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Small Molecule Screens to Identify Inhibitors of Infectious Disease

Elizabeth Hong-Geller and Sofiya Micheva-Viteva

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1. Introduction

In the 1940's, the development of penicillin as a potent broad-range antibiotic revolutionized the treatment of infectious disease and ushered in a prolific discovery period of natural small molecules produced by microorganisms that were antagonistic towards the growth of other bacteria. Antibiotics have generally been classified by their mechanism of action. For example, the β -lactam compounds, penicillin and cephalosporins, disrupt the synthesis of the peptidoglycan layer of the bacterial cell wall, whereas protein synthesis inhibitors, such as tetracycline and some aminoglycosides, bind to the 30S ribosomal subunit and block addition of amino acids to the growing peptide chain. By the 1960's, the majority of all antibiotics in use today had been isolated and developed for public consumption, leading the U.S. Surgeon General to declare in 1968 that the war on infectious disease had been won.

Unfortunately, nature has found a way to thwart mankind's effort to contain infectious disease. Under the selective pressure of antibiotics that target different cell processes, bacteria have evolved to become resistant to the lethal effects of many classes of antibiotics. One stark example that has emerged as a major public health threat is methicillin-resistant *Staphylococcus aureus* (MRSA), which is estimated to cause ~19,000 deaths in the US annually [1]. MRSA has become resistant to β -lactam antibiotics by acquiring the resistance gene *mecA*, which encodes for a unique penicillin binding protein PBP2A that can function as a surrogate for native staphylococcal PBPs normally inactivated by β -lactam antibiotics. In the last decades of the 20th century, MRSA has continued to evolve in response to a continually changing human environment, from a primary agent of hospital-acquired infections to a multi-drug resistant strain that has also acquired Tn1546 transposon-based vancomycin resistance. Furthermore, the appearance of MRSA strains in a community setting may be a stepping stone to the evolution of a completely drug-resistant strain.

There is no question that new strategies that target different aspects of pathogen function are urgently needed to combat multi-drug resistant bacteria. However, very few new scaffolds for drug discovery developed after the 1960s have been found to be effective [2]. To date, only four new classes of antibiotics, including mutilins and lipopeptides, have been introduced, but none of these have proven to be as effective as the panel of classic antibiotics. Instead, established scaffolds have been modified or re-purposed to develop successive generations of effective antibiotics. For example, the core structure of cephalosporins have been left intact to preserve activity, but the peripheral chemical groups have been modified to impart the molecule with the ability to penetrate the bacterial membrane more efficiently or be more resistant to β -lactamase [3]. Modifications of four classic antibiotics, cephalosporin, penicillin, quinolone, and macrolide, account for ~73% of the “new” antibiotics filed between 1981 and 2005 [4]. It is also important to note that small compounds need to exhibit not only anti-microbial activity, but also minimized cytotoxic properties to widen their therapeutic window.

Although advances in organic synthesis have extended the lifetime of classic antibiotics through synthetic modifications, new scaffolds are also needed. Recent efforts to search for new modalities amongst previously-overlooked natural sources, such as unmined bacterial taxa and ecological niches, have started to bear fruit. The increasingly rapid data acquisition and low cost of ultra high-throughput sequencing has provided rich coverage of bacterial genomes and transcriptomes. For example, genomic analyses of a vancomycin-resistant strain of *Amycolatopsis orientalis* revealed the presence of genetic loci that encode for at least 10 other secondary metabolites. One compound, ECO-0501, exhibited strong anti-bacterial properties against Gram-positive pathogens, including several strains of MRSA [5]. Mass spectroscopy (MS) is another primary methodology used to identify small molecule metabolites with potential anti-microbial properties. The polycyclic small molecule, abyssomicin C, from the marine actinomycete *Verrucosisspora* was characterized as an inhibitor of *p*-amino-benzoate biosynthesis by MS and also exhibited antimicrobial properties against MRSA strains [6].

2. Methodology for high-throughput screens (HTS) using small molecule libraries

The workhorse platform for anti-bacterial drug discovery is a chemical genetics HTS approach using small molecule compound libraries to identify candidates that inhibit bacterial growth or the function of key bacterial enzymes. Small molecules, generally <500 molecular weight, have the potential to enter cells and selectively perturb specific protein activity, thus functioning as therapeutic agents against disease. In general, the precise mechanism of inhibitor activity remains unknown in the initial screen. Subsequent identification of the molecular targets of small molecules will have to be performed to implicate the specific bacterial functions that were inactivated in the screen. Thus, HTS can sample a large unbiased collection of structurally diverse molecules to select compounds that perturb the defined cell phenotype of interest. (Fig. 1)

Various chemical compound libraries are now available through commercial and public resources that include FDA-approved bioactive compounds, therapeutic agents, and natural products. To maximize the structural complexity and diversity of small molecule libraries, scientists have also employed diversity-oriented synthesis,

Figure 1. General flowchart of high-throughput methodology to screen small molecule libraries for inhibitors of host-pathogen interactions

in which different scaffolds are modified with highly diverse functional groups. [7, 8]. To bolster academic research in chemical biology efforts for HTS-driven identification of bioactive compounds, the NIH launched the Molecular Libraries Program in 2005 to offer access to ten large-scale automated HTS centers in the Molecular Libraries Probe Production Centers Network, including diverse compound libraries through the Small Molecule Repository and information on biological activities of small molecules in the PubChem BioAssay public database.

