

Small molecule discovery



COLLABORATE. INNOVATE. ACCELERATE.

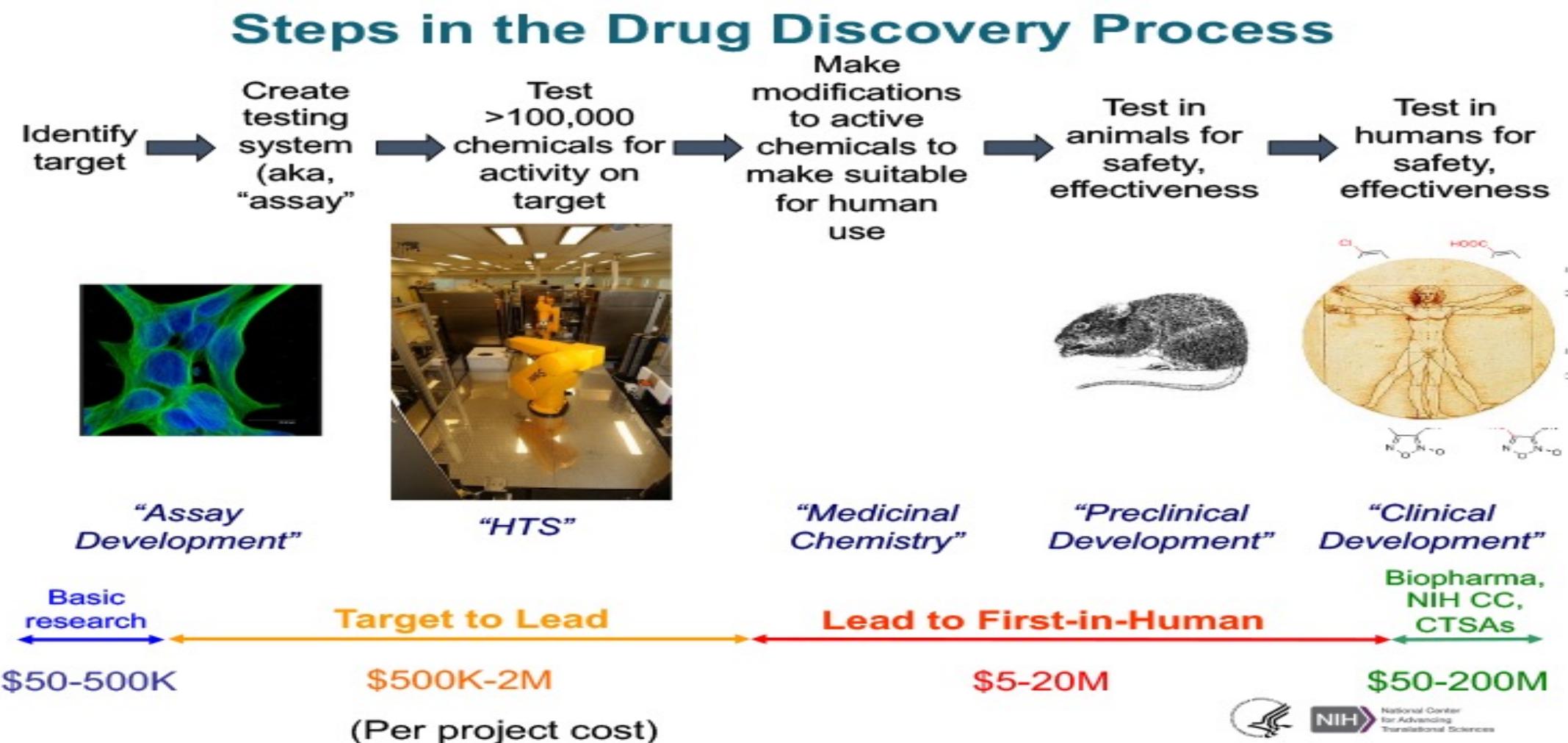
Small Molecule Discovery in Oncology and Beyond: Challenges and Opportunities

Anton Simeonov, Ph.D.

*Scientific Director, National Center for Advancing Translational
Sciences (NCATS), National Institutes of Health (NIH)*

TRACO Lecture
October 3, 2022

Drug discovery steps

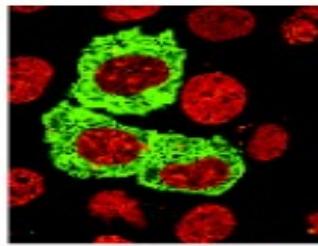


Screening assays

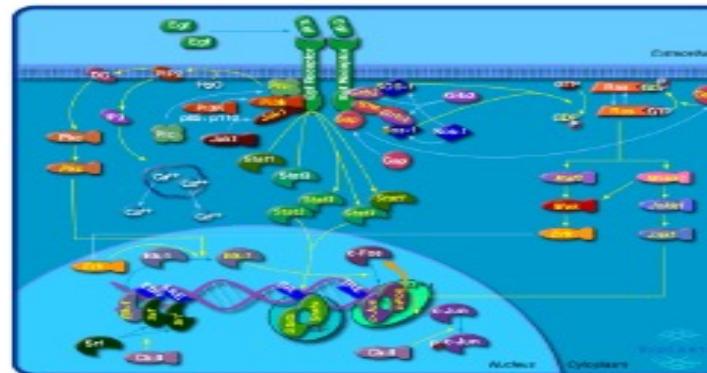
Range of Screening Assays

Extent of reductionism

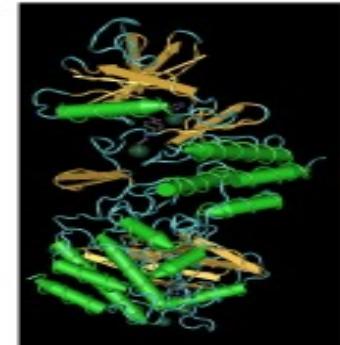
Phenotype
(Image-based
HCS, GFP, etc)



Pathway
(Reporters, e.g., luciferase, β -lactamase)



Protein
(Enzyme readouts, interactions, etc)

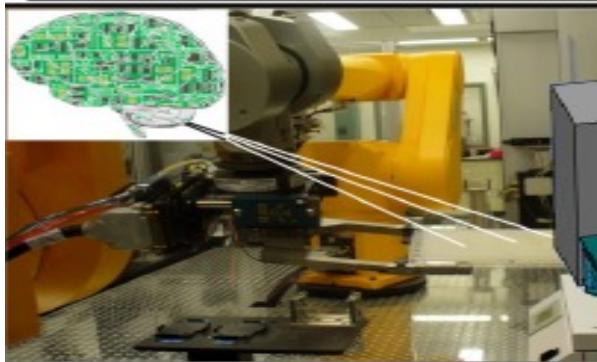


NIH

National Center
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Translational Sciences

