issues de tumeurs fraîches**
  - **Nombre de doublements limité**
  - **Hétérogénéité clonale**
  - **Difficultés de mise en culture en fonction de la provenance**

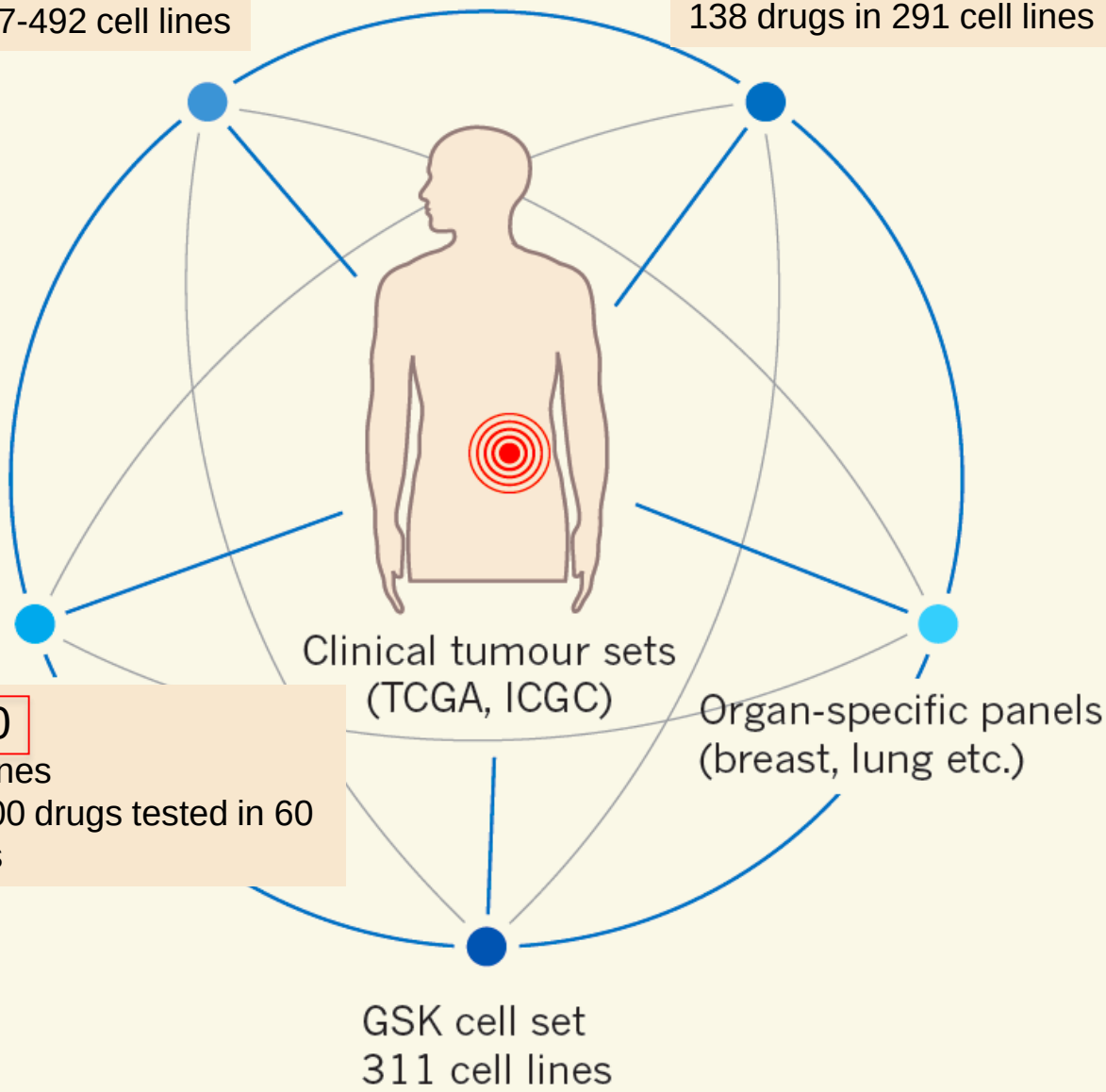
- ✓ **cultures en lignées continues**
  - **Clones spécifiques immortalisés**
  - **Temps de doublement de 24 à 48h**
  - **Culture en suspension ou en 2D sur plastique**
  - **« Facilement manipulables » (obtention de lignées isogéniques)**
  - **Modèles très variés mais très hétérogènes**

Cancer Cell Line Encyclopedia (MIT-Novartis)

1036 cell lines
24 drugs in 307-492 cell lines

CGP (MGH-Sanger)

727 cell lines
138 drugs in 291 cell lines



NCI-60

60 cell lines
> 100,000 drugs tested in 60 cell lines

Weinstein 2012

Hétérogénéité des modèles cellulaires de cancer de prostate

Cell line	Androgen receptor	Androgen sensitivity	5α-Reductase	PSA	PAMA	p53/other features	PTEN	TMPRSS2-ETS+
PC-93	?	AI	–	–	NR[a]	NR		
PC-3	–	AI	–	–	–	Deletion mutation of *p53*	del	ETV4
DU-145	–	AI	–	–	–	Mutated *p53*, pro to leu codon 223; val to phe codon 274 Has mutated p16 codon 84		
TSU-Pr1	–	AI	NR	–	NR	Mutated *p53* (has mutated H-ras)		
LNCaP	+ Mutated	AS	–	+	+	Silent mutation of *p53*	mut	ETV1
Sublines:								
C4		AI	++	+				
C4-2	AI + mets	+++	+			Low levels of functional *p53*		
C4-2B	AI + mets	+++	+					
P104-R2	Stimulated by finasteride							
LN3		Less sensitive than LNCaP	+++	++				
MDA PCa 2a	+	AS		+	NR	NR		
MDA PCa 2b	+	AS		+	NR	NR		
ALVA101	+	AS	+	+	NR	NR		
22Rv1	Grown from an AI xenograft	AS	NR	+	NR	–		
ARCaP	+	Androgen repressed	NR	+	NR	NR		
LACP-4	+	AS	NR	+	NR	AI sublines express high levels of HER/2-neu		
P69SV40T	NR	NR	NR	NR	NR	Express EGFR and TGFα		
RWPE-2	+	+	NR	+	NR	Express HPV-18 E7 protein; mutated *Ki-ras*		
CA-HPV-10	NR	NR	NR	NR	NR	Cytogenetic abnormalities		
VCap	+	+						ERG