A variety of different molecular and cellular methods have been developed for HTS using small molecule libraries. Automated microscopy has been utilized for high-content, image-based screens of cells exposed to small molecules. Acquired cell images can be analyzed by automated image analysis software to quantitate physiological changes at the single-cell level, including phenotypes such as morphology and cell toxicity. Small molecule microarrays, in which ~10,000 small molecules are covalently bound to a glass slide, has been generated to detect high affinity binding to a protein of interest, as a potential inhibitor of function. Binding of the protein of interest to specific compounds on the microarray was then detected with fluorescent antibodies [9].

3. Disruption of host-pathogen interactions for novel drug discovery

Given the innovation gap in the discovery of novel antibiotics post-1960, strategies to inhibit novel targets are greatly needed to combat infectious disease. Multiple studies have identified small molecule inhibitors that target gene expression of pathogen TTSS components in *P. aeruginosa*, enteropathogenic *E. coli*, and *Y. pestis* [10, 11, 12]. The small molecule virstatin, 4-[N1,8-naphthalimide)]-n-butyric acid, was identified as an inhibitor of the transcriptional regulator ToxT in *Vibrio cholerae* [13]. The small molecule, 2-imino-5-arylidene thiazolidinone, which blocks TTSS-dependent functions in *S. typhimurium*, was also found to inhibit virulence in *Yersinia*, *Pseudomonas*, and *Francisella* strains, indicating that compounds can be identified that target common processes in multiple pathogens [14].

Research efforts have recently begun to focus on disruption of host-pathogen interactions as a new approach to identify potential targets for drug discovery, rather than solely on specific pathogen targets or processes. In particular, the screening of small molecule libraries to identify inhibitors that block pathogen infection of the host, using such phenotypes as pathogen invasion, host morphology, and pathogen replication in the host, is a powerful approach for therapeutic development that may uncover fundamental mechanisms of pathogenesis and potentially lead to discovery of new classes of anti-infective agents. Here, we describe case studies of the use of small molecules in host infection screens to identify novel inhibitors against infectious disease, including bacterial, viral, parasitic, and fungal infections. We will discuss these studies in the context of re-purposing known drugs, inhibitor specificity, and discovery of basic mechanisms of host-pathogen interactions. The screen results are summarized in Table 1.

Screening for inhibitors of intracellular infection

Intracellular pathogens, including viruses, parasites, and some bacteria, manipulate specific host factors in order to downregulate the host immune response or modulate host actin cytoskeleton rearrangements to induce phagocytic uptake of the pathogen. *L. monocytogenes*, an intracellular Gram-positive bacteria, infects the human host primarily through ingestion of contaminated foods and causes gastrointestinal infection. In 2011, *Listeria* contamination of cantaloupes led to at least 30 deaths and ~150 illnesses in 28 states. Following internalization of *L. monocytogenes* in host membrane-bound vacuoles, the pore-forming cytolysin, listeriolysin O (LLO) and a phosphatidylinositol-specific phospholipase C (PI-PLC) mediates lysis of the vacuoles to release the pathogen into the host cell cytosol. *L. monocytogenes* then polymerizes host actin to propel itself into adjacent host cells to continue the infection process. To identify compounds that inhibited *L. monocytogenes* intracellular infection, a screen of 480 small molecules from the Biomol ICCB Known Bioactives library was performed using automated microscopy and image analysis [15]. Murine bone marrow-derived macrophages were infected with a GFP-expressing *L. monocytogenes* strain to assess efficiency of invasion, survival, and replication in the host. Twenty-one compounds, affecting cell functions such as actin polymerization, calcium signaling, and apoptosis, were identified that markedly decreased *Listeria monocytogenes* infection efficiency. In particular, the FDA-approved anti-psychotic drug pimozide, used to treat Tourette's syndrome and schizophrenia, was shown to

decrease internalization of not just *L. monocytogenes*, but other bacterial species as well, including *Bacillus subtilis*, *Salmonella typhimurium*, and *E. coli*. Furthermore, pimozone decreased vacuole escape and cell-to-cell spread of *L. monocytogenes* in the host. Thus, pimozone is an example of a small molecule that can be re-purposed to treat infectious disease with potential for broad spectrum anti-microbial applications.

