HHS Public Access

Author manuscript

Comb Chem High Throughput Screen. Author manuscript; available in PMC 2015 June 22.

Published in final edited form as:

Comb Chem High Throughput Screen. 2014 March ; 17(3): 256–265.

The University of New Mexico Center for Molecular Discovery

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Abstract

The University of New Mexico Center for Molecular Discovery (UNMCMD) is an academic research center that specializes in discovery using high throughput flow cytometry (HTFC) integrated with virtual screening, as well as knowledge mining and drug informatics. With a primary focus on identifying small molecules that can be used as chemical probes and as leads for drug discovery, it is a central core resource for research and translational activities at UNM that supports implementation and management of funded screening projects as well as “up-front” services such as consulting for project design and implementation, assistance in assay development and generation of preliminary data for pilot projects in support of competitive grant applications. The HTFC platform in current use represents advanced, proprietary technology developed at UNM that is now routinely capable of processing bioassays arrayed in 96-, 384- and 1536-well formats at throughputs of 60,000 or more wells per day. Key programs at UNMCMD include screening of research targets submitted by the international community through NIH’s Molecular Libraries Program; a multi-year effort involving translational partnerships at UNM directed towards drug repurposing - identifying new uses for clinically approved drugs; and a recently established personalized medicine initiative for advancing cancer therapy by the application of “smart” oncology drugs in selected patients based on response patterns of their cancer cells in vitro. UNMCMD discoveries, innovation, and translation have contributed to a wealth of inventions, patents, licenses and publications, as well as startup companies, clinical trials and a multiplicity of domestic and international collaborative partnerships to further the research enterprise.

Introduction

The University of New Mexico Center for Molecular Discovery (UNMCMD), directed by Dr Larry A. Sklar and located in Albuquerque, NM, is an academic research center that specializes in discovery using high throughput flow cytometry (HTFC) integrated with virtual screening, as well as knowledge mining and drug informatics. A primary focus is to identify small molecules that can be used as chemical probes and as leads for drug discovery. The HTFC platform in current use represents advanced, proprietary technology developed at UNM that is now routinely capable of processing bioassays arrayed in 96-, 384- and 1536-well formats at throughputs of 60,000 or more wells per day.

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The UNMCMD hosts a highly accomplished Cheminfomatics group led by Dr. Tudor O. Oprea, a pioneer of key concepts in current drug discovery such as lead-likeness, chemical space navigation, and computer-assisted drug repurposing. To complement HTFC technology, Oprea's group provides an integrated informatics platform, based on multiple adaptive technologies, that assembles information about compounds, biological targets and pathways using advanced knowledge management and modeling tools. Also provided is critical support for screening informatics and in silico evaluation of HTS hits, leads and advanced compounds.

Since 2005, UNMCMD has participated as one of the 9 academic centers funded to screen research targets submitted by the international community through NIH's Molecular Libraries Program. The ability to multiplex targets using HTFC has been recognized as a national resource for the Molecular Libraries Probe Production Center Network (MLPCN). Target classes have included gene expression, cell adhesion, signaling, and drug efflux among others for programs in cancer, infectious diseases, neuroscience and metabolic diseases.

As an academic screening center, UNMCMD has access within the university to a concentration of both basic scientists and clinicians with multi-faceted expertise in specific disease areas and a cogent understanding of key biological mechanisms and phenotypes that could be useful drug targets. The UNMCMD also benefits from strong collaborative interactions and support from three other nationally recognized research centers at UNM: the UNM Cancer Center, the UNM Clinical and Translational Science Center and the UNM Center for Infectious Diseases and Immunology. Together, these centers have partnered to promote a strong translational focus at UNM, a commitment to translating laboratory research findings into better treatments for patients. This has fostered pioneering, multidisciplinary projects at the UNMCMD in support of two recently trending translational paradigms: drug repurposing or repositioning, the finding of new uses for established drugs; and a personalized medicine approach to cancer therapy, the application of "smart" oncology drugs with high specificity and low toxicity in selected patients based on response patterns of their cancer cells in vitro.

Overall, it is worth noting that UNMCMD discoveries, innovation, and translation, have contributed to more than 60 inventions, 20 patents, 12 commercial licenses, 3 startups (Intellicyt, Azano, Biophagy), 3 clinical trials (Thioridazine, Ketorolac, Raltegravir), and more than 100 publications and many collaborative partnerships, both domestically and internationally. A brief video overview of the UNMCMD that introduces key technology and personnel may be found here.