Robotics

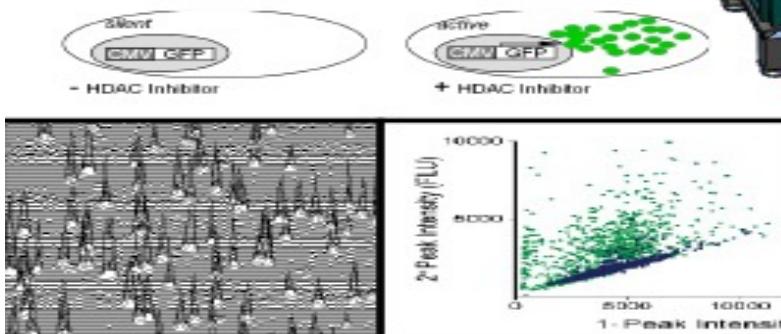
Robotics & Informatics



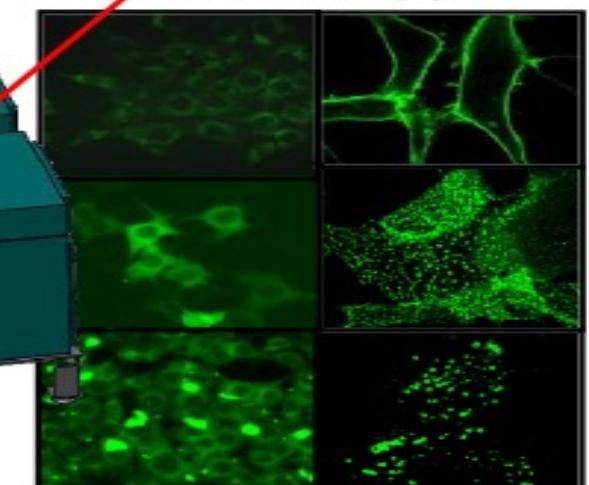
Microliter Dispensing



Laser Cytometry



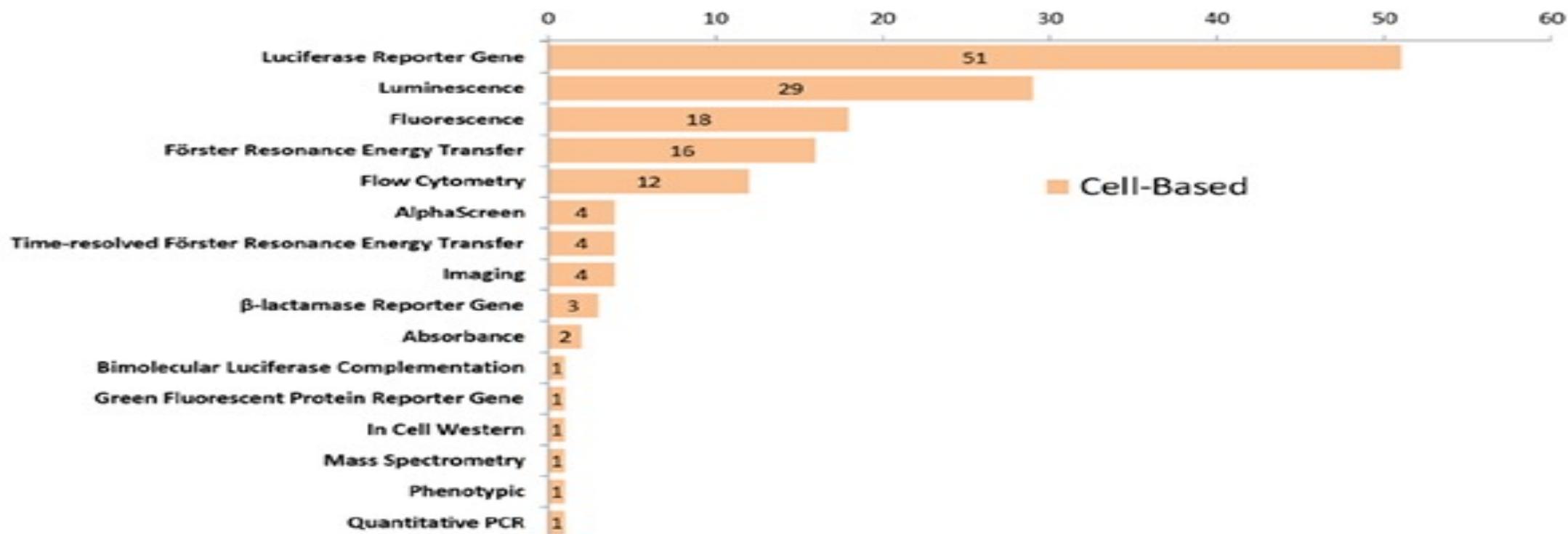
Microscopy



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Translational Sciences

HTS assays

149 Cancer Relevant Cell-Based HTS Assays from PubChem



Coussens, N. P., Braisted, J. C., Peryea, T., Sittampalam, S. G., Simeonov, A. and Hall, M. D. **Small Molecule Screens: A Gateway to Cancer Therapeutic Agents with Case Studies of FDA-Approved Drugs** *Pharmacological Reviews*, October 2017, 69 (4) 479-496

Assay expense

Important Considerations for Choosing an Assay

- **Assay expense**
 - Cost per well
 - Disposal cost(s)

Instrumentation

Important Considerations for Choosing an Assay

- Assay expense
 - Cost per well
 - Disposal cost(s)
- **Available instrumentation**
 - Select the best possible assays based on the available instrumentation

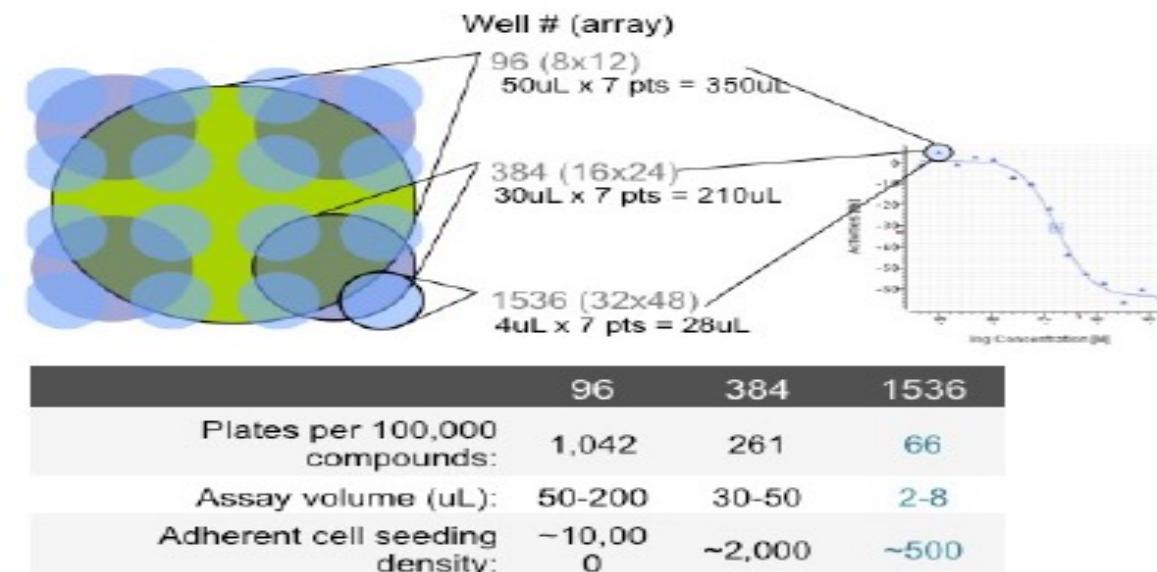
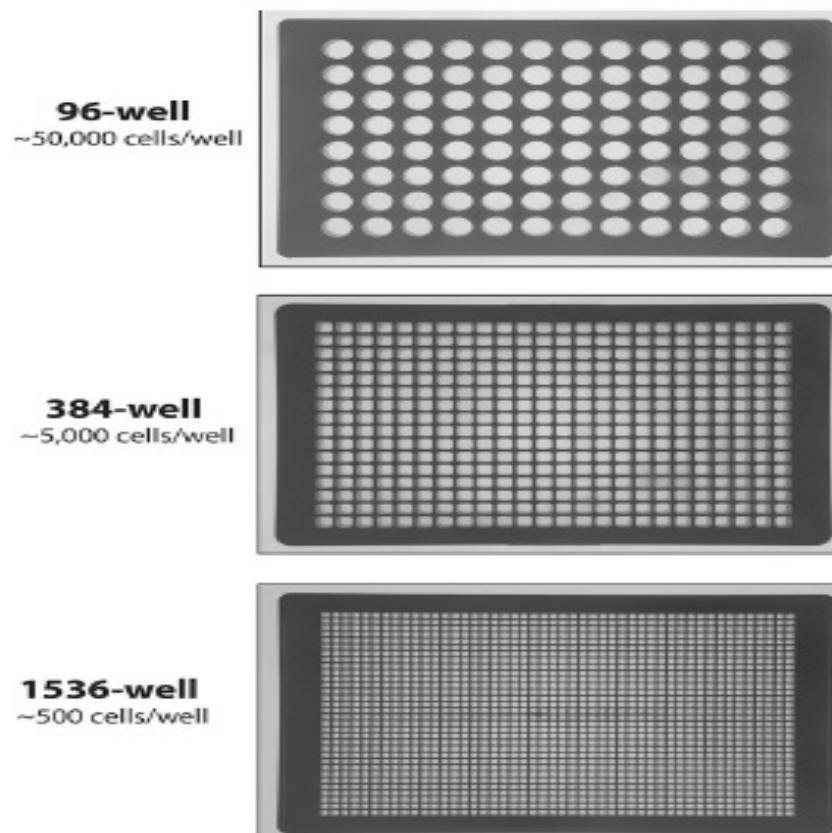
Throughput

Important Considerations for Choosing an Assay

- Assay expense
 - Cost per well
 - Disposal cost(s)
- Available instrumentation
 - Select the best possible assays based on the available instrumentation
- **Assay throughput**
 - Miniaturization reduces the cost per well

Miniaturization

Assay Miniaturization Saves Time and Reagents



Horman, Shane R. "Complex High-Content Phenotypic Screening." Special Topics in Drug Discovery. InTech, 2016.

Multiplex

Important Considerations for Choosing an Assay

- Assay expense
 - Cost per well
 - Disposal cost(s)
- Available instrumentation
 - Select the best possible assays based on the available instrumentation
- Assay throughput
 - Miniaturization reduces the cost per well
- **Ability to multiplex**
 - Can the response be measured by a single parameter; is multiparametric output possible?
 - Increased data per sample
 - Can guide hit selection by differentiating selectivity among related targets
 - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay

Reagents

Important Considerations for Choosing an Assay

- **Assay expense**
 - Cost per well
 - Disposal cost(s)
- **Available instrumentation**
 - Select the best possible assays based on the available instrumentation
- **Assay throughput**
 - Miniaturization reduces the cost per well
- **Ability to multiplex**
 - Can the response be measured by a single parameter; is multiparametric output possible?
 - Increased data per sample
 - Can guide hit selection by differentiating selectivity among related targets
 - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay
- **Reagents**
 - Stability for hours is important
 - Consistency is critical (ideally obtain a large quantity from a single lot)
 - All reagents need to be validated (cell lines, antibodies, enzymatic purity, etc.)