Parasites also employ a life cycle of host cell invasion, replication, and host cell lysis during onset of infection. *Taxoplasma gondii* is the protozoan intracellular human parasite of the phylum Apicomplexa and is related to *Plasmodium* and *Cryptosporidium*, the causative agents of malaria and diarrheal disease, respectively. To discover inhibitors of *T. gondii* invasion, a high-throughput

Pathogen	# Compounds	# Hits	Assay and methods	Ref
Bacteria				
<i>L. monocytogenes</i>	480	21	Host cell invasion, automated microscopy	15
<i>P. aeruginosa</i>	50,000	88	ExoU, edoated host cytotoxicity	18
	56,280	6	Cytotoxicity in yeast model	19
<i>Y. pseudotuberculosis</i>	100,000	45	Translocation of Yops into the host	20
<i>B. anthracis</i>	70,094	30	Lethal factor entry into host	21
	10,000	24	Interaction between edema factor and CaM	22
<i>P. syringae</i>	~200	3	Bleaching of <i>Arabidopsis</i> seedlings	23
	80	1	Bleaching of <i>Arabidopsis</i> seedlings	24
Parasite				
<i>T. gondii</i>	12,160	24	Host cell invasion, motility, adhesins	16
Virus				
HIV	~200,000	27	Induction of viral latency	49

Table 1 Small molecule screens using host-pathogen systems

microscopy assay was developed to distinguish between extracellular and intracellular parasites in a BS-C-1 epithelial cell model, using differential labeling with fluorescent dyes [16]. Out of a 12,160 structurally-diverse small molecule library, 24 non-cytotoxic inhibitors were identified that reduced parasite invasion to <20% compared to control wells. These molecules inhibited different aspects of the infection process, including gliding motility and secretion of host cell adhesins. One of these inhibitors, tachyplegins, was found to post-translationally modify TgMLC1, a myosin light chain component of the *T. gondii* myosin motor complex, which drives host cell penetration and parasite mobility [17]. TgMLC1 exposed to the small molecule exhibited a rapid and irreversible change in electrophoretic mobility on SDS-PAGE gels. Although the exact nature of the modification remains unclear, the modification has been mapped to amino acids V46-R59 by mass spectroscopy. These studies provide key mechanistic information on the importance of *T. gondii* motility in pathogenesis and illustrate the potential for small molecules to form covalent interactions with target proteins.

Targeting virulence toxin mechanisms of infection

Many Gram-negative bacteria, including *Pseudomonas* and *Yersinia*, utilize the TTSS as a primary mechanism of virulence to inject effector proteins into the host cytosol to downregulate

late the host immune response. A host cytotoxicity assay was designed to screen for small molecule inhibitors of *Pseudomonas aeruginosa*, a leading cause of hospital-acquired infections in cystic fibrosis patients. *P. aeruginosa* ExoU, a TTSS effector protein, is a member of the patatin family of phospholipase A₂ (PLA₂) that can lyse host cell membranes during infection. A high-throughput screen of 50,000 compounds from the Chembridge Microformat Library E was performed using a colorimetric live/dead assay to identify small molecules that protected Chinese hamster ovary (CHO) cells from cytotoxicity mediated by *P. aeruginosa* expressing ExoU as the sole TTSS effector [18]. A primary list of 88 compounds exhibited rescue of CHO cells from ExoU-mediated cytotoxicity. The most effective compound, pseudolipasin A, inhibited ExoU function downstream of TTSS delivery into the host. In addition to inhibition of CHO cytotoxicity, pseudolipasin A also protected the amoeba *Dictyostelium discoideum* from ExoU-mediated killing by *P. aeruginosa* and inhibited cytotoxicity in the yeast *Saccharomyces cerevisiae* expressing ExoU. Interestingly, pseudolipasin A did not affect eukaryotic PLA₂, suggesting that this small molecule may specifically target bacterial PLA₂. Pseudolipasin A is representative of small molecules that do not kill or inhibit the growth of pathogens, but instead attenuate their virulence.

Inhibitors of *P. aeruginosa* virulence have also been identified using a cell-based yeast phenotypic assay in combination with a large-scale small molecule screen. A total of 505 *P. aeruginosa* virulence factors and essential genes were individually overexpressed in *S. cerevisiae* to downselect genes that inhibited yeast growth [19]. Nine genes strongly or partially impaired yeast growth, including three TTSS effectors, ExoS, ExoT, and ExoY. ExoS has been previously shown to ADP-ribosylate multiple downstream targets, including vimentin, the Ras family of small GTP-binding proteins, and cyclophilin A. Given that ExoS is a critical mediator of *P. aeruginosa* chronic infections, a library of 56,280 compounds was screened to find inhibitors of ExoS ADP-ribosylation activity that rescued cytotoxicity in yeast. Six compounds were identified that restored yeast growth. The most promising compound, exosin, was found to modulate ExoS enzymatic activity *in vitro* and exhibited a protective effect against *P. aeruginosa* infection in mammalian CHO cells. This study demonstrates the effective use of a simple eukaryotic host, baker's yeast, as a tool for drug screening for applications in controlling infectious disease in humans.