High Throughput Flow Cytometry

Flow cytometry is a sensitive and quantitative method for the measurement of cell and particle fluorescence (Table 1). Unlike many analytical platforms that are informative only about collective properties of entire populations of cells, the flow cytometer can quantitatively characterize the differential behavior of selected individual cells within a group, capable of high-content characterization of a diversity of compound bioeffects at the

single-cell level. For years, the flow cytometer has represented a valuable instrument to rapidly examine single cells, with modern instruments capable of routinely measuring as many as 16 or more discrete fluorescence-encoded properties of each cell at tens-of-thousands of cells per second. However, the analysis of large numbers of cell samples such as required for high throughput screening applications in drug discovery has been technically and computationally challenging.

Initial high throughput flow cytometry capabilities developed at UNM were based on hardware and software developed by Dr. Larry Sklar, Dr. Bruce Edwards (Director of UNM's Flow Cytometry Shared Resource), and Fritz Kuckuck, and later upgraded to include a range of biological and computational capabilities. The first generation HTFC, patented in 2001, was based on reciprocating valve technology and called *Plug Flow Cytometry*. The second-generation HTFC technology, HyperCyt®, developed soon thereafter and patented in 2005, creates temporal gaps in the data collection process by introducing air bubbles between distinct samples, before delivering them to the flow cytometer [1]. Instead of collecting and saving data from each well individually during the analysis, HyperCyt continuously collects data for the whole plate and stores the information as a single file (Table 1). This approach eliminates delays that are otherwise caused when separately collecting data for each sample of large datasets. Samples can be as small as 1-2 μL to minimize cell and reagent requirements and maximize throughput. Alternatively, samples can be hundreds of μL in volume for detection of rare cell subpopulations. Distinguishing this from other automated flow cytometry sampling approaches is zero dead volume - the entire sampled volume is analyzed. Under a continuing program of development led by Edwards the HyperCyt platform has evolved through several subsequent generations, now enabled for routine analysis of bioassays in high-density, 1536-well format at rates of 160 wells/minute or more [2]. The high content analysis capabilities of the flow cytometer can also be exploited for bioassay multiplexing, the parallel evaluation of multiple drug targets or phenotypic endpoints in each well. By creating a suspension array of particles or cells, assays and responses can be highly multiplexed or performed on complex cell populations without compromising throughput. UNMCMD's novel sampling approach makes flow cytometry an attractive platform for a broad range of discovery applications.

Facilities, Equipment and Workflow

UNMCMD is currently housed in a 13,000 square foot facility which provides space for target development, high-throughput screening, cheminformatics and BSL2-level microbiology/virology work; UNM's Shared Flow Cytometry Resource (SFCR) and Translational Informatics Division also reside in the Center's facility. The administrative support staff for UNMCMD is located in proximity to the laboratory space in a shared administrative work area.

A wide range of flow cytometry instrumentation, as well as cell preparation, data analysis and storage services are available to support both HTFC screening project workflows as well as lower throughput analysis applications such as assay development, time-resolved cell physiological response analysis, and cell sorting, to name a few (Table 2). To complement flow cytometry resources, the UNMCMD houses conventional instrumentation for HT

bioanalysis as well as robotic platforms for manipulation of compounds, cells and other bioassay components in an automated workflow for high throughput screening (Table 3). Also available are computational and informatics resources to support virtual screening, data mining, scaffold analysis for structure-activity relationship (SAR) evaluation, SAR by commerce, and others (Table 4). Investigators are provided access to a variety of compound libraries of translational significance, natural products, the NIH Small Molecule Repository (pending approval via R01 or R21 funding mechanisms) as well as a diverse series of combinatorial libraries comprising more than 5 million small molecules (Table 2). The combinatorial libraries, available in collaboration with the Torrey Pines Institute for Molecular Studies, have recently proven to be a highly productive resource for identifying a diversity of selective, high affinity and functionally diverse probes for 2 members of a family of structurally related G-protein coupled receptors [3].

HTFC Projects

As a crosscutting resource for UNM, flow cytometry has a proven capability for design and implementation of bioassays that complement the interests of university signature programs and research centers Examples include bioassays for discovery and repurposing of drugs, biomarker evaluation in clinical populations, and basic research in cancer progression, adhesion receptor biology and cell signaling. The current pipeline for small molecule discovery includes molecular and cell phenotypic targets. These include bacterial virulence, prostate cell differentiation, efflux pump-associated multi-drug resistance in cancer treatment, as well as effector molecules associated with glioma metastasis, induction of TNF-related apoptosis inducing ligand for tumor suppression, chronic inflammatory disease, including Alzheimer's and prion diseases, vascular and stem cell adhesion biology, and cell signaling. Other receptor pathways include androgen and estrogen, and molecular complexes ranging from low molecular weight G proteins, RGS family proteins, Bcl-2 family proteins, proteasome, and yeast model systems [4]. The efflux pump project represents an example where: 1) UNMCC researchers developed cell-based systems for a single pump; and 2) UNMCMD researchers performed the screen on a drug library where molecules could be taken into animal models and clinical trials. The work has been expanded to include several ATP-binding cassette (ABC) transporters in a multiplex format.