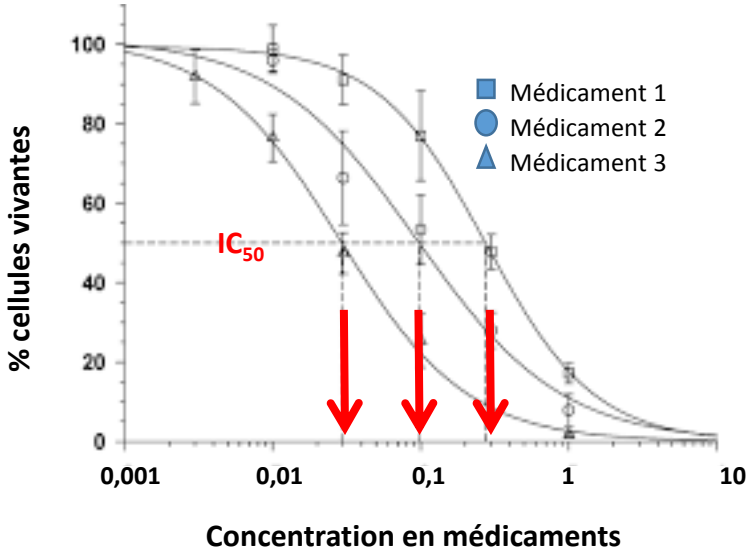
Ar-V7

L'utilisation des modèles *in cellulo*

Ce que l'on peut faire avec …

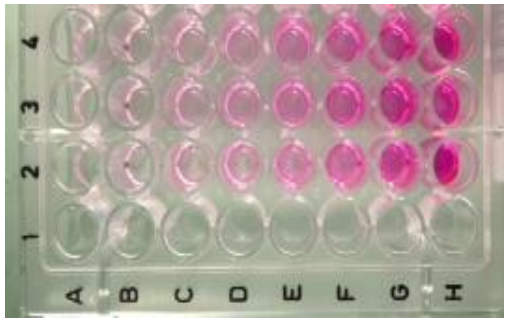
1- Evaluer l'activité antiproliférative d'une nouvelle molécule par différents tests

Comptage



Tests colorimétriques

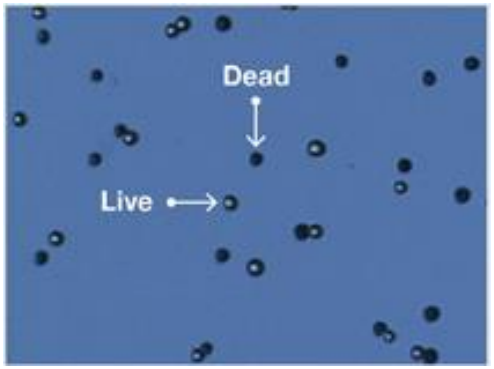
Sulforhodamine B (quantité de protéine)
MTT, XTT, MTS (activité mitochondriale)



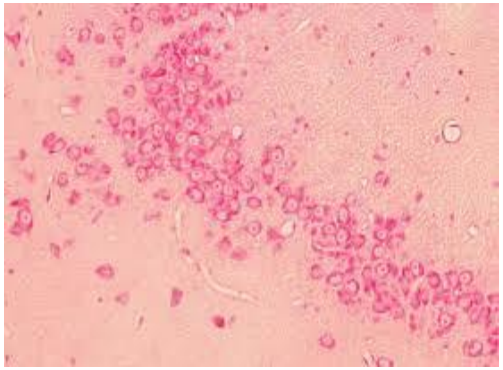
→ Impossible de distinguer un effet cytotoxique d'un effet cytostatique

autres tests de prolifération intégrant la mesure de la viabilité cellulaire

Test d'exclusion au bleu Trypan



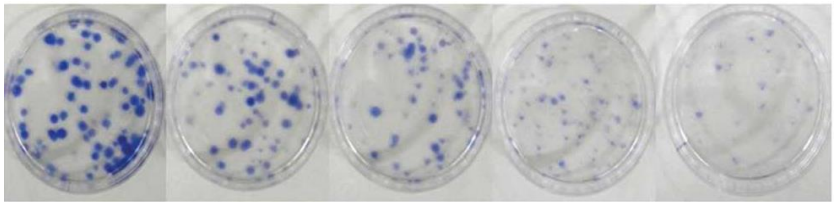
Test au rouge neutre



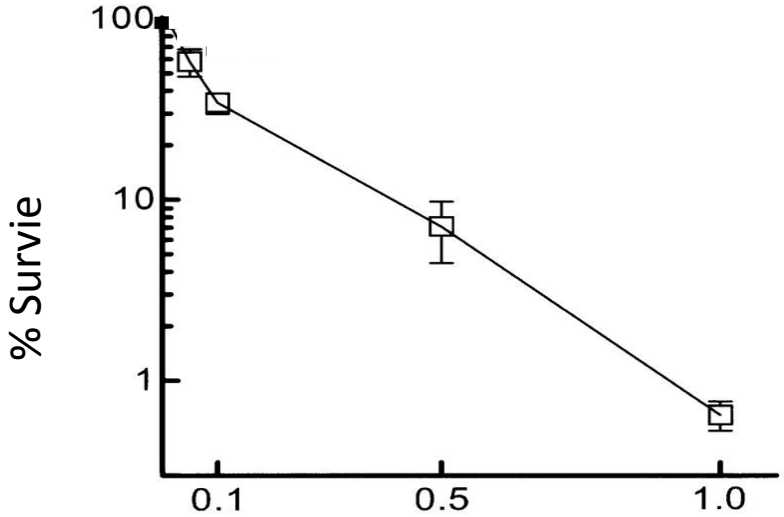
Donnent une indication sur les cellules vivantes/mortes

- Rapide
- Facile à mettre en œuvre
- Peu coûteux
- Idéal pour High Throughput Screening

Test d'efficacité de clonage (clonogenic assay)