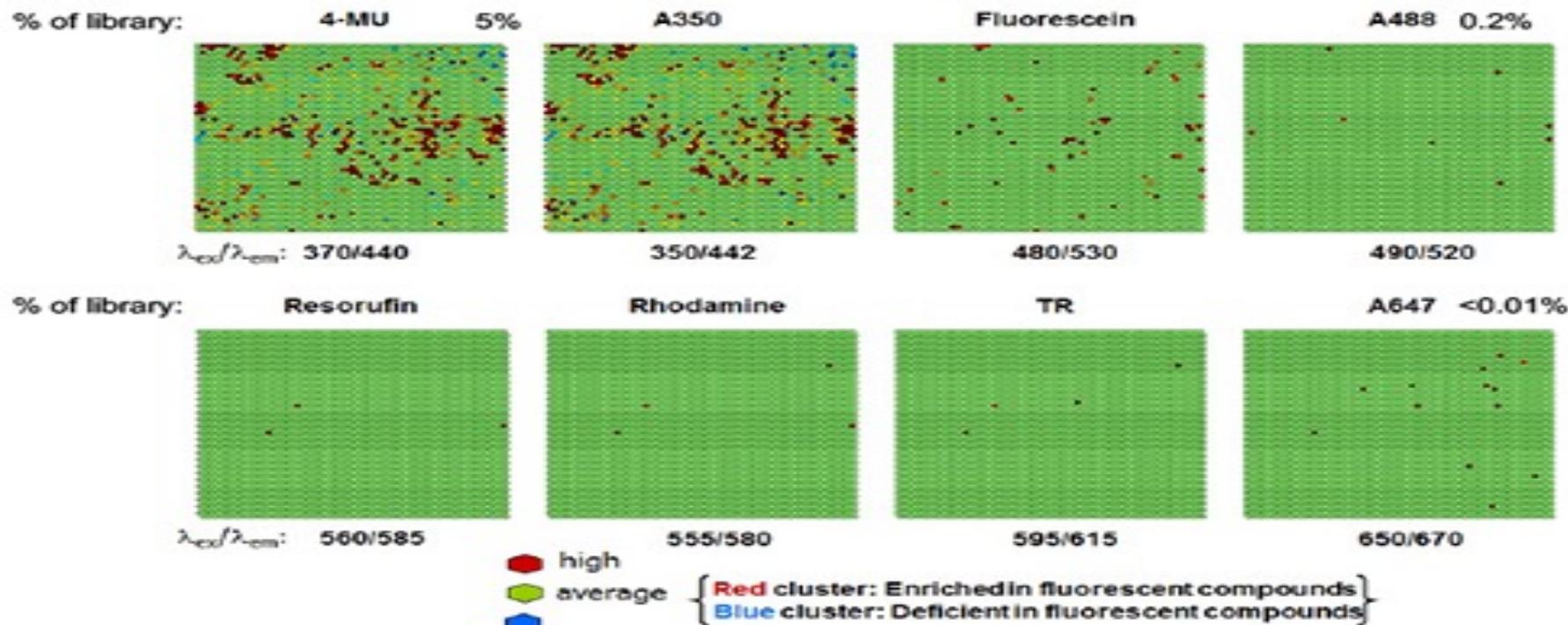
Interference

Important Considerations for Choosing an Assay

- Assay expense
 - Cost per well
 - Disposal cost(s)
- Available instrumentation
 - Select the best possible assays based on the available instrumentation
- Assay throughput
 - Miniaturization reduces the cost per well
- Ability to multiplex
 - Can the response be measured by a single parameter; is multiparametric output possible?
 - Increased data per sample
 - Can guide hit selection by differentiating selectivity among related targets
 - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay
- Reagents
 - Stability for hours is important
 - Consistency is critical (ideally obtain a large quantity from a single lot)
 - All reagents need to be validated (cell lines, antibodies, enzymatic purity, etc.)
- Potential for assay interference
 - Fluorescent compounds can interfere with fluorescent readouts
 - Colored compounds might interfere with luminescence

Spectroscopic profiling

Fluorescence Spectroscopic Profiling of Compound Libraries

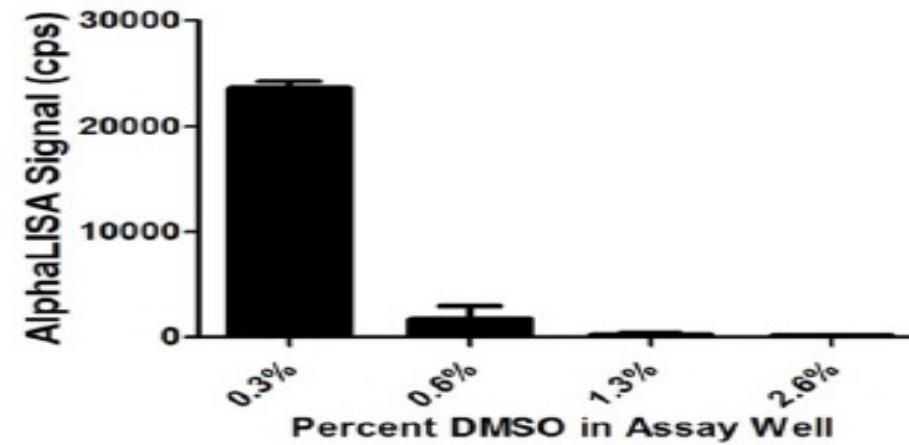
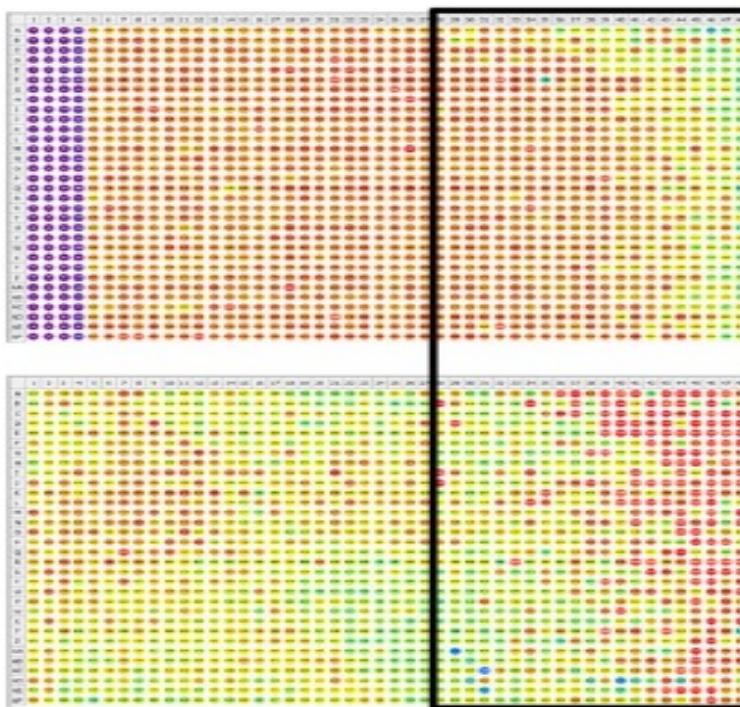


Simeonov, A., Jadhav, A., Thomas, C.J., Wang, Y., Huang, R., Southall, N.T., Shinn, P., Smith, J., Austin, C.P., Auld, D.S. and Inglese, J., 2008. **Fluorescence spectroscopic profiling of compound libraries**. *Journal of Medicinal Chemistry*, 51(8), 2363-2371.



Tolerance to DMSO

Determination of Assay Tolerance to DMSO/Vehicle is Important



Yasgar A., Jadhav A., Simeonov A., Coussens N.P., **AlphaScreen-Based Assays: Ultra-High-Throughput Screening for Small-Molecule Inhibitors of Challenging Enzymes and Protein-Protein Interactions.** *Methods Mol Biol.* 2016;1439:77-98.

Homogenous format

Important Considerations for Choosing an Assay

- **Homogenous assay format is preferred for screening**
 - Add reagents, mix and measure (no solution removal or wash steps)
 - Automation friendly
 - Reduces variability
 - Decreases hands-on time
 - Improves reproducibility

Time required

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
 - Add reagents, mix and measure (no solution removal or wash steps)
 - Automation friendly
 - Reduces variability
 - Decreases hands-on time
 - Improves reproducibility
- **Time required for assay**
 - Off-line reagent preparation
 - Is temperature equilibration required
 - Actual assay time
 - Kinetic versus end point read
 - Time required for data analysis and record keeping

Signal stability

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
 - Add reagents, mix and measure (no solution removal or wash steps)
 - Automation friendly
 - Reduces variability
 - Decreases hands-on time
 - Improves reproducibility
- Time required for assay
 - Off-line reagent preparation
 - Is temperature equilibration required
 - Actual assay time
 - Kinetic versus end point read
 - Time required for data analysis and record keeping
- Signal stability
 - Does the response occur rapidly or within a few minutes or hours?
 - Longer signal stability allows for flexibility in automated systems
 - Longer signal stability minimizes differences among plates within a stack