Another pathogen family that employs the TTSS is *Yersinia*, which secrete Yop effectors into the host cell. There are three *Yersinia* human pathogens, *Y. pestis*, the etiological agent of plague via intradermal fleabites or inhalation, and *Y. pseudotuberculosis* and *Y. enterocolitica*, which cause mild and self-limiting enteric disease by the oral route. HTS strategies have been developed to identify small molecules that inhibit translocation of the Yops into host cells. A recombinant *Y. pseudotuberculosis* strain was constructed to express a chimeric protein containing the first 100 amino acids of YopE, which contains the proper translocation signals to inject into the host, fused to a fragment of β -lactamase. [20]. This bacterial strain was used to infect HEp-2 host cells treated with a non-membrane-permeating, non-fluorescent dye CCF2-AM, which fluoresces green at 520nm, as a result of intramolecular FRET between the 7-hydroxycoumarin and fluorescein molecules, conjugated by a lactam ring. Upon cellular uptake, CCF2-AM is modified by cytoplasmic esterases and is trapped in the host

cell. If the YopE- β -lactamase fusion is introduced into the host, the β -lactamase will cleave the lactam ring in CCF2-AM and liberate the fluorescein, leaving the coumarin to fluoresce blue at 447nm. Using this differential fluorescence assay, 100,000 compounds from a number of sources, including the ChemDiv 2, ChemDiv 3, ChemDiv 4, Maybridge 3, Maybridge 4, and Biomol ICCB libraries, were screened for low ratios of blue-to-green fluorescence. In total, 200 compounds were deemed potential hits, and 45 were assessed further using secondary assays, including rounded host morphology in response to *Yersinia* infection. Finally, 6 compounds were found that inhibited translocation of effectors into the host without affecting expression and function of TTSS components. Several of these compounds also inhibited host cell rounding when induced by *Pseudomonas* effectors, suggesting that these compounds may have a broad-spectrum anti-infective effect.

A screen to identify small molecule inhibitors of *B. anthracis* also employed the CCF2-AM FRET assay. *B. anthracis*, the Gram-positive causative agent of anthrax, secretes three major toxins during infection, lethal factor (LF), protective antigen (PA), and edema factor (EF). A fusion protein between LF and β -lactamase was introduced into host cells by PA-directed endocytosis to hydrolyze the CCF2-AM fluorogenic substrate [21]. Out of 70,094 compounds tested, 1170 initial hits exhibited concentration-dependent inhibition of β -lactamase activity. Thirty compounds with known biological activities and/or were high confidence hits were selected for further analysis. Three compounds, NCGC00084148-01, diphyllin, and niclosamide, exhibited protective effects from anthrax LF, a LF fusion to *Pseudomonas* exotoxin, and diphtheria toxin in RAW264.7 murine macrophages and CHO cells, and are thought to interfere with toxin internalization in the host.

The interaction between *B. anthracis* EF and its cellular activator, calmodulin (CaM), became the basis of a two-step tandem screen to identify small molecule inhibitors of anthrax infection. A library of 10,000 compounds (Chembridge, Library # ET350-1) in pools of 8 was screened to identify small molecules that blocked an EF-induced flat to round morphology change in Y1 murine adrenocortical cells [22]. Twenty-four initial hits were then individually tested using surface plasmon resonance (SPR) to identify molecules that block interactions between EF and immobilized CaM. One compound, (4-[4-(3,4-dichlorophenyl)-thiazolylamino]-benzenesulfonamide) 10506-2A, efficiently inhibited EF-CaM binding in a dose-dependent manner, and was found to specifically target the CaM binding region of EF by fluorescence spectroscopy. Since this compound was found to be toxic in cultured mammalian cells, a series of structurally-related compounds was synthesized, and a new inhibitory compound with reduced toxicity was subsequently identified.

Small molecule discovery in plant-pathogen interactions

Discovery of small molecule inhibitors has also been extended to plant pathogen systems as an approach to develop commercially-relevant chemicals to protect crops assets from disease. The Gram-negative pathogen *Pseudomonas syringae* expresses a TTSS, enters plant tissues through the stomata or wounds, and infects a wide range of plant species. A major challenge in the application of small molecule screens to plant-pathogen interactions is the development of high-throughput methodology with a plant model system. A high-throughput liquid assay was developed based on *P. syringae*-induced bleaching of *Arabidopsis thaliana*.

ana cotyledon seedlings, which signifies a loss of chlorophyll from plant tissues and is indicative of bacterial pathogenesis [23]. A screen of ~200 small molecules active in *Arabidopsis* (LATCA, Library of Active Compounds in Arabidopsis) identified several sulfanilamide compounds, including sulfamethoxazole, sulfadiazine, and sulfapyridine, that prevented cotyledon bleaching upon *P. syringae* infection. The most potent compound, sulfamethoxazole, also inhibited *P. syringae* growth in mature soil-grown plants. A similar assay was used to implicate the same compound, sulfamethoxazole, and the indole alkaloid gramine as inhibitors of *Fusarium graminearum* fungal infection in *Arabidopsis* and wheat, indicating that this strategy represents a relevant surrogate system for identification of compounds that can prevent agriculturally-important infectious disease [24].