Colloration au crystal violet

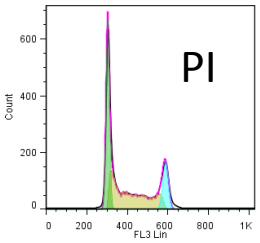


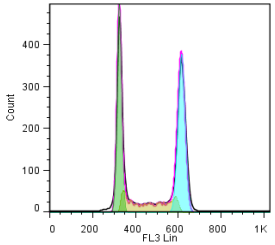
Concentration du médicament

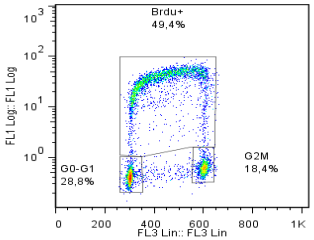
- A l'avantage d'évaluer exclusivement la mort cellulaire mais:
- Technique longue (~ 14j), difficilement miniaturisable et automatisable
- Ne s'applique qu'aux cellules ayant une bonne efficacité de clonage

L'apport de la cytométrie en flux

Analyse du cycle cellulaire

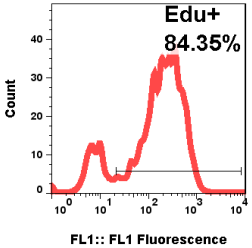


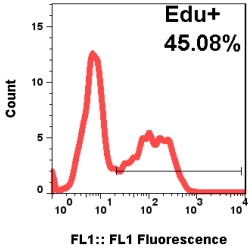


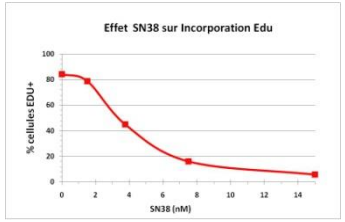


PI+BrdU

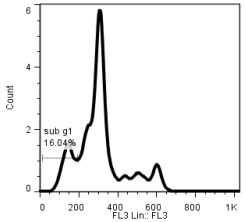
Prolifération