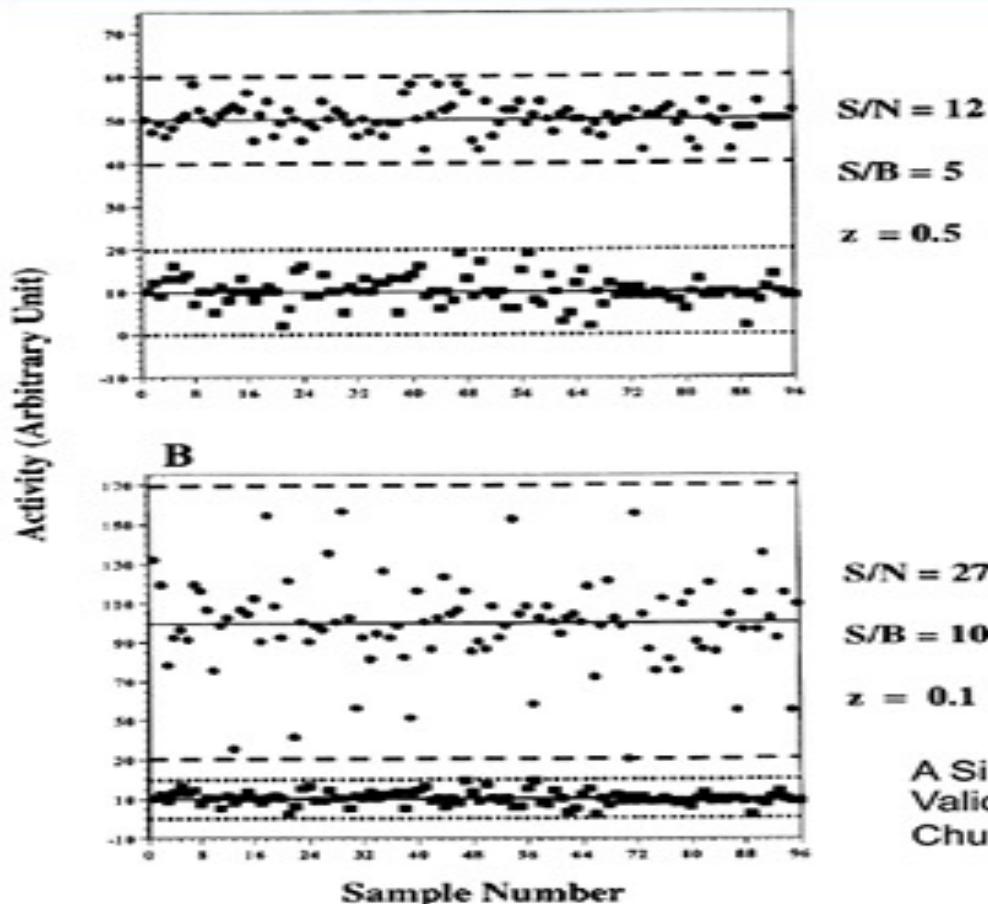
Sensitivity

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
 - Add reagents, mix and measure (no solution removal or wash steps)
 - Automation friendly
 - Reduces variability
 - Decreases hands-on time
 - Improves reproducibility
- Time required for assay
 - Off-line reagent preparation
 - Is temperature equilibration required
 - Actual assay time
 - Kinetic versus end point read
 - Time required for data analysis and record keeping
- Signal stability
 - Does the response occur rapidly or within a few minutes or hours?
 - Longer signal stability allows for flexibility in automated systems
 - Longer signal stability minimizes differences among stacks of plates
- Assay Sensitivity
 - Choice of readouts is important
 - Colorimetric < fluorescent < luminescent

Evaluating assay

Evaluating Assay Suitability for Screening



$$S/N = \frac{\text{mean signal} - \text{mean background}}{\text{standard deviation of background}}$$

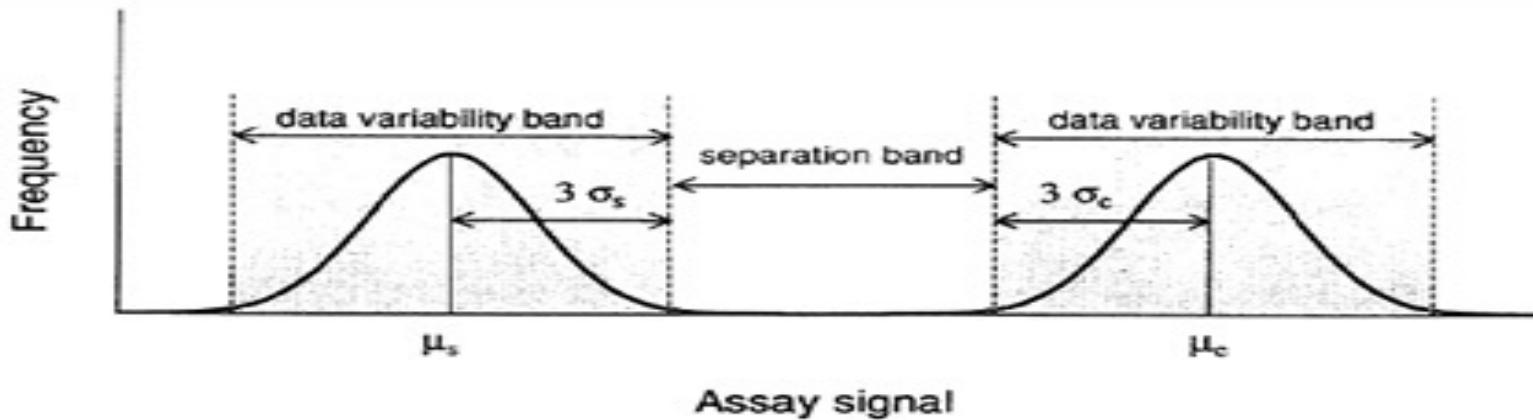
$$S/B = \frac{\text{mean signal}}{\text{mean background}}$$

$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{|\text{mean of sample} - \text{mean of control}|}$$

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. *J Biomol Screen.* 1999;4(2):67-73.

Suitability

Evaluating Assay Suitability for Screening



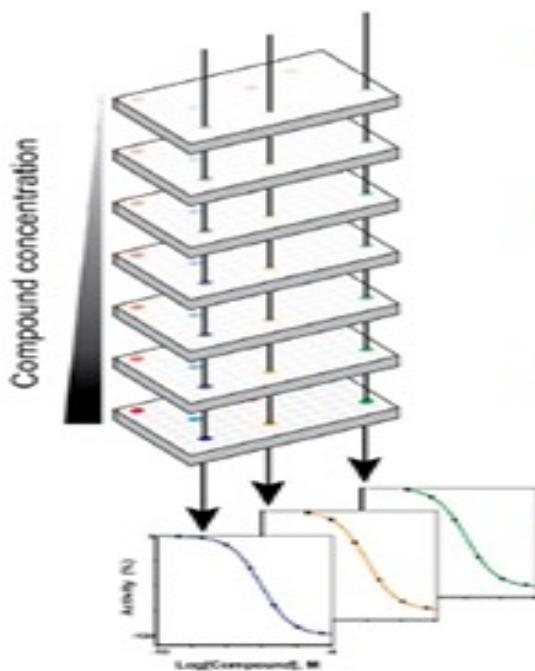
$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{|\text{mean of sample} - \text{mean of control}|} *$$

Z-factor value	Structure of assay	Related to screening
1	SD = 0 (no variation), or the dynamic range $\rightarrow \infty$	An ideal assay
$1 > Z \geq 0.5$	Separation band is large	An excellent assay
$0.5 > Z > 0$	Separation band is small	A double assay
0	No separation band, the sample signal variation and control signal variation bands touch	A "yes/no" type assay
<0	No separation band, the sample signal variation and control signal variation bands overlap	Screening essentially impossible

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. J Biomol Screen. 1999;4(2):67-73.

Quantitative HTS

Improving the Process of Early Discovery: Quantitative High-Throughput Screening (qHTS)



- Conventional screening done at one concentration
 - Not appropriate for potency testing – “dose makes the poison”
- qHTS tests compounds assayed at **multiple** concentrations (range: 4 logs)
- Enabled by miniaturized assay volumes (2-8 μ L per test) and informatics pipeline
- Generates *pharmacological actives* instead of statistical “hits”
 - Dramatically increases reliability
 - Dramatically reduces false positives and false negatives
- *To date, several hundred million datapoints from several hundred screens have been generated and deposited in the public domain.*