Combinational antiviral therapies for HIV

Given that viral pathogens are absolutely dependent on the host for propagation, even more so than bacterial pathogens, research in host-directed anti-virals has advanced at a faster pace than that for anti-bacterial agents. Human Immunodeficiency virus type 1 (HIV-1), a lentivirus of the retroviral family and the causative agent of AIDS, is the most-widely studied viral pathogen to date. HIV-1 infection causes a dramatic decline in host CD4⁺ T cell numbers and a progressive failure of the immune response, which makes the host susceptible to opportunistic infections and cancer. The highly glycosylated HIV-1 envelope, in combination with the extreme diversity of circulating viral strains, have presented daunting challenges for development of an effective vaccine. Furthermore, the virus establishes chronic infection that resists the highly active antiretroviral therapy (HAART). Conventional HAART for HIV-1 infection combines three main classes of anti-viral drugs:

1. nucleoside reverse transcriptase inhibitors (NRTIs),
2. non-nucleoside RT inhibitors (NNRTIs), which target the non-catalytic domain of RT, and
3. protease inhibitors (PIs).

HAART is usually patient-specific, and its formulation is determined by the viral load and drug resistance. A traditional HAART consists of two NRTIs and a NNRTI or a PI [25]. More advanced combination therapies include a fourth class of antiretroviral drugs, HIV entry inhibitors. HIV-1 entry into human cells is dependent on several sequential steps that include binding of viral envelope protein gp120 to the CD4 receptor, and conformational change in gp120 that increases its affinity to the chemokine co-receptors (CCR5 or CXCR4) and exposes gp41, an HIV envelope protein that executes the fusion of HIV and host cell membranes.

Currently, there are two approved inhibitors of HIV-1 entry:

1. enfuvirtide, a peptide fusion inhibitor that binds to gp41 and
2. maraviroc, a small molecule entry inhibitor that prevents interaction between gp120 and CCR5.

The β -chemokine receptor CCR5 was found to act as a major co-receptor for the macrophage-tropic HIV-1 R5 strains, predominant in the early asymptomatic stages of virus infec-

tion, whereas the T-cell-tropic strains (using the CXCR4 co-receptor) become prevalent in the symptomatic stages concomitant with the decline of CD4⁺ T-cells [26]. CCR5 is an attractive target for development of HIV-1 entry inhibitors, given the discovery that HIV-1 non-progressors, individuals homozygous for a 32-bp deletion in the coding region of CCR5 gene (CCR5 Δ 32) were naturally resistant to infection with R5 HIV-1 [27]. Natural and synthetic CCR5 ligands such as RANTES, AOP-RANTES, Mip-1 α , Mip-1 β , and Met-RANTES were found to efficiently protect against R5 HIV-1 infection [28, 29]. Thus, the first published high throughput screen (HTS) for discovery of non-peptide inhibitors of HIV-1 entry was performed in a virus-free cell-based system using [¹²⁵I]-labeled RANTES. A strong inhibitor of RANTES binding to CCR5 stably expressed on the surface of CHO cells was identified from the library of Takeda Chemical Industries. Further chemical modifications of the lead compound designated TAK-779 produced a potent (IC₅₀ 1.4 nM in CHO/CCR cells) and selective CCR5 antagonist capable of blocking R5 HIV-1 infection *in vitro* [30].

The number of CCR5 inhibitors has significantly grown since the discovery of TAK-779, but very few compounds have entered clinical trials, and only maraviroc has been approved for clinical use [31]. A radiolabeled-chemokine binding assay similar to one applied for the identification of TAK-779 was used in a HTS of a small molecule library at Pfizer for the discovery of UK-107,543, which had become a scaffold for intensive medicinal chemistry, producing ~1,000 analog compounds, from which maraviroc (UK-427,857) was selected for its excellent preclinical pharmacokinetics (90% inhibitory concentration of 2 nM in pool of PBMCs from various donors) [32]. Despite its proven efficacy against HIV-1 R5 infection, maraviroc is vulnerable to gp120 escape mutations [33]. Site-directed mutagenesis and molecular modeling studies have identified a common binding pocket on CCR5 that is shared by various small-molecule CCR5 inhibitors [34, 35, 36]. Emerging details on gp120 and CCR5 points of interaction and binding thermodynamics provide valuable information that can be applied in developing tools for rational design of novel HIV-1 entry inhibitors [37, 38]. Efficient block of HIV entry into host cells is essential to curtail virus dissemination and is a key step towards eradication of HIV infection. The current HAART regimen can reduce HIV replication to very low levels (below 50 copies/ml plasma) and can lead to recovery of CD4⁺ T-cell counts but not cure the infection. Patients that have been successfully treated with HAART for years have experienced a rapid virus rebound upon termination of the therapeutic regimen [39, 40]. Such clinical cases present evidence that HIV establishes a chronic infection that resists current HAART designed to target actively replicating virus. A deliberate and controllable induction of HIV-1 replication from its latent reservoirs in combination with HAART is a novel and actively pursued approach that aims to eliminate both active and latent viral pools [41].