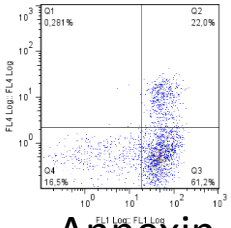


Apoptose

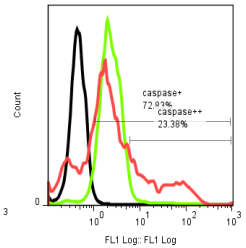


PI

PI

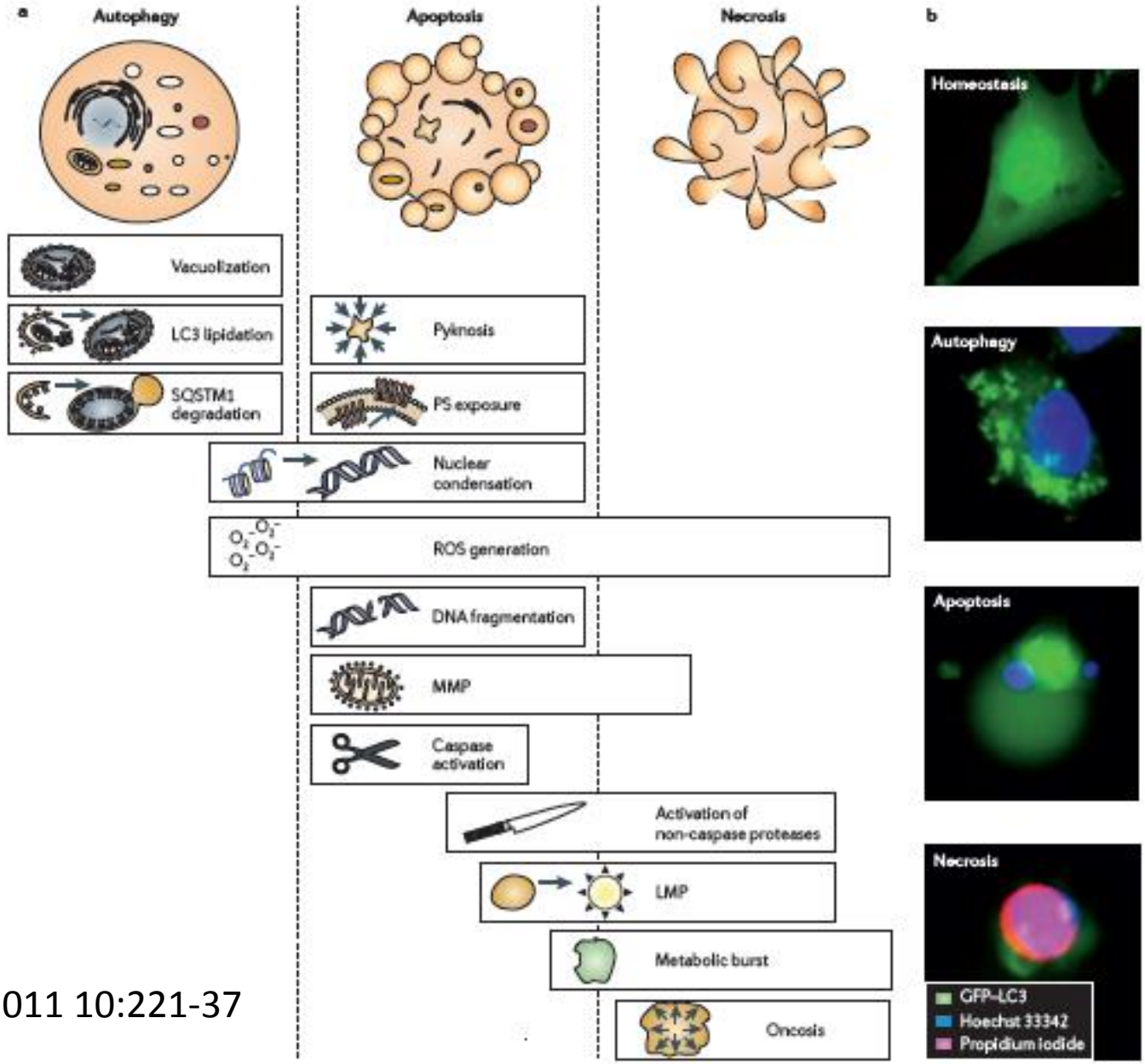


Annexin



Caspase 3

Beaucoup d'autres paramètres à considérer



Nat Rev Drug Discov 2011 10:221-37

Les limitations des modèles cellulaires 2D…

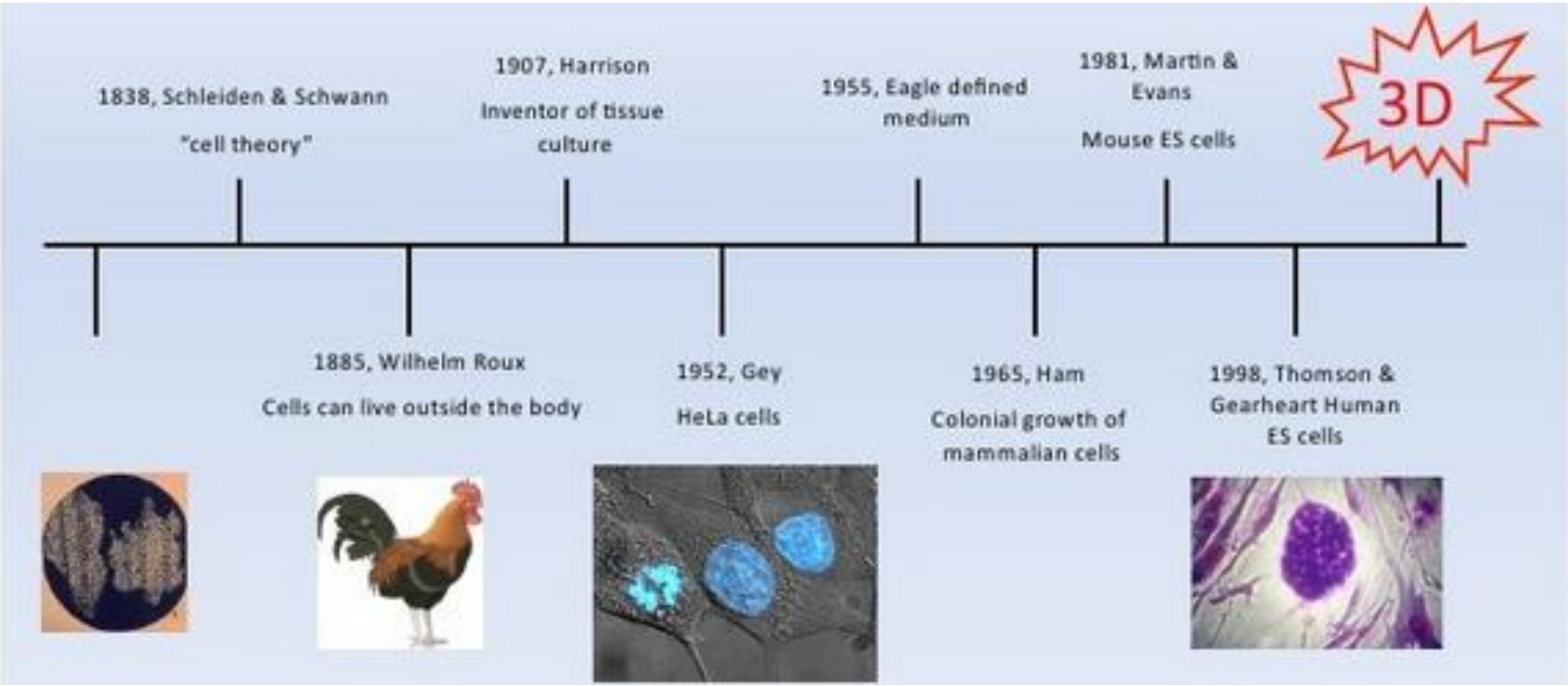
- ✓ **Liées à leur utilisation:**
  - **Possibilité de dérive clonale**
  - **Mycoplasmes**
  - **Problème de cross-contamination**

- ✓ **N'appréhendent pas la toxicité du médicament**
  - **Absence de système de métabolisation du médicament**

- ✓ **Absence « d'environnement tumoral »**
  - **Co-cultures**
  - **Cultures 3D**
  - **Organoïdes**