Operating since 2005, first as a Comprehensive Center and since 2008 as a Specialty Screening Center in the NIH-sponsored Molecular Libraries Probe Production Centers Network (MLPCN), UNMCMD has worked with a wide variety of research targets submitted by the international community through NIH's Molecular Libraries Initiative (Table 5). Target classes have included gene expression, cell adhesion, signaling, and drug efflux among others for programs in cancer, infectious diseases, neuroscience and metabolic diseases [5]. Results published by UNMCMD to the PubChem website detail more than 460 screening bioassays and 12.5 million tests of substances from the Molecular Libraries Small Molecule Repository, currently a collection totaling more than 350,000 compounds. To date, more than 100 peer-reviewed publications have been listed in conjunction with this activity (listed here). Supplemental funding was also awarded to support Center Driven Research Projects (CDPs) in yeast model systems, efflux transporters, and G-protein coupled receptors; the development of specialized informatics technologies for the NIH-funded

BARD (BioAssay Research Database); as well as translational Extended Probe Characterizations. The yeast model systems included expansion of mammalian transporter studies into yeast, as well as a pipeline in pathogenesis including fungus, virus, and bacteria. The third CDP was directed at collaboration with another NIH-sponsored Center at Carnegie Mellon University in the Technology Centers for Networks and Pathways which developed novel fluorescent tags for drug discovery based on protein transport.

The Extended Probe Characterization process was an effort to translate small molecule discoveries toward pre-clinical testing. This funding has contributed to expanding the work in estrogen receptors, mammalian transporters, and low molecular weight GTPases. For the mammalian transporter program, UNMCMD researchers developed cell-based detection systems for individual pumps and performed screens on a drug library where molecules could be taken into animal models and clinical trials. The work has been expanded to include several ATP-binding cassette (ABC) transporters in a multiplex format with the biological, informatics and technical expertise available at UNMCMD. The same technology is currently being applied to an $8M Defense Threat Reduction Agency contract “Targeting Multidrug Efflux Systems in Francisella tularensis & Burkholderia pseudomallei”. To date, 17 high quality chemical probes have been produced in conjunction with these MLPCN projects (Table 5), 8 in the pilot phase from 2005-2008 (reviewed in Supplementary Document 1) and 9 in the subsequent production phase with more in development.

We have collected a library of FDA approved drugs, along with natural products and the NIH clinical collection, for use in discovery. Moreover, these projects have led to a total of more than 2 dozen patent applications in technology and small molecule discovery with more than a dozen patents already awarded, and available for licensing and commercialization. Taken together, the biological and technical expertise available in the flow cytometry resource provides state-of-the-art research and educational opportunities for biomedical research, target development, discovery, and commercialization, opportunities not available elsewhere. Investigators can find more information at http://nmmlsc.health.unm.edu/ and http://hsc.unm.edu/research/flowcyt/.

Translational Partnerships

Critical to the translational mission of the UNMCMD are research and clinical partnerships with several nationally and internationally recognized centers at UNM as well as the presence of an established organization for licensing and commercialization of intellectual property.

The UNM Clinical Translational Science Center

The Clinical Translational Science Center at the Health Sciences Center (PI: Richard Larson; http://hsc.unm.edu/research/ctsc/) is advancing scientific discovery into improved health outcomes. The CTSC makes connections, finds best practices, bridges gaps, engages the community and builds on an already impressive HSC foundation of medical research and education expertise.

The CTSC is a member of the national Clinical Translational Science Award (CTSA) consortium. The National Institutes of Health (NIH), with the CTSA promotes this emphasis on clinical and translational research, to facilitate the expansion of clinical research, the training of clinical researchers, and the emergence of community voice in the direction and implementation of clinical research.

Our CTSC provides resources and infrastructure to enable investigators to perform cutting-edge clinical research. The staff, facilities, and resources of the CTSC are available to everyone involved in research at the UNM HSC and their collaborators.

The UNM Cancer Center

UNM Cancer Center scientists are working diligently every day to discover the causes and cures for cancer. As the state's only National Cancer Institute-designated cancer center (PI: Cheryl Willman; http://cancer.unm.edu/) and the Official Cancer Center of the State of New Mexico, the UNM Cancer Center is committed to fighting cancer on all fronts. Our researchers have been recruited from outstanding institutions across the nation, including Harvard University, Fred Hutchinson Cancer Research Center and Memorial Sloan-Kettering Cancer Center, and are supported by $60 million in federal and private funding every year.