Medicinal chemistry

Medicinal Chemistry, an Integrated Process



Tier 1: Synthesis & validation

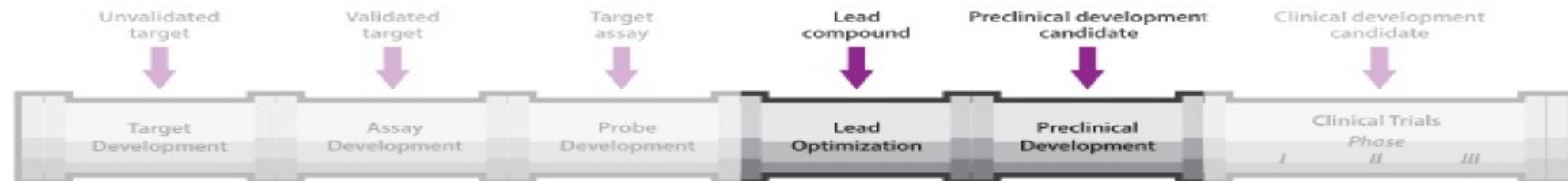
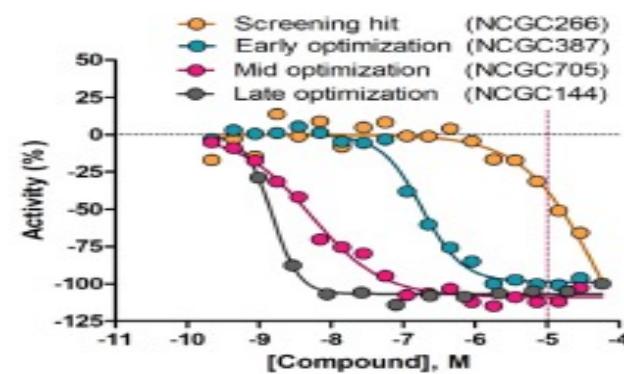
- Medicinal chemistry
- Purification
- In vitro ADME

Tier 2: Compound profile expansion

- Met ID/CYP studies
- In vitro toxicology

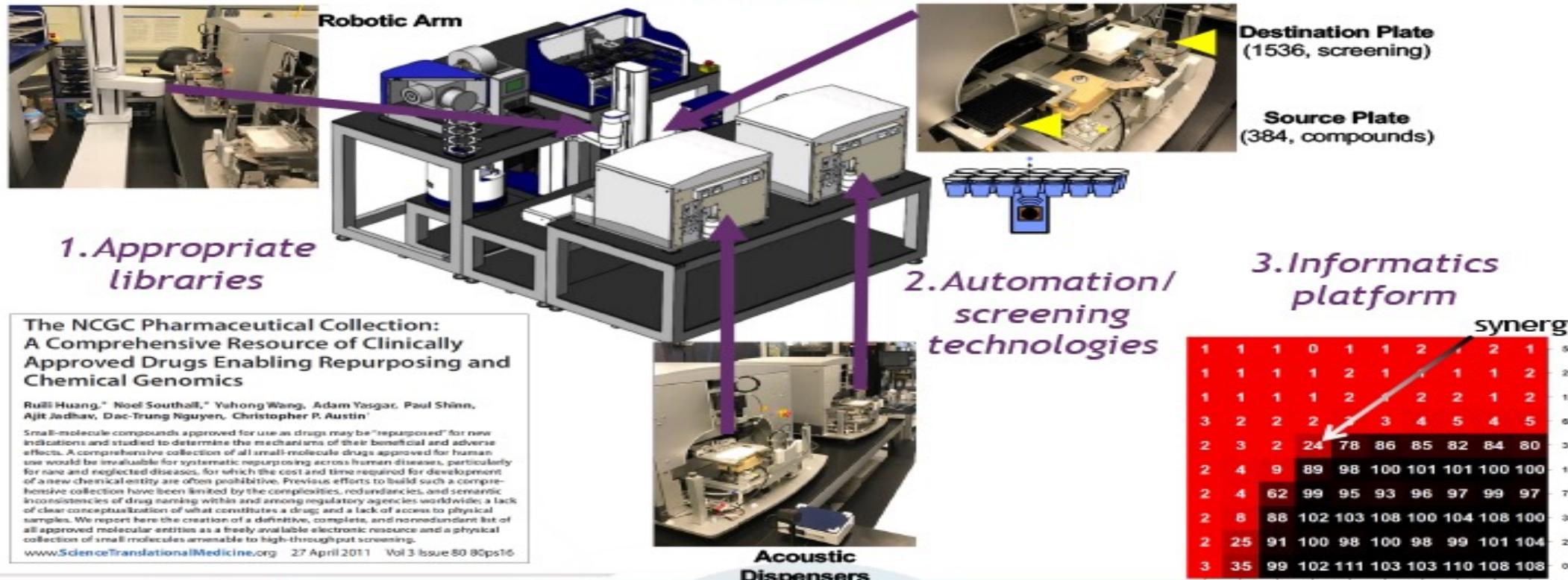
Tier 3: Advanced Preclinical studies

- Formulation
- Scale-up
- In vivo PK/PD
- Preclinical toxicology



Drug combinations

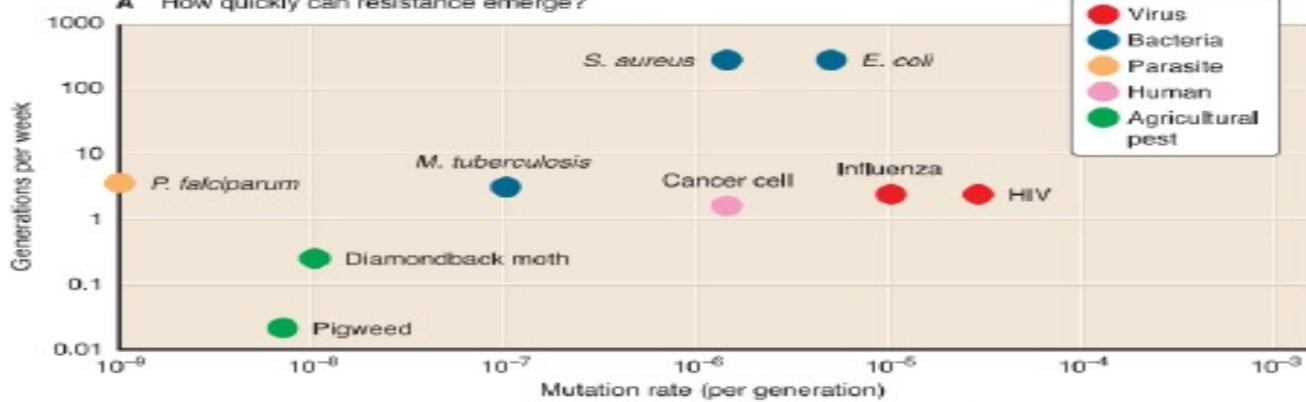
Translation Challenge: Rapid Discovery of Drug Combinations



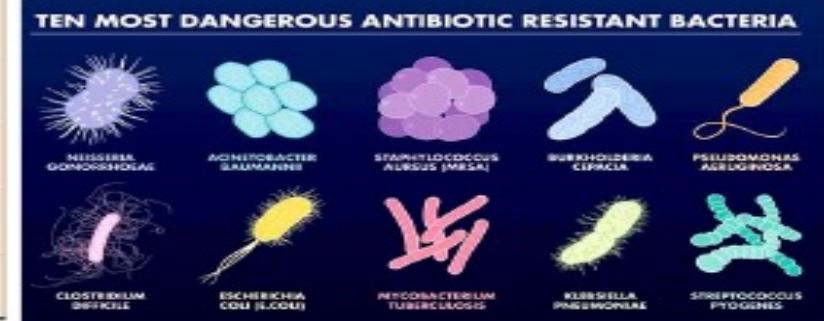
Resistance

Application of Drug Combinations to Address Resistance

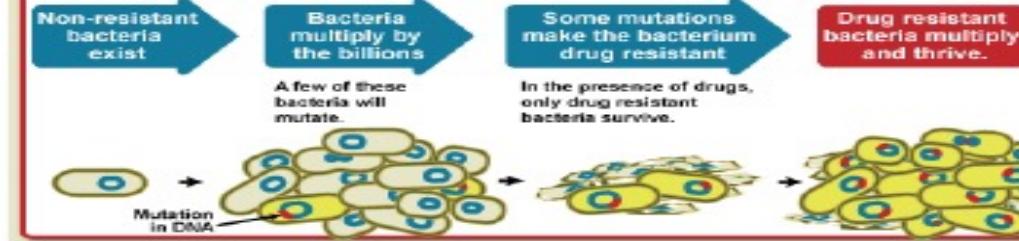
A. How quickly can resistance emerge?