Good Bye Flat Biology…
Welcome to 3D Culture Model

Cell culture Evolution



The 3D culture as a tool to reduce animal testing and evaluate drug candidate

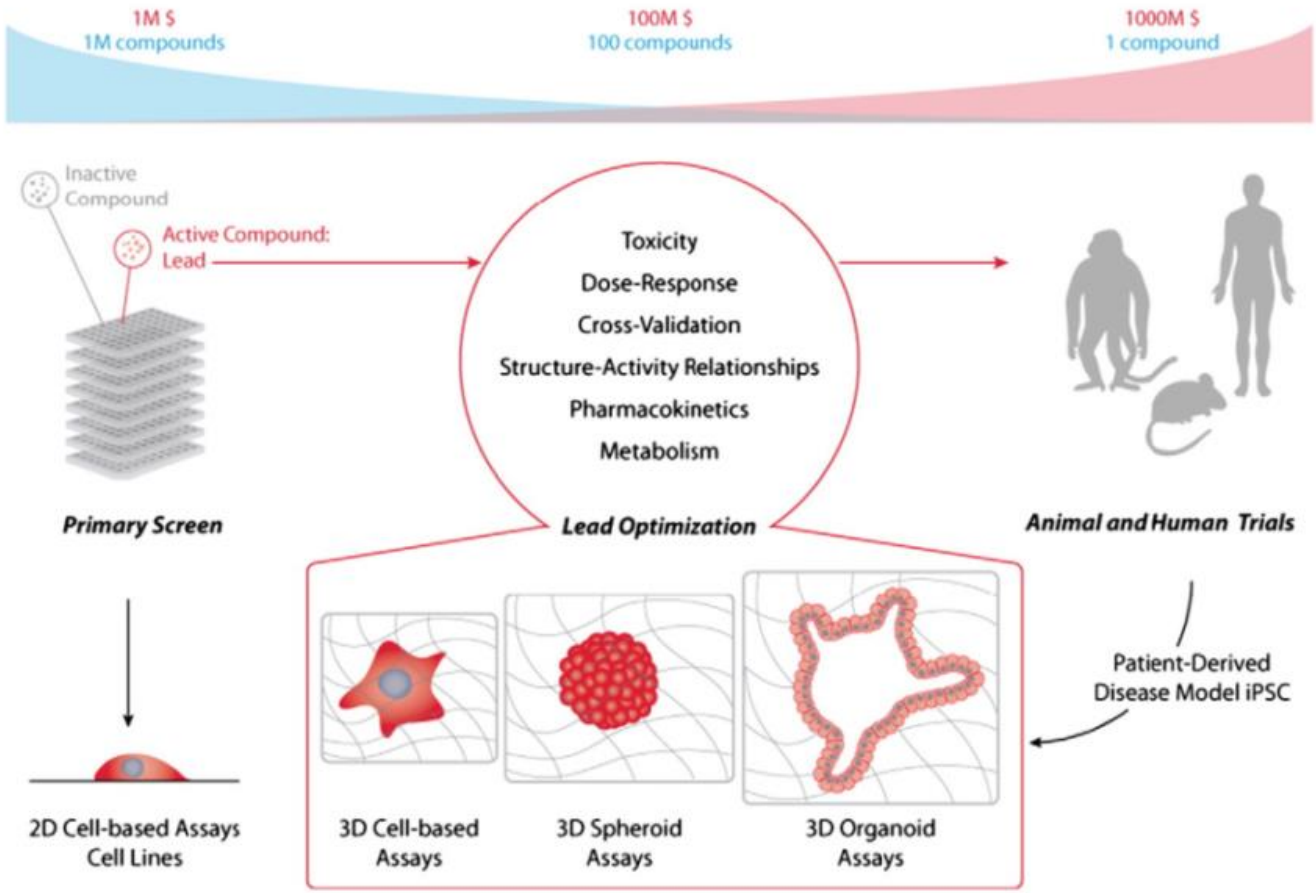
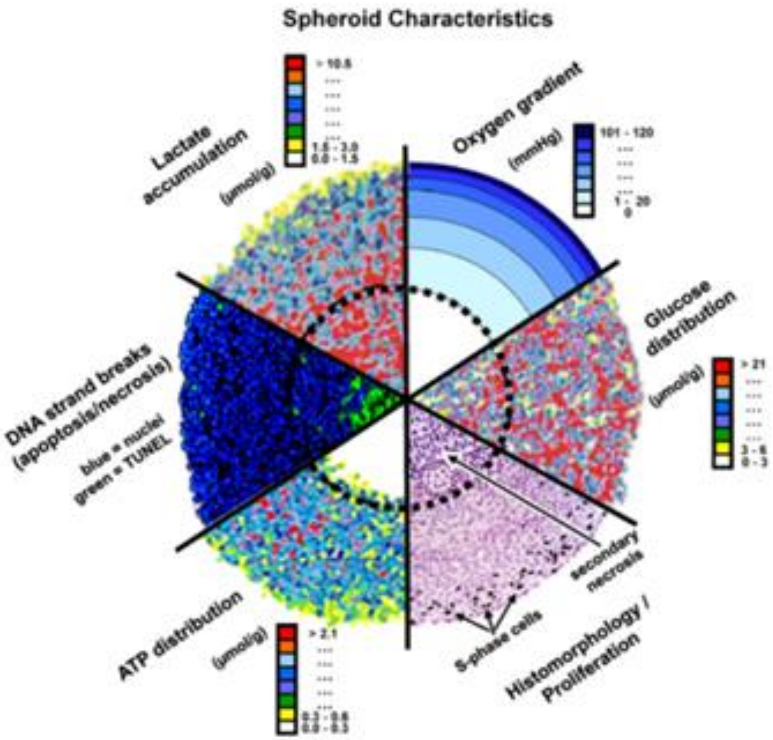
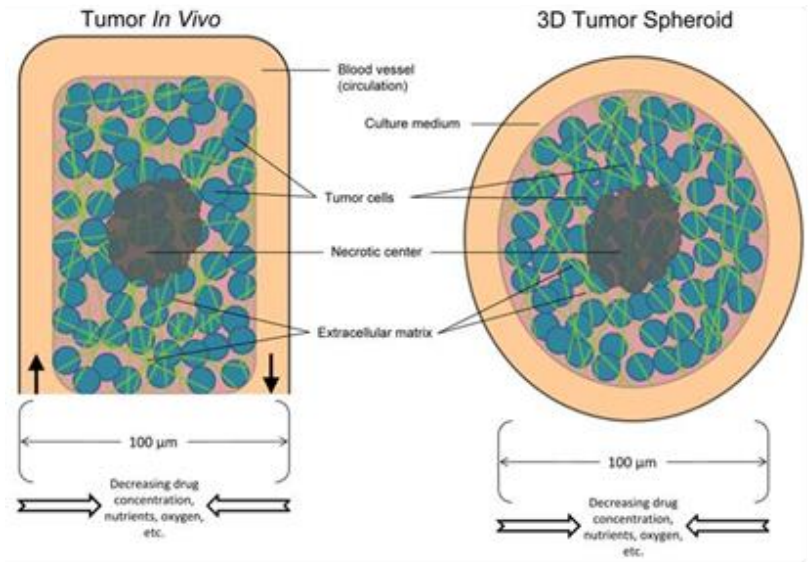


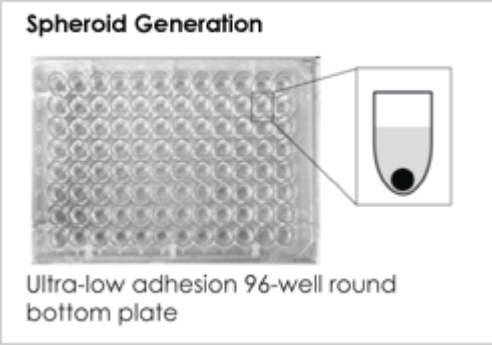
Fig. 1. 3D assays could bridge the gap between primary screening and animal and human trials. Drug discovery pipeline typically proceeds from multiple compounds tested at relatively low cost to few compounds in high-cost high-risk trials. The process of lead optimization and validation can benefit from increasingly representative in vitro technologies.