The UNM Cancer Center's team of 126 researchers is making significant progress in developing new cancer drugs, genome sequencing, cancer prevention and cell cycling and signaling. Our scientists are clustered into four National Cancer Institute (NCI) research programs: Cancer Population Sciences, Cancer Biology & Biotechnology, Hematologic Malignancies, and Women's Cancers. A large fraction of patients treated at the UNM Cancer Center participate in cancer clinical trials, which include prevention, screening, diagnosis and treatment trials.

The UNM Center for Infectious Diseases and Immunology

The mission of the CIDI (http://hsc.unm.edu/som/programs/cidi/) is to develop and enhance collaborative programs among researchers, physicians and businesses in New Mexico that address the threat of infectious and immunologically mediated diseases in New Mexican populations and the world. This will be achieved by characterizing epidemiologic issues, studying basic host-pathogen mechanisms, developing new vaccines, therapeutics, and diagnostics, and testing the preventive, therapeutic and diagnostic efficacy of these discoveries in clinical trials.

The UNM Center for Infectious Disease and Immunity is affiliated with the following national and international organizations: International Collaboration In Diseases Research: Hantavirus Ecology, located in Chile and Panama; MTb Programs; Duke University; Los Alamos National Laboratory; National Center for Genome Resources; Research Center of Excellence at University of Texas Medical Branch, Lovelace Respiratory Research Institute (LRRI); National Institute for Allergy and Infectious Diseases (NIAID).

The UNM Science and Technology Corporation

The licensing arm of UNM, the UNM Science and Technology Corporation (http://stc.unm.edu/) supports and enables deployment of UNMCMD technologies for repurposing screens, as well as for investigator-initiated discoveries related to drug repurposing

Drug Repurposing

Drug repurposing, finding new uses for established drugs, is a major recent trend in drug development. The rationale behind this approach is that *de novo* drug discovery is a lengthy and costly process, whereas already approved drugs are more likely to be repurposed for another indication - a potentially faster and less expensive route to meeting urgent medical needs. Translational, target and disease foci are strategic advantages fostered by close proximity and frequent interactions between basic and clinical scientists, circumstances conducive to discovering new modes of action for approved drugs. Unique opportunities have presented themselves with respect to the integration of basic research, conducted at the UNMCMD and the clinical research that is conducted at the UNM Clinical and Translational Science Center (CTSC) as well as the UNM Cancer Center, UNMCC, and the UNM Center for Infectious Diseases and Immunology (CIDI). Each of these centers has supported repurposing: UNMCMD through pilot screens of approved drug collections; UNMCC through support of core resources and grant support for pilot screens as well as early phase animal studies; UNM CTSC through requests for applications linking clinical trials to repurposing screens; UNM CIDI through collaborative studies of host pathogen interactions. This has fostered a multi-year effort across these nationally recognized research centers, investigating new uses for a dozen different drugs [6].

Repurposing projects have been approached in 2 ways (Table 6). The first is a computational approach that emphasizes computational analysis of known drugs for potential interactions with defined biological targets. An example is the recent identification of Raltegravir, an HIV-1 integrase inhibitor approved by the FDA in 2006, as an inhibitor of metnase, a DNA repair enzyme proposed by a team of basic and clinical scientists at UNM to be a potential target for adjuvant therapy in cancer. The connection was initially made on the basis of structure-based virtual screening studies conducted by the cheminformatics group at UNMCMD, subsequently confirmed in the laboratory and is now under investigation in a pilot clinical study at UNMCC evaluating the potentiation of cisplatin chemotherapy in head and neck squamous cell carcinoma.

The second approach emphasizes physical screening of collections of known drugs using a biologically relevant assay adapted for high throughput screening. An example is the recent use of a multiplex HTFC assay designed to identify inhibitors of protein-small molecule interactions between a fluorescent guanine nucleotide analog and an array of 6 individual low molecular weight GTPases displayed on color-coded sets of beads. R-naproxen and the follow-up hit ketorolac (identified via subsequent virtual screening) were found to inhibit activation of Rho family GTPases, in particular Rac and Cdc42. Later confirmed in laboratory studies to inhibit downstream cellular responses that depend on these activated GTPases (cell proliferation, migration, adhesion and tumor growth in xenograft models)[7], these these two drugs are under consideration as potential candidates for use in adjuvant

therapy following debulking surgery to prevent ovarian tumor growth and dissemination during post-surgical recovery prior to administration of chemotherapy.

