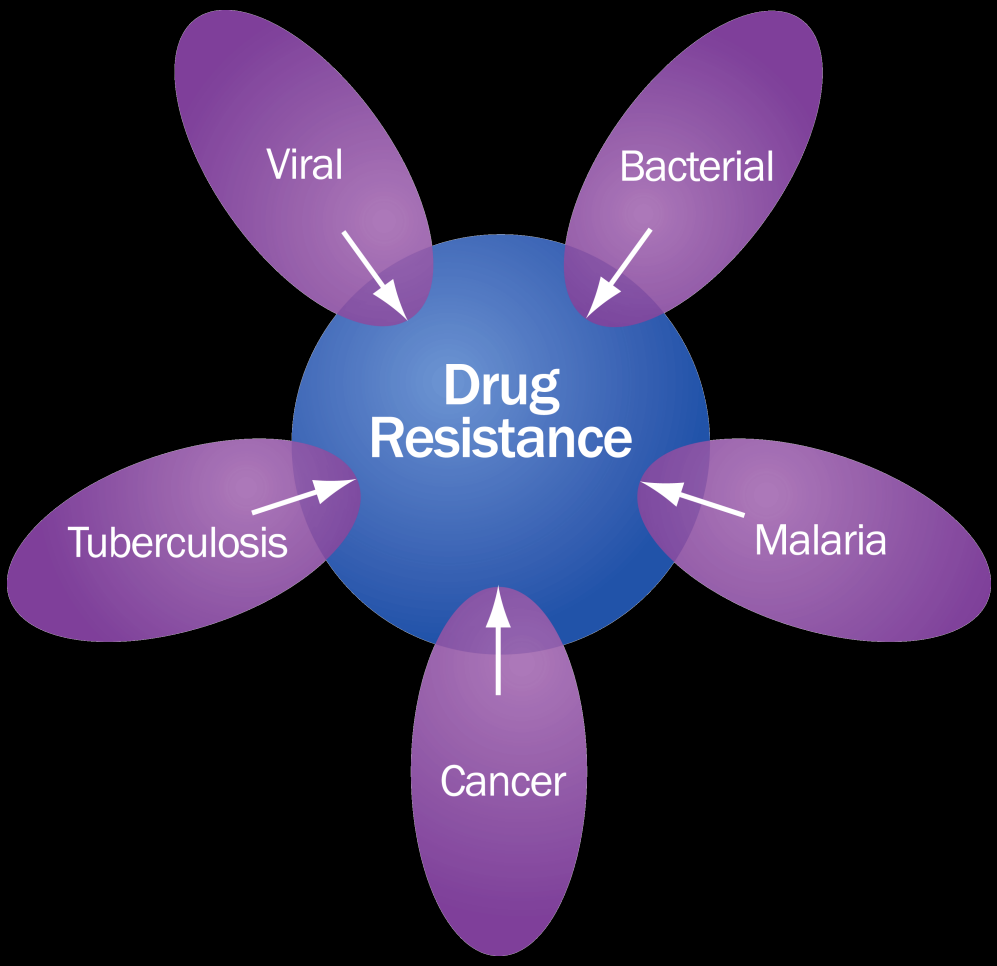

Approaches and Techniques to Avoid Drug Resistance

A Workshop Sponsored by

The Institute for Drug Resistance

May, 2010

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Sponsored by

The Institute for Drug Resistance

May 18, 2010

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University of Massachusetts: The President's Science and Technology Award, 2009

Institute for Drug Resistance

http://www.drug-resistance.org

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INTRODUCTION

The Institute for Drug Resistance (IDR) was created in 2009 to serve the drug resistance research and clinical communities with funds from the Science and Technology Award from the President's Office of the University of Massachusetts. Founded upon the observation that close parallels can be often be drawn between resistance seen in one disease state and resistance seen within another, the IDR believes deeply that cross-disciplinary collaboration and discussion could fill important gaps in current research and form the basis of novel solutions by putting drug resistance considerations first in structural and therapeutic drug design.

The IDR recognizes the fact that collaborations and innovation can be fostered more rapidly by bringing together the best minds from the drug resistance research community in the form of live interactive meetings. As a result, one of the most appreciated activities of the IDR is the sponsorship of workshops that promote interaction and intellectual engagement among scientists and clinicians representing academia, industry, and government sectors as well as a wide range of disciplines. What unites this group is their individual and collective focus on drug resistance research. No other forums currently exist that focus solely on inter-disciplinary drug resistance. In fact, this is a newly emerging field and there is very little research focused specifically on the question of how to leverage drug resistance research from fields as disparate as bacterial, viral, cancer, and parasites.