- Virus
- Bacteria
- Parasite
- Human
- Agricultural pest



Genetic Mutation Causes Drug Resistance



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Dissemination

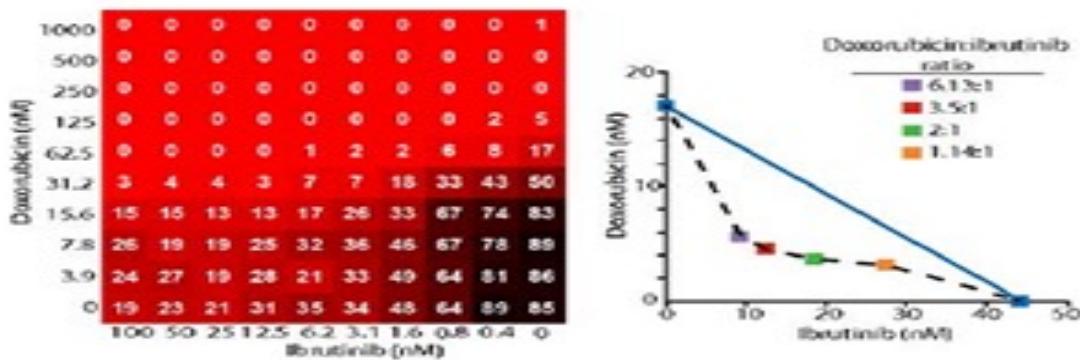
Dissemination of technology: combination screening to overcome drug resistance in cancer cells

- Applied to ABC subtype of Diffuse Large B-Cell Lymphoma (ABC-DLBCL)
- Ibrutinib is a BTK inhibitor that has activity against ABC DLBCL
- Lead investigators: Craig Thomas (NCATS) and Louis Staudt (NCI)
- Study evaluated 459 drugs *in combination* with Ibrutinib
 - » 6 x 6 concentration-response “matrix blocks”, validation in 10 x 10 concentration-response matrix blocks
- DNA-damaging agents identified as synergizing with Ibrutinib in killing ABC DLBCL cell lines
- **Dissemination:**
 - » Protocols
 - » Source code for dispense

High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells

Lindsey A. Mathews*, Grilmer*, Rajanish Gubhaju*, Paul Shimoni*, Ryan M. Young*, Jonathan M. Kather*, Donghui Liu*, Ian S. Goldkurst*, Adam Yang*, Crystal McKnight*, Matthew B. Becker*, Damien Y. Deneuve*, Ram-Kang Kang*, Sean Michael*, Tim Mikrawa*, Wanwei Huang*, Martin A. Whelchel*, Brynn T. Most*, Panesma Patel*, William Leiserson*, David J. McKinney*, Christopher A. Leclair*, Ganendra Rao*, Agit Jacob*, Brian D. Prysner*, Christopher P. Austin*, Scott E. Martin*, Anton Simonson*, Marc Perner*, Louis M. Staudt*, and Craig E. Thomas*

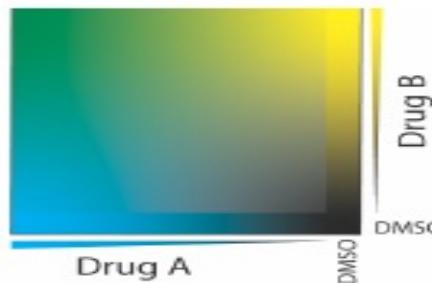
*Equal contribution. National Institutes of Health Chemical Genomics Center, National Center for Advancing Translational Sciences, Bethesda, MD 20892; Center for Cancer Research, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and Basic Science Programs, NCI, Bethesda, MD, Chemical Biology Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD 21702



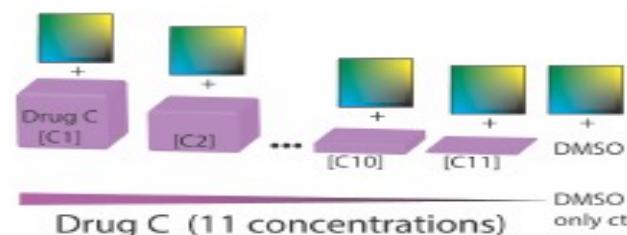
Triple drug combination

Example: triple drug combination screening to tackle resistance against artemisinin-based combination therapies in malaria

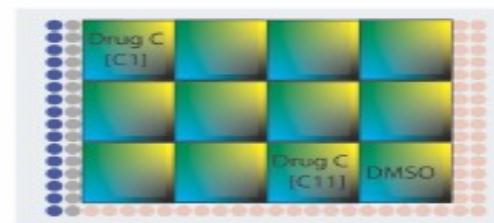
ACS Pharmacol. Transl. Sci. 2020, <https://dx.doi.org/10.1021/acsptsci.0c00110?ref=pdf>



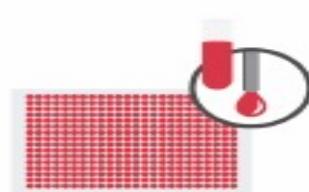
① Drugs A and B are acoustically dispensed in a 10x10-well matrix, 12 replicate blocks per plate. Single drug responses, bottom row (Drug A) and right column (Drug B).



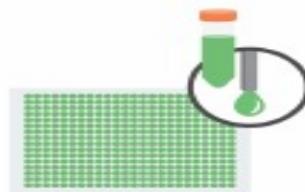
② To each replicate block, serial dilutions of Drugs C is acoustically dispensed, with the final block serving as a DMSO control



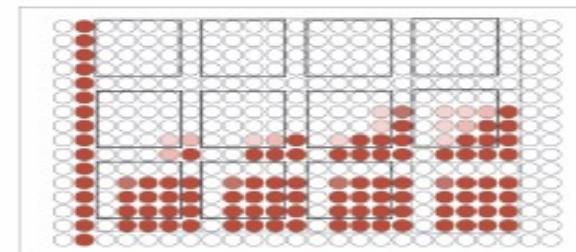
③ Plate view of triple combination screening plate with positive control (artesunate, blue) and neutral controls (DMSO, grey) also shown.



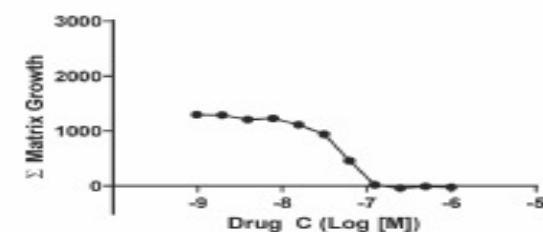
④ Dispense *P. falciparum* and erythrocytes, incubate 72 hr



⑤ Dispense 2 μ L of SYBR Green 1 and lysis solution, incubate overnight. Fluorescence quantified



⑥ Parasite proliferation response is normalized to artesunate and DMSO controls. For each concentration Drug C block, response of Drug A + Drug B wells is summed.



⑦ Triple drug response is analyzed as a function of Drug C concentration.

ViPOR combination

 U.S. National Library of Medicine
ClinicalTrials.gov

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Home > Search Results > Study Record Detail

Save this study

Venetoclax, Ibrutinib, Prednisone, Obinutuzumab, and Revlimid (ViPOR) in Relapsed/Refractory B-cell Lymphoma

⚠ The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT03223610

Recruitment Status: Recruiting
First Posted: July 21, 2017
Last Update Posted: July 7, 2022
[See Contacts and Locations](#)

Sponsor:
National Cancer Institute (NCI)
Information provided by (Responsible Party):
National Institutes of Health Clinical Center (CC) (National Cancer Institute (NCI))

ViPOR Regimen Is Safe, Shows Impressive Activity in Relapsed/Refractory DLBCL

December 27, 2020

Gene Hunt



December 27, 2020 — The 5-drug regimen of venetoclax, ibrutinib, prednisone, obinutuzumab, and lenalidomide showed a tolerable safety profile and encouraging antitumor activity with complete responses (CRs) in patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL), according to phase 1/2 findings that were presented during the 2020 ASH Annual Meeting and Exposition.



Christopher J. Hensel, MD

The 5-drug regimen of venetoclax (Venclexta), ibrutinib (Imbruvica), prednisone, obinutuzumab (Gazyva), and lenalidomide (Revlimid; ViPOR) showed a tolerable safety profile and encouraging antitumor activity with complete responses (CRs) in patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL), according to phase 1/2 findings that were presented during the 2020 ASH Annual Meeting and Exposition.

Results showed that of 52 evaluable patients, the best overall response was 71%, with a complete response (CR) rate of 52% and a 19% partial response (PR) rate. Specifically, in relapsed patients ($n = 36$), the objective response rate (ORR) was 60% with a 20% and 13% CR and PR rate, respectively. The ORR was 50% in refractory patients ($n = 23$), with a 27% CR rate and a 27% PR rate.

<https://www.onclive.com/view/vipor-regimen-is-safe-shows-impressive-activity-in-relapsed-refractory-dlbcl>

ViPOR Regimen Signals Benefit in Patients With Mantle Cell Lymphoma

February 1, 2022
Nichole Turner



An interview with Targeted Oncology (Christopher Hensel, MD) discusses the ongoing ViPOR study exploring a 5-drug regimen for relapsed and refractory mantle cell lymphoma.

Treatment with the ViPOR regimen consisting of venetoclax (Venclexta), ibrutinib (Imbruvica), prednisone, and lenalidomide (Revlimid), has thus far appeared safe for use in patients with mantle cell lymphoma (MCL) and has demonstrated preliminary activity.

Results from the phase 1 portion of the ViPOR study (NCT03223610) were presented during the 63rd American Society of Hematology (ASH) Annual Meeting & Exposition. Of the 111 patients who were treated, the ORR was 70% and the complete response (CR) rate was 60%. Nine patients were evaluable for safety and no dose-limiting toxicities were observed. There were five grade 3 and 4 adverse events (AEs), but the hematologic grade 3/4 AEs included neutropenia (1.3%), anemia (3.7%), and thrombocytopenia (3%). The non-hematologic grade 3/4 AEs included hypotension (3.0%) along with fatigue, hypotension, blurred vision, atrial fibrillation, lung infection, and syncope occurring in 1.1% of patients each.