Personalized Medicine

Cancer therapies increasingly apply drugs with high specificity and low toxicity in selected patients based on a precision medicine paradigm. This paradigm evolved from the concept that cancers that appear similar from one patient to the next may instead be heterogeneous with respect to a single oncogene or signaling pathway that is aberrant. Drugs specifically targeting such defects can achieve dramatic therapeutic effects when applied to the right patients. Exemplified by the drug imatinib, or Gleevec®, that effectively targets chronic myelogenous leukemias with BCR-ABL mutations, hundreds of similar drugs have received approval for clinical use or are undergoing clinical trials. Such “smart” oncology drugs are particularly attractive because they also result in a major improvement in quality of life for patients due to their low toxicities compared to conventional chemotherapeutics. However, despite the progress in developing new targeted agents and increased knowledge of cancer genomics, there exist numerous cancers with mutations for which smart oncology drugs remain to be identified and developed.

Acute leukemias represent cancers with a long tradition of clinical biomarker use in the form of immunoprofiling, chromosomal rearrangements, and mutations. For this reason, they are well-suited to a precision medicine paradigm. Most patients with acute lymphocytic leukemia (ALL) have multiple somatic mutations in the dominant ALL clone and it is likely that the clonal heterogeneity of these mutations contributes to the observed clinical responses. Key mutations have been identified in pediatric ALL that are targets for new therapeutic approaches involving smart oncology drugs. These considerations are the basis for a precision medicine screening program recently initiated in the UNMCMD in which cell lines and ultimately primary tissues from patients are screened for responsiveness in vitro to both old and new agents and their combinations. Single cell analysis capabilities of flow cytometry are ideal for assessing the degree to which heterogeneity of the original tumor is retained in such an in vitro therapy model and how different subpopulations of cancer cells are affected by the treatment regimen. This also facilitates finding drug combinations that affect all cell populations and might result in more durable tumor responses than therapies that have large average effects but leave certain cancer cell populations unharmed (e.g., cancer stem cells). The ability of HyperCyt to work with very small volumes of cells together with zero dead volume in the analysis step is also a major consideration for efficient utilization of limited patient samples. This effort is expected to ultimately lead to an understanding of the basis of durable responses and translation to clinical therapy.

Future Perspectives

The UNMCMD is a central core resource for research and translational activities at UNM. In addition to partnerships with UNMCC, CTSC and CIDI research centers highlighted above, UNMCMD supports (and will continue to do so) research activities of signature programs and areas of disease focus at the UNM Health Sciences Center that include childhood health, environmental health, global health, metabolic diseases (cardiovascular,

diabetes, kidney diseases), neurodegenerative diseases and stroke. This includes implementation and management of funded screening projects as well as “up-front” services such as consulting for project design and implementation, assistance in assay development and generation of preliminary data for pilot projects in support of competitive grant applications. Expansion is underway for future development in several areas. Of particular emphasis is expansion of capabilities for working with primary tissues from patients and xenografts derived from such tissues. Also underway are outreach activities to explore new possibilities for international translational collaborations and expanded domestic extramural collaborations, both academic and industrial that would benefit from unique capabilities provided by UNMCMD. It is noteworthy that in addition to the current focus on small molecule screening, the UNMCMD is well positioned to provide unique capabilities for high throughput, high content, single cell screening of a wide range of biologics (e.g., siRNA, antibodies, peptides, sera, biomarkers, etc.) that might be sourced by collaborators or customers for a project.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Unique features of High Throughput Flow Cytometry

Flow Cytometry
• Single cell analysis
• Cells or particles in suspension
• Detection of as few as hundreds to thousands of fluorescent molecules on a cell or bead
• Detection of fluorescent molecule concentrations as low as 10–100 pM
• Superior discrimination of bound vs. free fluorescence (the laser excites only a very small volume of the sample fluid immediately surrounding the cell, diminishing the background signal by limiting the excitation of excess, unbound fluorescent molecules in solution)
• Analysis rates of up to tens of thousands of cells per sec
• Discrimination of up to 16 or more distinct fluorescent signals from each cell
• Broad dynamic range of optical signal detection (4-6 orders of magnitude)
HyperCyt High Throughput Flow Cytometry Technology
• Uses a peristaltic pump in combination with an autosampler to boost endpoint assay performance to rates in excess of one sample per second
• Sampling probe of the autosampler moves from one well to the next of a multiwell microplate
• A peristaltic pump sequentially aspirates sample particle suspensions from each well
• Between wells, a bubble of air is drawn into the sample line to generate a tandem series of bubble-separated samples for delivery to the flow cytometer
• Single data file produced for each plate
• Small sample volumes (1 μL/well) for efficient use of cells and reagents
• Alternatively, large sample volumes (hundreds of μL) for detection of rare cell subsets (stem cells)
• Zero dead volume (entire sample is analyzed)
• Superior multiplexing capabilities with the potential for hundreds of distinct measurements or assays per well.
• Homogeneous, no-wash assays to simplify assay assembly and analysis
• For a 1536-well plate, 10 min for sampling and data acquisition (100-10,000 cells/well) plus 5 min for analysis results summary and QC statistics