Researchers often seek new anti-infective agents amongst small molecules that have previously been approved for the treatment of cancer and neurological diseases, since they have well-established pharmacokinetics and in most cases, known molecular mechanisms of action. One example of this is the histone deacetylase (HDAC) inhibitor, valproic acid (VA), which had previously been approved for treatment of neurological and psychiatric disorders. HIV-1 has been shown to enter dormancy using epigenetic silencing via deacetylation

of histones in the vicinity of the integrated viral genome [42]. Thus, VA was tested as a potential agent to disrupt HIV-1 latent infection. However, years of VA treatment in combination with HAART showed no clearance of the latent HIV reservoir [43]. A more potent HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), approved for treatment of cutaneous T-cell lymphoma, was subsequently tested as a potential agent that could 'flush out' HIV-1 from latently infected cells, based on its superior effect to VA in cell culture models [44, 45]. A substantial effort has also been invested in the design and synthesis of bryostatin chemical analogs, small molecules that activate protein kinase C (PKC) with single nanomolar concentration [46]. PKC activation leads to phosphorylation of nuclear factor κ B (NF κ B), a key transcription factor regulator of HIV-1 gene expression [47]. However, modulation of NF κ B activity requires great caution, since abnormal NF κ B signaling has been related to the pathophysiology of inflammatory diseases and neurodegenerative disorders [48].

A HTS of a small molecule library recently identified novel HIV latency activators [49]. The screen was performed using a lymphoma CD4⁺ T-cell line (SupT1) harboring latent recombinant HIV-1 and two reporters that reflect early and late virus gene expression incorporated in the HIV-1 genome [50]. A luminescent assay based on secreted alkaline phosphatase (SEAP) activity, incorporated in the late virus gene transcripts, was applied to screen a chemical library of ~200,000 compounds. Validation of 27 hits with diverse chemical structures demonstrated induction of latent virus from various cell models. Compounds with a selective index (CC₅₀/EC₅₀) above 25 were chosen for downstream medicinal chemistry modifications. Moreover, the lead compounds were shown to reactivate latent HIV from primary resting CD4⁺ T-cells with no induction of cell proliferation. Small molecule activators of latent HIV that act in concert using different mechanisms have a better chance of purging the virus out of infected cells [49]. Such pre-clinical data strongly suggests that successful treatment of HIV infection can be achieved only through combinational therapy consisting of diverse class of antiviral drugs.

4. Whole animal small molecule screens using *C. elegans*

In vitro high-throughput screens have several limitations for the discovery of therapeutic inhibitors with high efficacy. Synthetic compound libraries often contain toxic compounds with poor pharmacokinetic properties, and many *in vitro* assays are not physiologically-relevant in the context of which a specific drug is expected to function. In a previous section, we had described the use of a HTS whole organism-based assay based on *Arabidopsis* seedlings as the host system. Here, we detail the use of a whole animal model, the nematode worm *C. elegans*, in chemical screens that permit simultaneous assessment of the immunomodulatory effects, potential toxicity of compounds, and drug efficacy in a host with a functioning immune system. The results of these screens are summarized in Table 2. Whole animal screens have the distinct advantage of being able to directly discard compounds that induce organismal toxicity and can identify compounds that target host-pathogen interactions in a relevant physiological context.

C. elegans, a hermaphroditic nematode normally found in soil, is a versatile, more ethically-acceptable whole animal system for high-throughput analysis of host response to pathogen infection. *C. elegans* contains a fully sequenced genome that facilitates both genetic and genomic analysis, offering an ideal compromise between organismal complexity and experimental tractability. *C. elegans* offers other experimental advantages, including a rapid 2-3 week life span, simple growth conditions, target-selected gene inactivation, and a relatively low cost of maintenance compared to other whole animal systems. A wealth of experimental data has demonstrated that many developmental, neurological, and biochemical processes have been highly conserved between *C. elegans* and mammals. For example, cellular functions as diverse as innate immunity, the first line of defense against pathogen infection, and RNA interference to downregulate gene expression via double-stranded RNA, are found in both *C. elegans* and higher eukaryotes, suggesting the existence of a common ancestor of these diverse species. Thus, anti-infective compounds identified using a *C. elegans* infection model may also be translatable in humans.

C. elegans as a model host system has been well-studied for numerous bacterial pathogens, including the Gram-positive *S. aureus*, *S. pneumoniae*, and *B. thuringiensis*, and the Gram-negative *B. pseudomallei*, *P. aeruginosa*, and *S. marcescens*. In general, different types of bacteria are fed to *C. elegans* in place of their normal *E. coli* food source to provoke detectable symptoms of illness, such as locomotion dysregulation, intestinal cell lysis, and shortened life span.