> *The cross-fertilization between otherwise disparate areas of research is really, really cool.*
>
> *~Respondent to Workshop Survey*

Building upon the success of the first inaugural meeting in Oct 2009, on May 18, 2010 the IDR conducted its second workshop titled, *Approaches and Technologies to Avoid Drug Resistance*. The workshop was very well attended and can be characterized as enthusiastic and collegial. Forty-two participants attended the "invitation-only" event. The second workshop examined approaches and technologies commonly used by drug researchers in an effort to explore the parallels in use between different disease states. The participants truly appreciate the multidisciplinary nature of these sessions as documented in the post-meeting evaluations.

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SUMMARY OF PRESENTATIONS

Dr. Celia Schiffer, Professor at University of Massachusetts Medical School and a Director of the IDR kicked off the meeting by reiterating the goal and mission of the IDR and issued a challenge to the attendees and speakers to think collaboratively outside of the box in order to find collective solutions to the problems of drug resistance. Dr. Schiffer reviewed the work and accomplishments of the IDR over the past year and reminded attendees that organization of the workshops for cross-disciplinary discussion was only half of the effort required for the IDR to fulfill its mission. She challenged attendees to complete the effort by identifying:

- How the techniques and approaches highlighted in the workshop could be used in new ways within each person's research;

- What parallels might be able to be drawn in the use of techniques and approaches between disease states;

- Who, or what groups attending the meeting might be possible collaborators for a near term project; and

- When (and how) can the IDR be of assistance in supporting such collaboration?

Dr. Daria Hazuda, Vice President, Worldwide Discovery Franchise Head, Infectious Diseases, Merck Research Laboratories presented the first of two Keynote Addresses on "HCV Antiviral Drug Resistance - Lessons & Opportunities" that highlighted parallels and lessons learned between HCV and HIV. HCV shares the characteristic of significant genetic variation within the patient with HIV; a characteristic known to limit the success of therapies. In fact, it is estimated that the problem is ten-fold more difficult in HCV and that failure to cure HCV always results in resistance. Dr. Hazuda reported that all first generation anti-viral agents require only one mutation to be neutralized. Unlike HIV however, Dr. Hazuda believes that HCV can be cured with therapies that combine agents with non-overlapping resistance. This combination therapy should prevent

replication of pre-existing variants and can lead to a sustained response and eradication of infection.

Since HCV drug development is at its peak with a large number of compounds representing multiple mechanisms in various clinical research stages the response to Dr. Hazuda's talk was very enthusiastic and productive in that she triggered and challenged the researchers and clinicians to explore the issues around drawing parallels resistance from the virology lessons learned by Merck to other quickly evolving diseases. The reception Dr. Hazuda's talk highlighted the values of the IDR and served as great beginning to the meeting.

Dr. Amy Anderson, Associate Professor of Medicinal Chemistry at University of Connecticut discussed her work on structure-based predictions of resistance mutations using computational algorithms. Dr. Anderson reported on exciting proof of concept work that uses a protein design algorithm to predict mutations that would confer resistance to the biphenyl propargyl-linked inhibitors. The challenges of such an approach include the need to be able to predict mutations that confer resistance while maintaining activity. The results of Dr. Anderson's research lead to the conclusion that computational predictions are indeed possible and applicable to any system that is subject to resistance by mutation; however, results are dependent upon knowledge of the protein structure. Dr. Anderson's work has help further the understanding of resistance mechanisms in Staphylococcus aureus.

Dr. Michelle Butler, Senior Research Scientist at Microbiotix, Inc., provided a perspective on the rise of three classes of resistant pathogens emerging as a public health issue and the simultaneous decrease in the rate of new antibiotic approvals. She presented data supporting the observation that since 1960s all antibiotics approved have been synthetic modifications of existing scaffolds. In light of the need for new and novel antibiotics Dr. Butler discussed approaches with significant potential currently including:

- Exploration of new essential targets in an effort to avoid pre-existing mechanisms of resistance;
- Creation of two antibacterial agents within one molecule to decrease the probability that mutation would compromise the agents;
- Identification and inhibition of virulence factors as these factors appear to be non-essential, thus less subject to mutation; and
- Identification and inhibition of SOS response factors as an indirect method of decreasing virulence and persistence and increasing the potency of accompanying antibiotics.

Dr. Kim Lewis, Professor of Biology at Northeastern University and Director of the Antimicrobial Center discussed the Center's research into persister cells and the mechanisms supporting their formation. Dr. Lewis described research using cell sorting and transcriptome analysis that resulted in a finding that chromosomally encoded "toxin" genes act to shut down cellular functions creating a dormant state. The mechanisms of dormancy are modulated by HipA protein and can be neutralized by HipB. Persister cells represent a challenge to certain therapies equal to that of resistant cells. Indeed in chronic conditions such as cystic fibrosis the therapies seem to select for persister cells in particular.