Table 2

UNMCMD Workflow Elements

Workflow capacity

- Resources managed across project teams and project manager
- One screening campaign plus one or more screening follow up projects and at least one outreach/assay development project per biologist
- 20 projects at any given time ongoing in CMD
- Capable of two 350,000 compound screening campaigns over a 10-12 week period

Reagent Production

- Labeled bead preparation
- Labeled peptide receptor ligands

Cell Production

- Primary leukocytes
- Cell lines (U937, Jurkat), large scale production in flasks or spinners

Sample Logistics

- Automated assay plates production
- Preparation of compound daughter plates from mother plates; preparation of dose-response plates
- 96, 384, and 1536 well format
- Programmed cherry picking

Data Analysis

- Full-time data manager to oversee data analysis for each screen
- Z' of 0.5 or better required for acceptance of each screening plate
- Multiple flow cytometry gating scenarios evaluated for deconvoluting responses in complex cell populations or multiplexed targets
- Removal of fluorescent or cytotoxic compounds
- Data formatted for review by the sponsor

Data Storage

- Lab information system for tracking compounds and compound identifiers per project
- Annotation, analysis and archiving of assays and screening data
- Data storage, retrieval, backup and recovery of raw data files

Informatics

- Knowledge-enabled frontload screening:
    - ○ In-depth, adaptive evaluations of chemical libraries, thorough annotation of drugs-targets-indications-contraindications-side effects
    - ○ In-depth knowledge relating bioactive chemicals to targets and pathways
    - ○ Proactive correlation of toxicity with biochemical targets
    - ○ Advanced knowledge management system comprised of a database, a network generation tool, and a Cytoscape plugin for KEGG pathways integrated with specific disease information.
- Network-based modeling for systematic mining of literature (and patents) and commercial libraries for scaffold-based evaluation of SAR-by-commerce and SAR-by-chemistry
- Access to a wide variety of in silico models, developed at UNM or commercial, or in conjunction with the Technical University of Denmark (DTU).
- Via DTU, direct access to the OpenPHACTS platform, and to ChemProt, a design-enabling in silico platform

Compound Libraries

- Prestwick Chemical Library
- NIH Molecular Libraries Small Molecules Repository
- NIH Clinical Collection
- Microsource Spectrum Collection
- Tocriscreen Collection
- Torrey Pines Institute for Molecular Studies Combinatorial Libraries

Table 3

UNMCMD Equipment

Agilent BioCel System - A fully automated and highly flexible solution for complex cell handling

- Bravo automated liquid handling platform capable of 96 – 1536 well formats
- VPrep precision pipetting system for submicroliter liquid transfer.
- VSpin automated microplate centrifuge
- Thermo Teleshake for automated microplate controlled-frequency shaking.
- BioTek EL406 bulk liquid dispenser for handling multiple
- Multiple Liconic high-capacity (hundreds) plate storage cabinets provide a full range of humidity and temperature controlled environments for compound storage
- Storage hubs and chilled plate hotels
- Automated barcoding and microplate-based tracking.
- PlateLoc plate sealer
- Nexus Xpeel plate desealer
- Docking carts for integrating automated reader platforms (plate reader, Hypercyt cluster)

Beckman Coulter Biomek FXp Workstation

- Integrated gripper, Span-8 and Multichannel heads,
- Span-8 and 384 tip-wash stations
- 4×3 and 1×1 Automated Labware Positioners
- Static Peltier Labware Positioner and Shaking Labware Positioner
- Cytomat24 and 44 compound plate storage incubators for storage of up to 504 and 900 plates, respectively (flushed with nitrogen to reduce oxygen level to 5%)
- Cytomat2 cell incubator for up to 42 plates
- CytomatHotel for up to 63 tip boxes or 252 plates
- V&P Scientific pin tool sets (384×100 nL, 384×200nL, 1536×100 nL) for compound transfer

Additional Workstations

- Beckman Coulter Biomek NX Span-8 Workstation with integrated gripper
- Beckman Coulter Biomek NX Multichannel Workstation with integrated gripper, P30/384 dispense head
- Eppendorf epMotion 5070 automated pipetting system with TS50 and TS1000 single channel and TS50-8 and TS1000-8 eight-channel tools