Small molecule inhibitors of bacterial infection

A small manual screen of 6000 synthetic compounds and 1136 natural extracts were analyzed in an immunocompromised mutant of *C. elegans* infected with *Enterococcus faecalis* to identify compounds that promoted host survival. [51]. A total of 16 compounds and 9 extracts were identified that either modulated bacterial growth *in vitro*, impaired pathogen virulence, or boosted host innate immunity. Furthermore, 15 out the 16 compounds did not kill *C. elegans* or mammalian erythrocytes, indicating that the compounds are not toxic.

The development of automated sorting and handling of *C. elegans* rapidly enabled high-throughput screening of small chemical libraries to identify compounds that enhanced survival of *C. elegans* in response to bacterial infection. This methodology was enabled by the Complex Object Parametric Analyzer and Sorter (COPAS) BioSort worm sorter (Union Biometrica) to dispense a defined number of living worms into multi-well plates, which were then imaged using automated microscopy to quantify worm survival. A library of 37,200 compounds and natural product extracts was screened using the same *C. elegans*-*E. faecalis* infection system described above [52]. Twenty-eight compounds and extracts were identified that enhanced survival of infected *C. elegans*. Six structural classes of identified compounds did not affect the growth of *E. faecalis* itself, suggesting that the small molecules inhibited a specific aspect of the host-pathogen interaction. Interestingly, two structural classes are similar to compounds previously identified in a high-throughput screen to identify inhibitors of *P. aeruginosa* biofilm development, indicating the presence of common molecular targets across multiple bacterial species for drug discovery [53].

A *P. aeruginosa* infection model of *C. elegans* has also been developed to screen for novel anti-infective compounds. The high-throughput assay was based on *P. aeruginosa*-induced slow

killing of *C. elegans* in the presence of 1300 bioactive extracts produced by endophytic fungi associated with medicinal plants [54]. The screen identified 36 extracts that promoted the survival of the infected worms, while 4 extracts were found to inhibit *P. aeruginosa* growth using a disc diffusion assay. Given that these extracts contain a mixture of metabolites, the specific compound against *P. aeruginosa* remains to be determined. Nevertheless, this study illustrates the rich reservoir of small molecules in natural symbiotic organisms with antibacterial activity.

Pathogen	# Compounds	# Hits	Assay and methods	Ref
<i>E. faecalis</i>	7136	25	Host survival	51
	37,214	28	HTS, automated microscopy of host survival	52
<i>P. aeruginosa</i>	1300	40	Host survival	54
<i>C. albicans</i>	1266	15	Host survival, inhibition of <i>C. albicans</i> filamentation	55
	3228	19	HTS, co-inoculation of worms with <i>C. albicans</i>	58

Table 2 Small molecule screens using *C. elegans* as host model for infection

Discovery of novel antifungal agents

The *C. elegans* infection model was also used to screen for compounds that prolonged host survival following infection with the human pathogenic fungus *Candida albicans*. [55]. Given that most compounds that have antifungal activity are also toxic to the human host, high-throughput methods can greatly increase the likelihood of discovering specific antifungal inhibitors. From a screen of 1266 compounds with known pharmaceutical activities, 15 small molecules were identified that increased survival of *C. albicans*-infected nematodes and inhibited *in vivo* filamentation of *C. albicans*, a mechanism of pathogenesis seen during mammalian infection. Two compounds, caffeic acid phenethyl ester (CAPE), a natural component of honeybee propolis, and the fluoroquinolone agent enoxacin, were further shown to exhibit antifungal activity in a mouse model, validating the use of a *C. elegans* model for potential targets in a mammalian system. Interestingly, CAPE is known to inhibit the mammalian transcription factor NF- κ B and to induce immunomodulatory effects in mice [56, 57]. Since *C. elegans* does not express a NF- κ B homolog, it may be the case that CAPE affects alternative targets to achieve antifungal activity.

An automated high-throughput screen using the COPAS Biosort was also applied to *C. albicans* infection of *C. elegans* to assess a library of 3,228 compounds consisting of 1948 bioactive compounds and 1280 small molecules derived from diversity-oriented synthesis [58]. In total, 19 compounds were identified that increased *C. elegans* survival in response to *C. albicans* infection, 7 of which are currently used antifungal agents. Several immunosuppressant agents identified in this screen, including ascomycin, cyclosporin A, and FK-506, were previously found to exhibit weak antifungal activity against *Cryptococcus* and *Aspergillus*, in addition to *C. albicans* [59, 60]. Other hits were predicted to affect an array of biological activities,

such as dequalinium chloride, a potent anti-tumor and protein kinase C inhibitor, and triadimefon, an inhibitor of ergosterol biosynthesis.