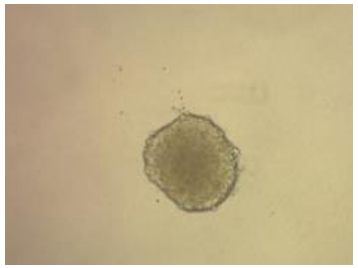




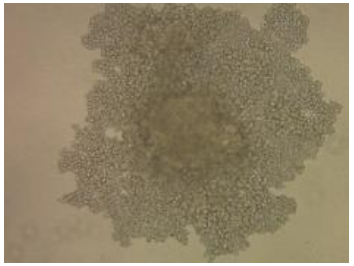
* **Speroids exhibit many features of tumor microencironement :**
- Hypoxia
- large between blood vessel
- High instestinal fluid pressure
- Structure and composition of the extracellular matrix
- Cell-cell adhesion
- 3D gene expression profiles reflect clinical expression profiles

* **3D culture models :**
- allow for a simple diffusion of nutrients and oxygen and may better reflect the tumour microenvironnement compare to a fully oxygened and nourished 2D cell layer
- offers the promise of improving clinical eficacy prediction





SW80 tight spheroid



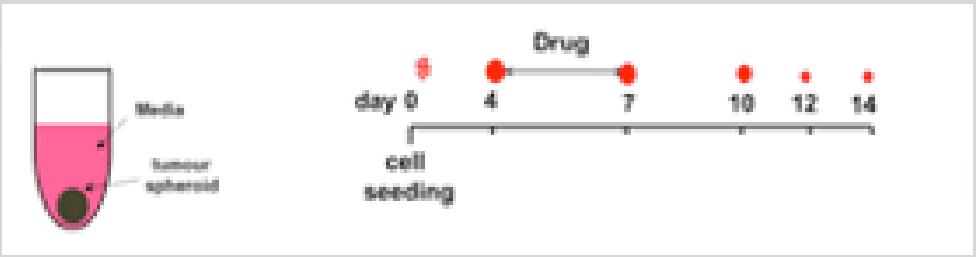
SW620 loose aggregate

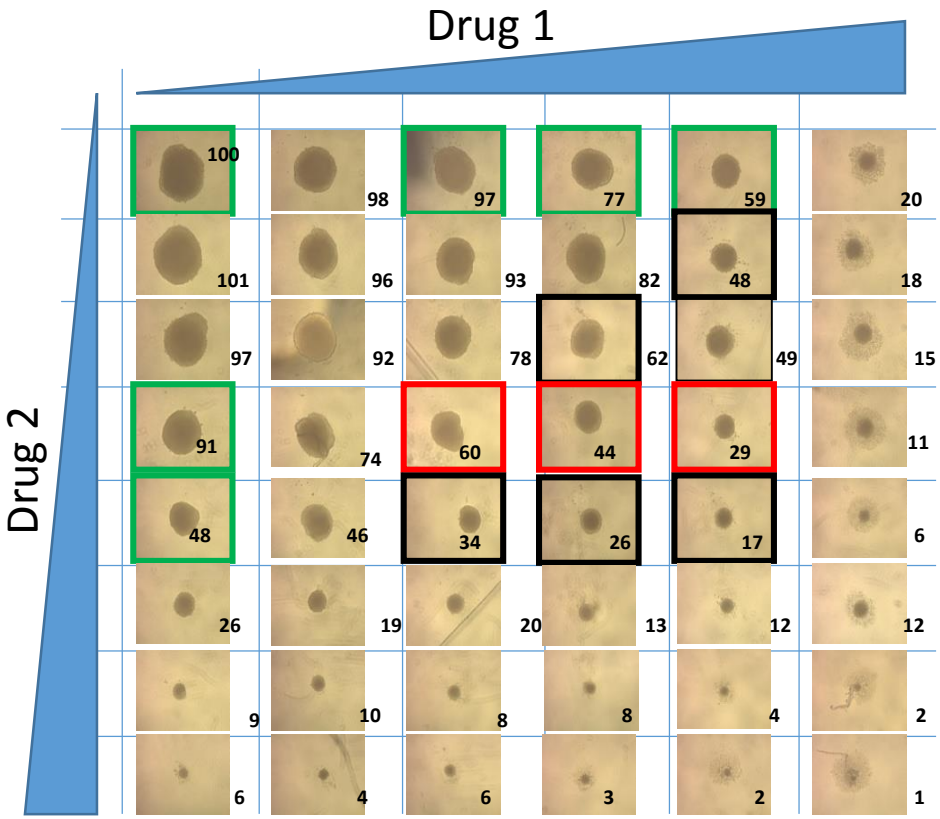
•96-well round ULA plate. A single spheroid / well is generated in 24h-48h in the center of the well .

•Classification of spheroids according to their morphology (tight, spherical and regular, compact aggregate, loose aggregate that could be improved by a addition of matrigel at the time of initiation)

•Optimal seeding density should be established for each cell line until a size range of 300-500 µm diameter on day 4 considered appropriate for initiating experimental .

• A size that is sufficient to develop hypoxia in the center. The smaller size may not develop hypoxia and larger spheroids develop a in their centers.


Media
Tumour spheroid
Drug
day 0
4
7
10
12
14
cell seeding


Drug 1
Drug 2
100
98
97
77
59
20
101
96
93
82
48
18
97
92
78
62
49
15
91
74
60
44
29
11
48
46
34
26
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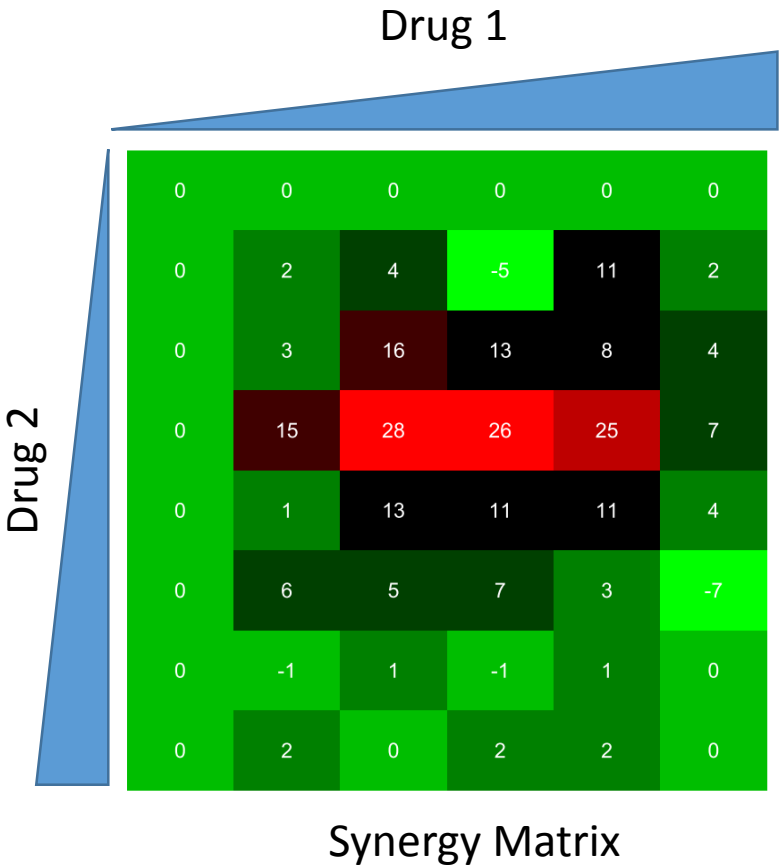
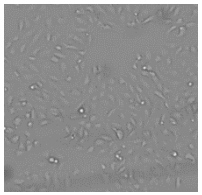
4
6
3
2
1