Dr. Lewis also discussed that fact that lack of source compounds represents the most significant bottleneck in the development of new antibiotics. Currently, less than 1% of microorganisms can be grown in the petri dish; the rest are unculturable. Research from the Antimicrobial Center has successfully replicated a natural environment in a culture chamber with recovery of associated microorganisms of 25-40%. This approach presents researchers with a new source of microorganisms for domestication and potential antibiotic production.

Dr. Roy Kishony, Associate Professor of Systems Biology, Harvard Medical School gave the second Keynote Talk to begin the afternoon session. Dr. Kishony is known for his work in understanding the mechanisms that lead to ecological-balance in the natural setting, we might be able to identify new strategies for clinical treatment that would be less likely to generate rapid resistance. He discussed the difference in the emergence of antibiotic resistance in the natural and clinical settings. In the clinic, use of antibiotics has been followed by rapid spread of resistance; a pattern not replicated in the natural environment. In the natural environment, resistant and sensitive bacterial strains live in an ecological balance in which collections of antibiotics and other toxins are present in the soil, whereas in the clinic one or a few drugs are used. Dr. Kishony described research that suggests that the ecological collection within the natural environment keep the evolution of resistance in check. Antibiotic combinations, termed "suppressive" are those in which the effect of a drug combination is lower than the effect of one of the individual drugs alone, actually *invert* selection for resistance. Because the combination of drugs reduces growth less than does a single drug, mutants that acquire resistance to one drug actually grow *slower* than their non-resistant ancestors.

Dr. Kishony also discussed the finding that natural antibiotics have much more time to degrade chemically into a range of degradation compounds, which can affect selection for antibiotic resistance. Using tetracycline, research showed that

while tetracycline selects for tetracycline resistance, degradation products actually makes tetracycline resistant bacteria lose in competition with tetracycline sensitive strains.

Dr. Kishony's talk prompted a significant question and answer period in which the entire audience was engaged in the fascinating discussion around the differences between the clinical and ecological evolution of antibiotic-resistance.

Dr. Richard Ellison, Professor of Medicine, Molecular Genetics & Microbiology at University of Massachusetts Medical School described the efforts of University of Massachusetts Medical Center to manage multi-drug resistant organisms (MDROs) in healthcare settings. Dr. Ellison described the need for research to produce adequate data on epidemiology of MDROs across the spectrum of healthcare organizations and the need for evidence-based information on appropriate approaches to control infections in different healthcare environments. Dr. Ellison's talk helped bridge the gap between the science of drug resistance and its clinical implications.

Dr. Li-Jun Ma, Principal Research Scientist at the Broad Institute provided an overview of sequencing technologies. Central to many research protocols for drug resistance, deep sequencing has undergone significant technological change in the last decade. Dr. Ma provided an overview of the best uses and limitations of the five most available technologies to accomplish this task as well as a look into new sequencing technologies expected within the next five-years.

Dr. Dan Bolon, Assistant Professor, Biochemistry and Molecular Pharmacology at University of Massachusetts Medical School discussed the applicability of systemic fitness analysis to drug resistance research. The systematic identification of drug-resistant mutations is important both for understanding the molecular mechanisms of resistance and for improving our ability to predict and mitigate against future resistance. Dr. Bolon presented results from a high throughput experimental approach to generate all possible point mutations in a gene and quantify the fitness of each mutant by measuring its abundance in a mixed culture as a function of time in competition. Performing the growth competition in the presence of a drug will enable the systematic identification of resistant mutations.

Dr. Joseph Jerry, Associate Professor of Veterinary and Animal Sciences, University of Massachusetts Amherst discussed regulation of stem cells and the risk of breast cancer. While mutations in p53 are common in breast cancers, they are enriched in the basal-like subtype of breast cancers with 82% of tumors carrying mutations. These tumors are aggressive and develop resistance to standard chemotherapies frequently. Mouse models that are deficient in p53 develop spontaneous mammary tumors that exhibit features of the basal-like breast tumors and stem cell markers. We now show that loss of p53 function results in an expansion of the population of progenitor cells in the mammary epithelium. The progenitors provide a vulnerable pool of cells that can accumulate mutations resulting in mammary tumors. The stem/progenitor cell origins of this subtype of mammary tumors may explain the phenotypic plasticity and ability to acquire resistance to chemotherapeutics.

WORKSHOP EVALUATION

A post-workshop survey quantified the enthusiasm of participants that was so evident during the workshop. The ability to network and brainstorm with other researchers focusing on drug resistance research is highly valued. Almost 80% of respondents reported leaving the meeting having identified a person or group with whom to explore new collaborations.

Attendees were split evenly between those who have attended other IDR events and those who attending their first event. They were united in their strong interest in future IDR events, expressing appreciation for the following characteristics of IDR events:

- Quality of the speakers and the diverse topics covered;
- Collaborative nature and variety of institutions represented at events;
- Cross-fertilization between disparate areas of research;
- Ability to get researchers from academia and industry together.