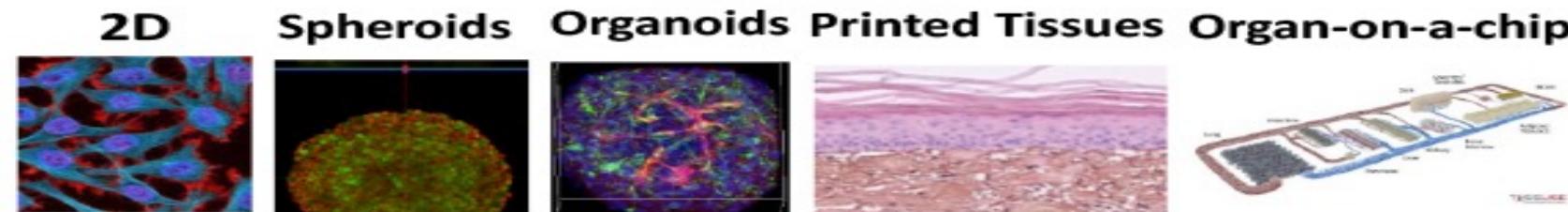


Christopher J. Hensel, MD

<https://www.targetedonc.com/view/vipor-regimen-signals-benefit-in-patients-with-mantle-cell-lymphoma>

3D models

Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues



HTS compatibility

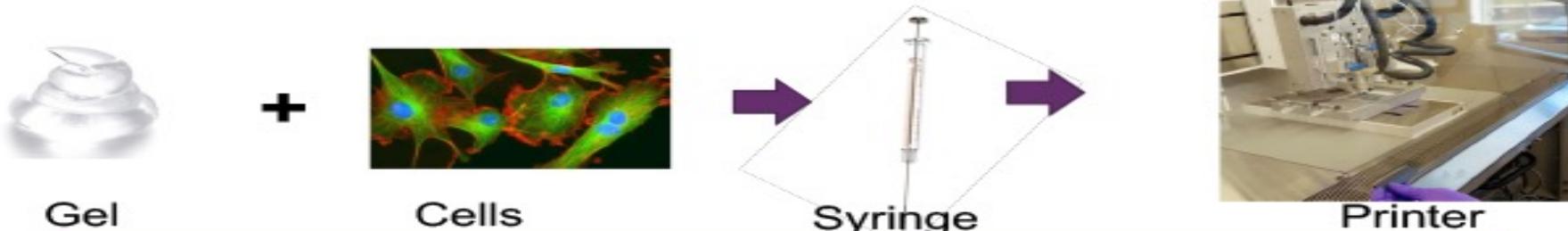
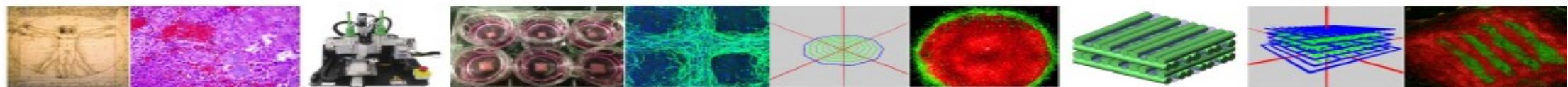
Physiological complexity



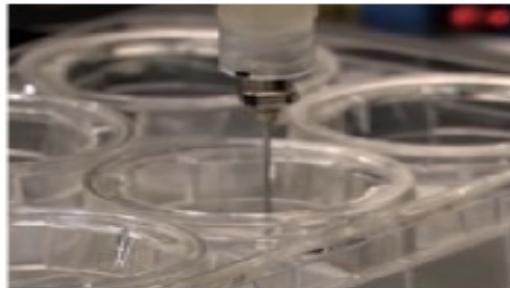
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Tissue bioprinting

3D Tissue Bioprinting



Hydrogel polymer is mixed with cells and loaded into syringe.



Printed construct

1 day

1 week

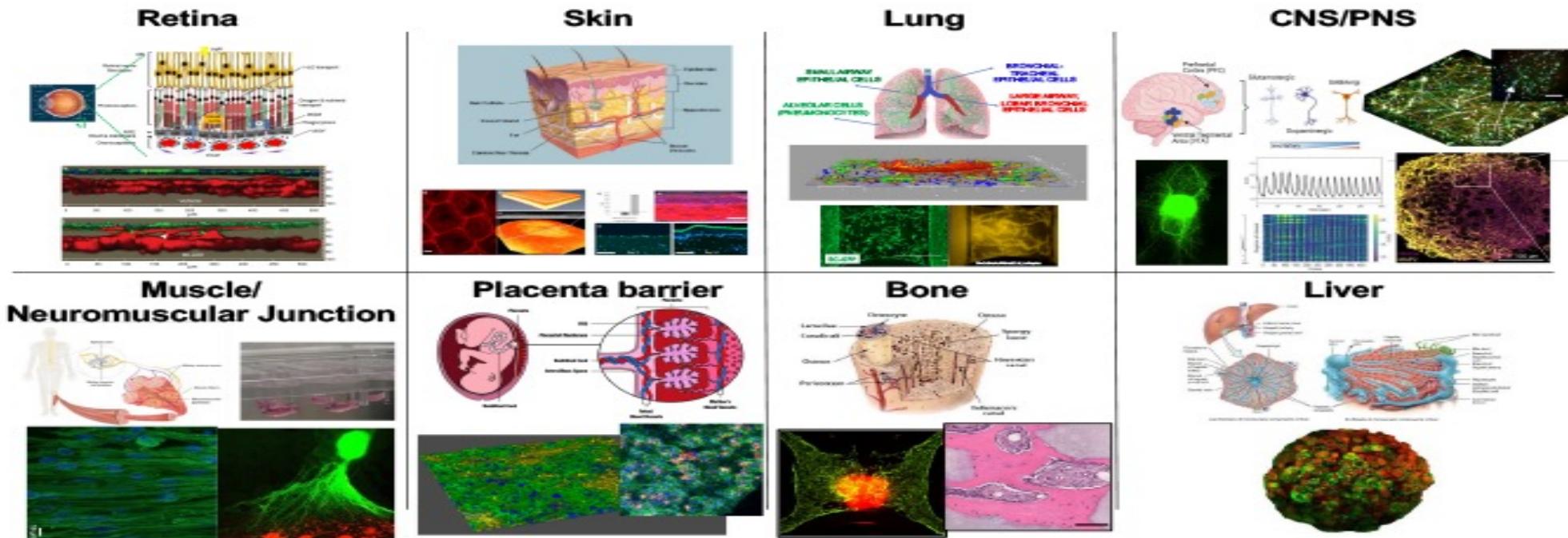
2 weeks

The printer “3D prints” the cell/gel mixture in a layer by layer approach.

The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.