Drug 1
Drug 2
0 0 0 0 0 0
0 2 4 -5 11 2
0 3 16 13 8 4
0 15 28 26 25 7
0 1 13 11 11 4
0 6 5 7 3 -7
0 -1 1 -1 1 0
0 2 0 2 2 0
Synergy Matrix

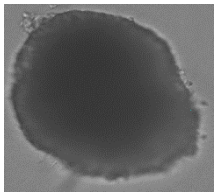
The Celigo® is a benchtop *in situ* cellular analysis system that rapidly provides high integrity whole well images for routine brightfield and fluorescent cellular analysis with a simple workflow.

Brightfield (Segmentation software)

2D-culture

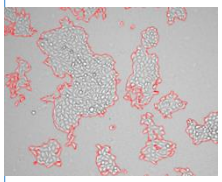


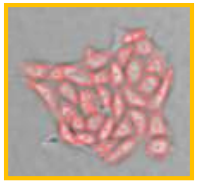
3D-culture

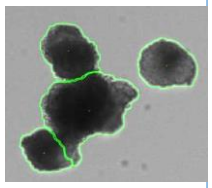


Proliferation /growth tracking

-number of cells/confluence (2D culture)
-number/size/volume (3D culture)

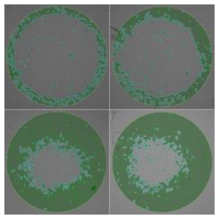


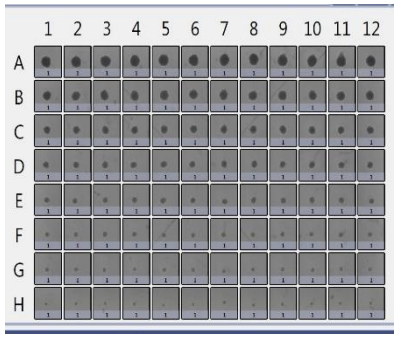


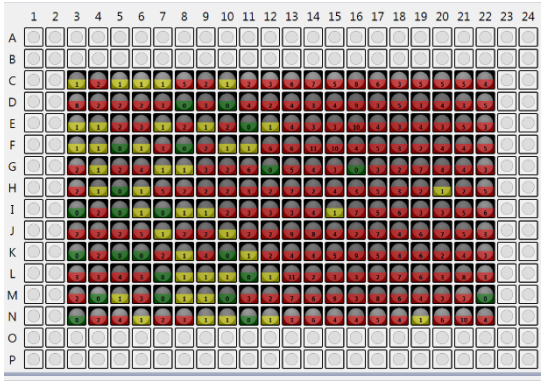


Migration

Wound Area Fill View

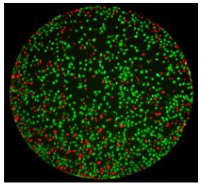




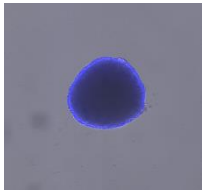


Fluorescence

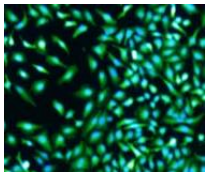
2D-culture

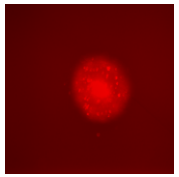


3D-culture



Cell health assay





-viability/apoptosis (2D culture/3D culture)