Reader Platforms

- 2 BD Biosciences Facscan Flow Cytometers (1 laser, 5 detectors)
- 4 Beckman Coulter CyAn Flow Cytometers (3 lasers, 12 detectors)
- 7 Accuri C6 Flow Cytometers (2 lasers, 6 detectors)
- Wallac 1420-040 Victor3V Multilabel Plate Reader
- BioTek Synergy 4 Plate Reader
- Biotek PowerWave HT Microplate Spectrophotometer
- 4 custom HyperCyt instrument clusters (each with a CyAn flow cytometer)
- Custom HyperCyt Quad Cytometer cluster with 4 Accuri C6 flow cytometers operating in parallel for 1536-well plate processing

Liquid Dispensing Automation

- BioTek Nanoquot nanoliter dispenser

- 4 BioTek Microflow nanoliter dispensers with 1536-well capability

Sample Preparation

- Microsonic Hendrix SM100 Ultrasonic Fluid Processor
- Labconco Centrivap Concentrator
- Beckman Coulter Optima XE 100K Ultracentrifuge

Cell Preparation

- 2 dedicated cell culture laboratories
- 7 Class II biological safety cabinets
- 4 Thermo 3110 cell culture incubators
- 2 inverted light microscopes
- Olympus inverted fluorescent microscope

Table 4

Supplementary Resources Available at UNMCMD

UNM Shared Flow Cytometry Resource

- Sony Biotechnologies Synergy 3200 high speed cell sorter (5 lasers, 17 detectors)
- BD Biosciences FACScan flow cytometer (1 laser, 5 detectors)
- BD Biosciences FacsCalibur flow cytometer (2 lasers, 6 detectors)
- BD Biosciences LSRFortessa flow cytometer (4 lasers, 15 detectors)
- Zeiss fluorescence, phase-contrast microscope
- Beckman Coulter Z1 particle counter

Computational Resources

- 384 Core Linux Cluster with
  - ○ 32 nodes
  - ○ 32×2 Intel Xeon E5-2620 Sandy Bridge 2 GHz 6 Core 32nm processors, 15MB L3 Cache
  - ○ 32×64 GB RAM
  - ○ 2 TB SATA hard-drives on master + 32 × 250 GB on each other node
- 144 core Linux cluster with
  - ○ 18×2 Intel Xeon E5620 with 12MB L3 Cache, 18×24GB RAM, 6TB disk space
  - ○ 16×2 AMD Opteron with 1MB L2 Cache, 16×2GB RAM, 16×250 GB SATA (4 TB) hard drives on master + 15×80 GB on each node
  - ○ 16-way IBM 590 AIX, 256 GB RAM
- 10 Tera Byte Dell server
  - ○ 2 Dual Core Xeon Processors 5130
  - ○ 4LTO-3 tape drives and ML6000 tape library, which can hold up to 402 400 GB cartridges for remote backup

Data Mining Software

- Spotfire Lead Discovery - data visualization;
- Umetrics SIMCA - linear multivariate analysis
- WeKa non-linear modeling
- MatLab - general mathematical modeling

Chemoinformatics Software

- ADMET Predictor - advanced pharmacokinetics and toxicity prediction
- Chemaxon software for chemical structure/query parsing, processing and depiction, chemical database management
- Openeye Scientific programs for chemical library filtering, docking, conformer generation, similarity searching, electrostatics scoring and substructure search programming
- Mesa Analytics & Computing LLC software for 2D fingerprinting, similarity searches, and fingerprint studies;
- Chemical database systems from various vendors (MDL-Elsevier, Cambridgesoft, and Chemaxon) for storage, retrieval and analysis of bio-activity data.

Accelrys Data Management Software

- Compound registration
- Compound tracking
- Data formatting (annotation, storage, transformation) compatible with PubChem depositions and files for communicating data sets with customers, collaborators and chemistry centers