3D tissue models

Current portfolio of engineered 3D tissue models

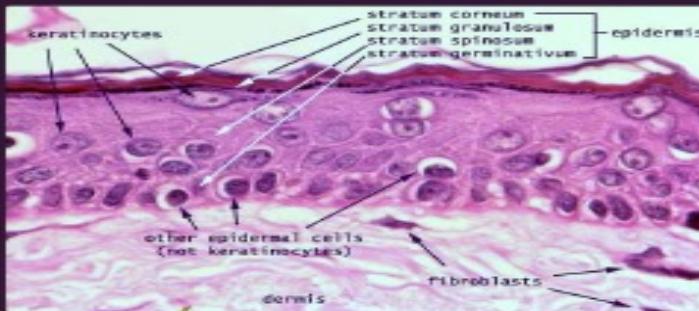


Program Director Marc Ferrer, Ph.D. <https://ncats.nih.gov/bioprinting>

Skin biofabrication

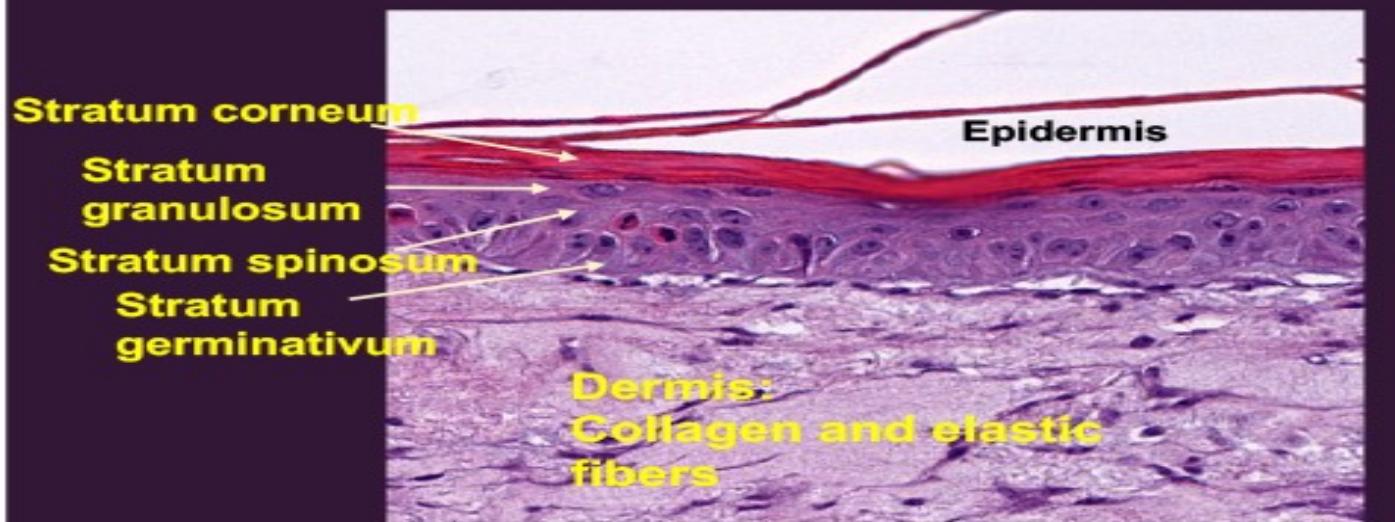
Skin biofabrication

Native Skin



<http://www.siumed.edu/~dking2/intro/IN005b.htm>

3D-Bioprinted Skin



NIH

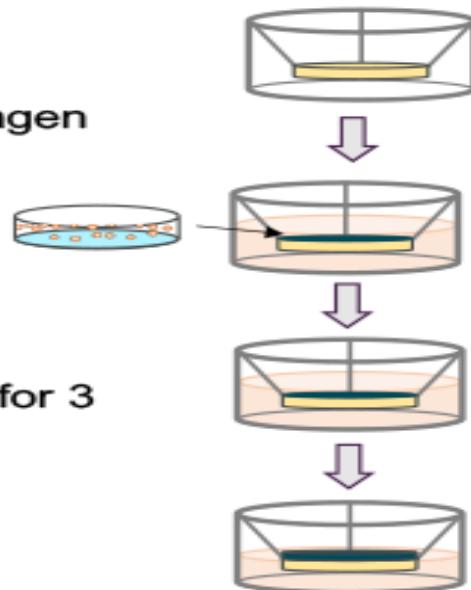
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Skin tissue generation

Generation of bioprinted skin tissues

Reconstructed human epidermis (RhE)

1. Coat the 96-well transwell insert membrane with collagen
2. Add keratinocytes
3. Submerge culture for 3 days
4. Air-liquid interface culture for 8 days



Full thickness skin tissue (FTS)

1. Suspend fibroblasts in bioprinting gel
2. Bioprint fibroblast bioink to a 3-layer U shape on bottom side of 96-well transwell insert membrane
3. Add bioprinting gel to cover the U shape
4. Submerge bioprinted tissue in medium for 7 days
5. Add keratinocytes and submerge culture for 3 days
6. Air-liquid interface culture for 8 days

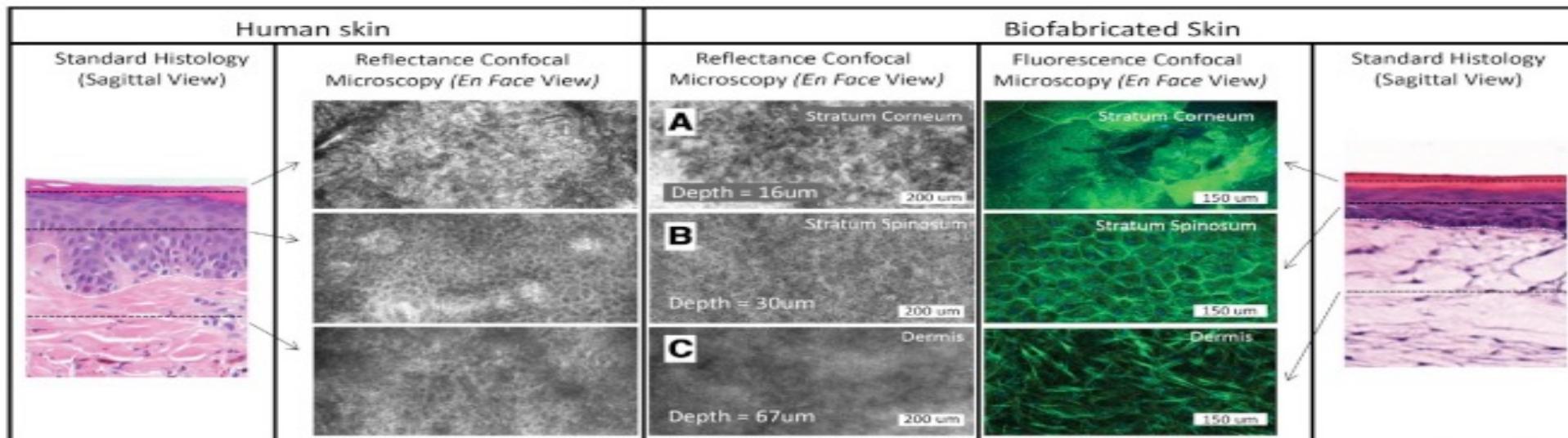
Tissue model

www.oncotarget.com

Oncotarget, 2020, Vol. 11, (No. 27), pp: 2587-2596

Research Paper

A 3D biofabricated cutaneous squamous cell carcinoma tissue model with multi-channel confocal microscopy imaging biomarkers to quantify antitumor effects of chemotherapeutics in tissue



Collaboration between NCATS (Marc Ferrer) and Rockefeller University (Daniel Gareau)

Information

**Where do I go for more
information about assay
development?**



Book

Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)

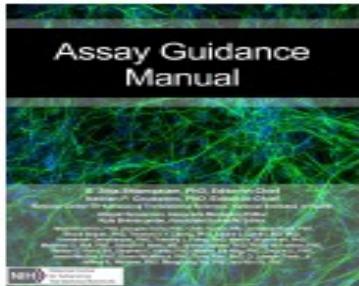


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Website: <https://ncats.nih.gov/expertise/preclinical/agm>

Email us: NCATS_AGM_Editors@mail.nih.gov



Facebook: www.facebook.com/assayguide



LinkedIn: www.linkedin.com/groups/7427244

<https://ncats.nih.gov/agm-video>

August 7th Videos

1. Austin, CP: Welcome to the Assay Guidance Manual (AGM) Workshop
2. Coussens, NP: Strategies for Assay Selection & Robust Biochemical Assays
3. Riss, T: Treating Cells as Reagents to Design Reproducible Screening Assays
4. Trask, OJ: Assay Development Considerations for High Content Imaging
5. Auld, DS: Studies in Mechanisms and Methods in Assay Interferences
6. Dahlin, JL: Assay Interference by Chemical Reactivity
7. Chung, TDY: Basic Assay Statistics, Data Analysis & Rules of Thumb
8. Devanarayan, V: Reproducibility & Differentiability of Potency Results
9. Sittampalam, GS: Avoiding Artifacts & Interferences in Assay Operations

March 26-27th Videos

1. Austin, CP: Welcome to the Assay Guidance Manual (AGM) Workshop
2. Coussens, NP: Robust Assays Define Success in Preclinical Research
3. Lal-Nag, M: Target Identification & Validation in Translational Discovery
4. Foley, TL: Development & Validation of Cell-Based and Biochemical Assays
5. Riss, T: Treating Cells as Reagents to Design Reproducible Screening Assays
6. Trask, OJ: Assay Development for HCS & Best Practices for 3D HCS
7. Roth, KD: Mass Spectrometry for Drug Screening and Lead Optimization
8. Dahlin, JL: Bioassay Interference by Aggregation and Chemical Reactivity
9. Patnaik, S: Lead Selection and Optimization by Medicinal Chemistry
10. Xia, M: *In Vitro* Toxicological Testing Using a qHTS Platform
11. Xu, X: *In Vitro* Assessment of ADME Properties of Lead Compounds
12. Kahl, SD: Statistical Design of Experiments for Assay Development
13. Guha, R: Pharos Application to Target Evaluation and Drug Discovery
14. Weidner, JR: Assay Operations: Keeping Assays Robust and Reproducible



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Assay guidance manual

Assay Guidance Manual Training Workshops

- [Online Training Modules](#)
- [Upcoming Workshops](#)
- [Past Workshops](#)

NCATS offers a variety of [Assay Guidance Manual \(AGM\)](#) training workshops throughout the year designed to share best practices and advice on robust assay design, development and implementation for researchers involved in the drug discovery process.



Online Training Modules

NCATS offers an online AGM training workshop in addition to the in-person AGM workshops held throughout the year. The online training workshop also features experts sharing best practices and expert advice on assay design, development and implementation. [View the video modules.](#)

<https://ncats.nih.gov/expertise/preclinical/agm/training>



Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development

⌚ Tue Jun 7, 11:00 AM - Wed Jun 8, 5:15 PM (EDT)

📍 Zoom

[Add to calendar](#)

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THIS EVENT HAS ENDED

Video of the workshop is available at the links below:

[Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development \(Day 1\) \(June 7, 2022\)](#)

[Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development \(Day 2\) \(June 8, 2022\)](#)

About the Workshop

The National Center for Advancing Translational Sciences (NCATS) Assay Guidance Manual (AGM) program is hosting a two-day workshop that will cover a broad range of critical concepts, including practical approaches and best practices, for developing standardized 3D cellular assays with the hope of helping the community to successfully develop therapeutics for future pandemic threats. This workshop is jointly organized by NCATS, the National Institute of Allergy and Infectious Diseases (NIAID) and the Bill & Melinda Gates Foundation. The overall goal of this workshop is to help scientists establish robust, reproducible, scalable, consistent, advanced 3D tissue models to study pandemic threat viruses.



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