-DNa synthesis/cell cycle

-internalisation

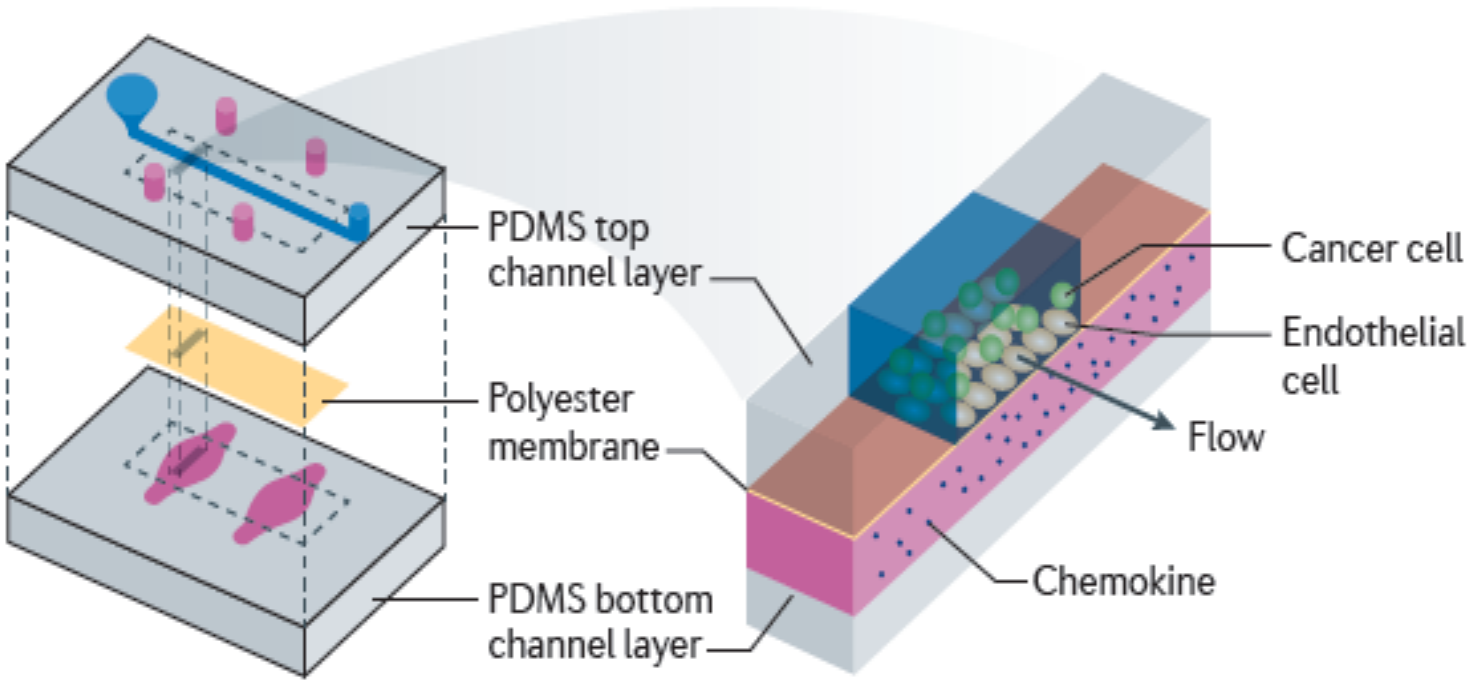
-phospho protein detection

L'avenir :

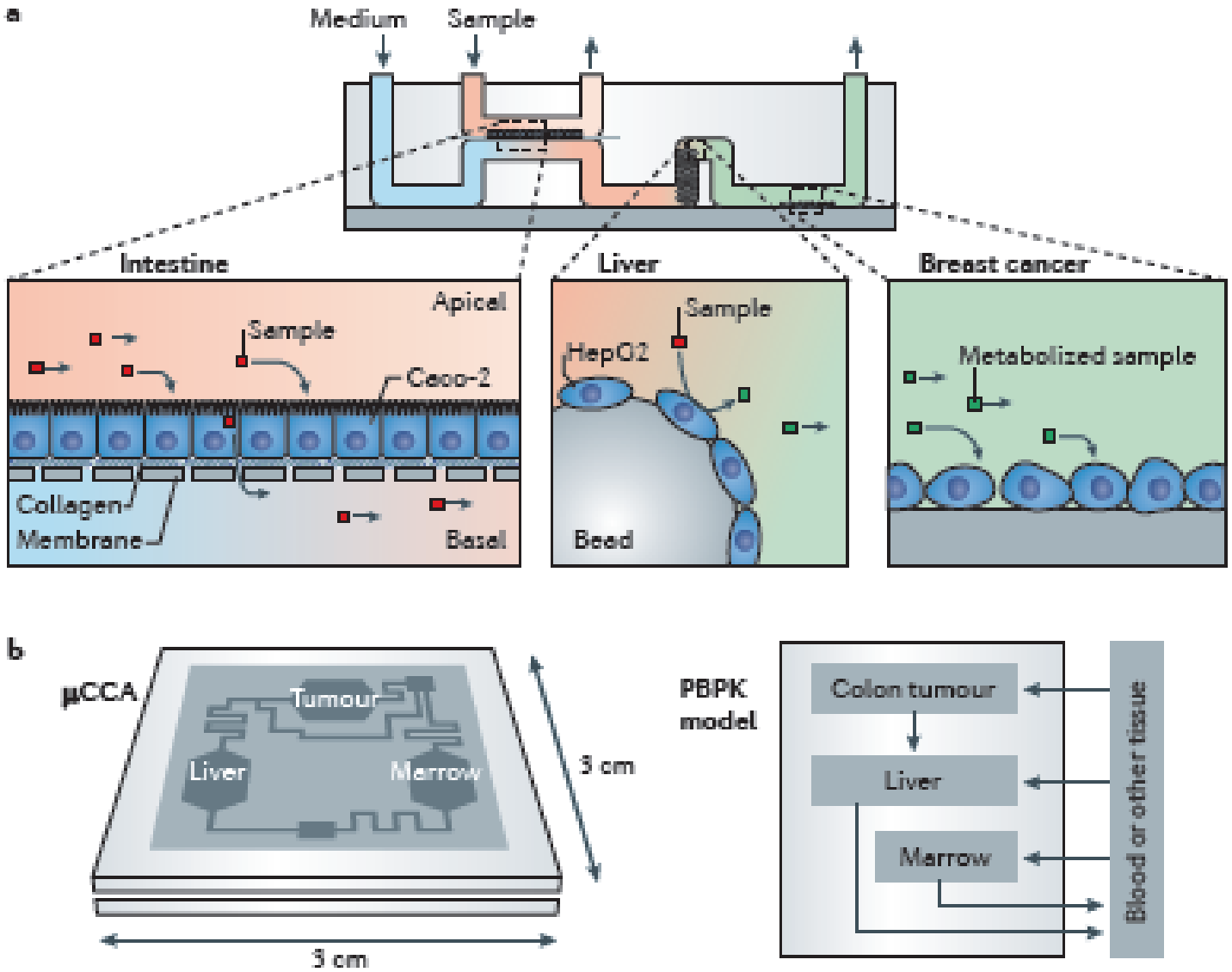
Organs-on-chips at the frontiers of drug discovery

Eric W. Esch, Anthony Bahinski and Dongeun Huh

Nat Rev Drug Discov 2015 14:248-260



Vers le body-on-chip ?



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Quelques conclusions

- ✓ Les modèles in vitro sont incontournables pour l'identification de nouvelles molécules anticancéreuses mais ne remplacent pas les modèles animaux

- ✓ Ils sont indispensables pour l'étude des mécanismes d'action et/ou de résistance à ces molécules

- ✓ Ils doivent être choisis sur une base rationnelle prenant en compte leur spécificités génétiques

- ✓ Les tests utilisés doivent être judicieusement choisis en fonction de la question posée