Looking to the future, attendees suggest longer workshop schedules (full day meetings) and increased efforts to create collaborative opportunities.

Thank you again for the invitation. It was really a great meeting; I learned so much and left with lots of new ideas.

~Daria Hazuda

On October 4, 2010 the IDR will hold a full day scientific conference to further explore the issues that have arisen from workshop efforts to discover the parallels between disciplines and disease states and to understand how shifting the paradigms of drug resistance will open up novel ways to challenge the problem of drug resistance.

WHAT'S NEXT FOR THE IDR?

A Science and Technology Award from the President's Office of the University of Massachusetts funded the efforts of the IDR between 2009-2010. To continue the work of the Institute will require additional funding as well as the on-going enthusiasm of researchers and clinicians. To this end, the IDR will focus its efforts on the following activities:

- Broaden the participant base to include a larger geographical, scientific community

- Target a set of research funding opportunities and provide logistical and technical support to enhance and accelerate collaborations

- Expand the recognition of Drug Resistance as an emerging discipline in its own right

- Establish a 2010-2011 Workshop and Webinar Conference schedule to support continued networking of researchers with a drug resistance focus.

Institute for Drug Resistance
"Approaches and Technologies to Avoid Drug Resistance"
Hoagland-Pincus Conference Center, UMass Medical School, Shrewsbury Campus
www.umassmed.edu/conferencecenter/Directions.aspx
May 18th, 2010

11:30-12:00	**Registration & Lunch**
Welcome and Introductions: **12:00 – 12:30**	**Danielle Federa – Welcome & Participants - Brief Introductions** **Celia Schiffer – Overview of IDR where we are, what we have planned**
Key Note: "HCV Antiviral Drug Resistance – Lessons & Opportunities"	
12:30 – 1 pm	**Daria Hazuda – Merck** **Vice President Worldwide Discovery, Franchise Head for Infectious Diseases, MRL, Merck**
Molecular Strategies	
Peg Riley Facilitator **1:30 – 2:30 pm**	**Amy Anderson – University of Connecticut** **Michelle Butler – Microbiotix** **Kim Lewis – Northeastern**
2:30-2:45	**Panel Discussion**
2:45 Coffee/Dessert Break	
Key Note: "Inverting Selection for Antibiotic Resistance"	
3:15-3:45	**Roy Kishony – Harvard Medical School Associate Prof of Systems Biology, Harvard Medical School**
Tien Bui Facilitator **3:45 – 5:05 pm**	**Dick Ellison – U Mass Medical School** **Li-Jun Ma – Broad Institute** **Dan Bolon – U Mass Medical School** **Joe Jerry – U Mass Amherst**
5:05 – 5:20	**Panel Discussion**
Summary and Close	
5:20-5:30	**– Closing Thoughts**
5:30-6:30pm	**Social & Networking Hour**

LIST OF PARTICIPANTS

Dr. Akbar Ali	UMASS Medical School
Dr. Amy Anderson	University of Connecticut
Dr. Matthew Baevsky	Prime Organics, Inc.
Dr. Dan Bolon	UMASS Medical School
Dr. Stephen B. Brecher	VA Boston Healthcare System
Ms. Tien Bui	Biozen Consulting
Dr. Michelle Butler	Microbiotix, Inc.
Dr. Hong Cao	UMASS Medical School
Dr. Jen Carr	Brown University
Dr. Alfred DeMaria, Jr.	MA Department of Public Health
Dr. Richard T. Ellison, III	UMass Memorial Hospital Center
Ms. Danielle Federa	Institute for Drug Resistance
Dr. Jon Goguen	UMASS Medical School
Dr. Ronen Hazen	Harvard Medical School
Dr. Daria Hazuda	Merck
Dr. George Jacoby	Lahey Clinic
Dr. Joe Jerry	UMASS Amherst
Dr, Geunhwa Jung	UMASS Amherst
Dr. Romy Kirby	US Army RDECOM
Dr. Roy Kishony	Harvard Medical School
Dr. Kim Lewis	Northeastern University
Dr. Li-jun Ma	Broad Institute
Dr. Nagraj Mani	Vertex Pharmaceuticals
Dr. C. Robert Matthews	UMASS Medical School
Dr. Michael Mesleh	Cubist Pharmaceuticals
Dr. Donald Moir	Microbiotix, Inc.
Dr. Tim Opperman	Microbiotix, Inc.
Dr. Debasis Patnaik	Independent Consultant
Dr. Norton Peet	Microbiotix, Inc.

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Dr. Laurence Rahme	Harvard Medical School
Dr. Celia Schiffer	UMass Medical School
Dr. Donald Soja	Merck
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