Table 5

MLPCN Screening Projects at UNMCMD

Title	Assay Endpoint Class[a]	Assay Substrate	# Compounds Screened	PubChem Summary AID	Probe ML ID[b]
GPR30 and Classical Estrogen Receptor Inhibitors	Target-Based	Cells	640	1989	050; 051
Formylpeptide Receptor Family Inhibitors	Target-Based Multiplex × 2	Cells	25,000	805; 1202	047; 048; 049
Regulators of Disassembly of the 26S Proteasome	Target-Based	Beads	25,000	1824	
VLA-4 Integrin Modulators	Phenotypic	Cells	25,000	1998; 2617	
Activators of Prostate Cell Differentiation	Phenotypic	Cells	25,000	1260	052
Bacterial Quorum Sensing Inhibitors	Phenotypic	Cells	25,000	1206	053; 054
BcI-2 Family Regulators	Target-Based Multiplex × 6	Beads	225,000	1693	258
Regulators of Ras and Ras-related GTPases	Target-Based Multiplex × 6	Beads	225,000	1772	097, 098, 099; 141; 282
RGS Family Protein Interactions	Target-Based Multiplex × 5	Beads	225,000	1504	
ABC Transporter Inhibitors	Phenotypic Multiplex × 2	Cells	200,000	1818	230
MEKK2-MEK5 PB1 Domain Interactions	Target-Based Multiplex × 3	Cells	300,000	1683	
T Cell Immune Modulators	Phenotypic	Cells	325,000	2087	
Regulators of TOR pathway in S. cerevisiae	Phenotypic Multiplex × 5	Cells	325,000	1908	231
Caloric Restriction Mimetics Inhibiting Age-related Superoxide	Phenotypic	Cells	325,000	2714	
Antifungal Efflux Inhibitors	Phenotypic Multiplex × 3	Cells	325,000	485335	
VLA-4 Allosteric Modulators	Phenotypic	Cells	350,000	2617	
RNA aptamer-based Screen for Selective GRK2 Inhibitors	Target-Based	Beads	350,000	488855	
Inhibitors of Pemphigus Autoantibodies	Phenotypic	Cells	350,000	588367	
Arrestin-AP2 Inhibitors	Phenotypic	Cells	350,000	504493	
ABCC6 Transporter Inhibitors	Phenotypic	Cells	350,000	588561	
Regulators of V-ATPase Proton Transport in Yeast	Phenotypic	Cells	350,000	504622	
Non-cannonical Ligands for β Adrenergic Receptor Internalization	Phenotypic	Cells	350,000	504448	342
Lytic Granule Exocytosis Inhibitors	Phenotypic	Cells	350,000	651682	
Toxin Protease Inhibitors	Target-Based Multiplex × 3	Beads	350,000	588467; 588469 588470	

Title	Assay Endpoint Class[a]	Assay Substrate	# Compounds Screened	PubChem Summary AID	Probe ML ID[b]
Myeloid Differentiation Promoters	Phenotypic	Cells	350,000	588701	

[a] red highlight, HTS performed as multiplex with indicated number of assay endpoints/targets measured in each well.

[b] Blank indicates no probe produced or probe production still in progress. A review of UNMCMD probes produced in the NIH screening center pilot phase (IDs 047-054) is provided as supplementary document 1.

Table 6

Drug Repurposing Projects at UNMCMD

Repurposed Drug	Existing Target/Application	New Target/Application	Screening Approach
Raltegravir	HIV-1 Integrase; antiviral treatment for HIV patients	Metnase; adjuvant therapy in cancer	virtual screening based on metnase
Cyclobenzaprine	skeletal muscle relaxant	mono-amine transporters and serotonin receptors	virtual screening based on serotonin receptors
Benzbromarone	xanthine oxidase; uricosuric for treatment of gout	quorum sensing pathway; antibacterial	physical screening based on GFP reporter pathway activation
Mometasone Furoate	glucocorticoid receptors; treatment for seasonal allergy	P-glycoprotein transporter; adjuvant therapy in cancer	physical screening based on transport of fluorescent P-gp substrate
Astemizole	histamine H1 receptors; antihistamine treatment for seasonal allergy	inducer of autophagy; adjuvant therapy in prostate cancer	physical screening based on autophagy-associated granular phenotype of cells
R-Naproxen	cyclooxygenases; non-steroidal anti-inflammatory drug for short term treatment of pain	RAC and CDC42 GTPases; adjuvant therapy in cancer	physical screening based on binding of fluorescent guanine nucleotide analog to bead-bound GTPases
Ketorolac	cyclooxygenases; non-steroidal anti-inflammatory drug for short term treatment of pain	RAC and CDC42 GTPases; adjuvant therapy in cancer	virtual screening based on R-Naproxen
Tolfenamic acid	cyclooxygenases; non-steroidal anti-inflammatory drug for short term treatment of pain	binding of hantavirus to DAF receptor; antiviral for Sin Nombre virus	physical screening based on binding of inactivated, fluorescent virus to DAF receptor on cells
Phenothiazines	prototype for neuroleptic drugs; antipsychotics for management of schizophrenia	VLA-4 cell adhesion receptor; cell adhesion inhibitor for inflammation and cancer	physical screening based on fluorescent antibody detection of VLA-4 activation epitope
Methylergonovine maleate	oxytocic; treatment of post-partum uterine hemmorhage	BCL-2 family proteins; anti-apoptotic as adjuvant therapy in cancer	physical screening based on binding of fluorescent ligand to bead-bound BCL-2 family proteins
Beta-adrenergic receptor drugs	beta-2 adrenergic receptor; agonists for therapeutic management of asthma	non-cannonical G-protein coupled receptor ligands	physical screening based on receptor internalization detected with antibody-activated fluorogens