5. Conclusion

Chemical library screens are a potent and valuable molecular tool for HTS identification of potential inhibitors of infectious disease. The long-standing paradigm to treat pathogen infection with small molecules that specifically target pathogen growth or metabolism has led to our current dilemma of microbial drug resistance and re-emergence of once-contained infectious diseases. Thus, new approaches to target pathogen virulence or host response factors rather than essential pathogen functions have become increasingly more attractive strategies that are less likely to induce microbial resistance. Some compounds, such as the FDA-approved anti-psychotic, pimozide, exhibited inhibitory properties against infection by several pathogens, suggesting that small molecules can potentially be developed as broad-spectrum anti-infectives. Although the molecular mechanism of inhibition by small molecules remains unknown in most cases, it may be possible to make an educated guess if targeted pathogens share a common virulence strategy, such as the Type III secretion system in Gram-negative bacteria. In other cases, identification of an inhibitor can lead to a molecular understanding of the infection mechanism. For example, the small molecule, tachypleglinA, was found to post-translationally modify TgMLC1, a myosin light chain component, to drive host cell penetration by the parasite *T. gondii* [17].

From the various studies detailed in this review, it is apparent that the library screens represent a first step on the road of drug discovery. There has been a growing realization that fundamental discovery of biological mechanisms oftentimes reaches a 'valley of death', in which potential translation avenues into clinical therapies and diagnostics for disease treatment comes to a standstill and is lost. NIH is addressing this widening gap between basic and clinical research with the establishment of Clinical and Translational Science Centers across the country. The research community will have to remain pro-active to move promising leads from the initial screen stage into downstream validation and development modes in a timely manner. As with any drug development strategy, there still remain multiple technical challenges that need to be overcome before small molecule inhibitors can successfully transition into the clinic. Researchers will need to assess such parameters as compound toxicity, pharmacokinetics and pharmacodynamics, and validation in animal models. However, FDA-approved small molecule libraries can be applied to HTS as a cost-effective method to identify existing licensed drugs for repurposing from diseases unrelated to microbial infection. Furthermore, the development of the *C. elegans* whole organism model for small molecule screening provides a novel methodology to simultaneously assess compound toxicity and host response to pathogen infection. It would be informative to determine whether small molecules identified from conventional host cell culture studies can also inhibit pathogen infection in the *C. elegans* model. Future anti-infective treatments will most likely be comprised of combination therapies that produce additive or synergistic effects to target key processes in both the pathogen and the host. The overall promise of discovering novel anti-

infective compounds has generated great hope in the biomedical community for discovery of new countermeasures against infectious disease.

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Author details

Elizabeth Hong-Geller* and Sofiya Micheva-Viteva

*Address all correspondence to: ehong@lanl.gov

Bioscience Division, Los Alamos National Laboratory, Bioscience Division, Los Alamos, NM, USA

References

- [1] Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L. H., Lynfield, R., Dumyati, G., Townes, J. M., Craig, A. S., Zell, E. R., Fosheim, G. E., Mc Dougal, L. K., Carey, R. B., & Fridkin, S. K. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*, 298, 1763-71.
- [2] Fischbach, M. A., & Walsh, C. T. (2009). Antibiotics for emerging pathogens. *Science*, 325, 1089-93.
- [3] Neu, H. C., & Fu, K. P. (1978). Cefuroxime, a beta-lactamase-resistant cephalosporin with a broad spectrum of gram-positive and-negative activity. *Antimicrob Agents Chemother*, 13, 657-64.
- [4] Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *J Nat Prod*, 70, 461-77.
- [5] Banskota, A. H., Mc Alpine, J. B., Sorensen, D., Ibrahim, A., Aouidate, M., Pirae, M., Alarco, A. M., Farnet, C. M., & Zazopoulos, E. (2006). Genomic analyses lead to novel secondary metabolites. Part 3. ECO-0501, a novel antibacterial of a new class. *J Antibiot (Tokyo)*, 59, 533-42.
- [6] Bister, B., Bischoff, D., Strobele, M., Riedlinger, J., Reicke, A., Wolter, F., Bull, A. T., Zahner, H., Fiedler, H. P., & Sussmuth, R. D. (2004). Abyssomicin C-A polycyclic antibiotic from a marine *Verrucospora* strain as an inhibitor of the p-aminobenzoic acid/tetrahydrofolate biosynthesis pathway. *Angew Chem Int Ed Engl*, 43, 2574-6.

- [7] Schreiber, S. L. (2000). Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science*, 287, 1964-9.
- [8] Tan, D. S. (2005). Diversity-oriented synthesis: exploring the intersections between chemistry and biology. *Nat Chem Biol*, 1, 74-84.
- [9] Kuruvilla, F. G., Shamji, A. F., Sternson, S. M., Hergenrother, P. J., & Schreiber, S. L. (2002). Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature*, 416, 653-7.
- [10] Aiello, D., Williams, J. D., Majgier-Baranowska, H., Patel, I., Peet, N. P., Huang, J., Lory, S., Bowlin, T. L., & Moir, D. T. (2010). Discovery and characterization of inhibitors of *Pseudomonas aeruginosa* type III secretion. *Antimicrob Agents Chemother*, 54, 1988-99.
- [11] Gauthier, A., Robertson, M. L., Lowden, M., Ibarra, J. A., Puente, J. L., & Finlay, B. B. (2005). Transcriptional inhibitor of virulence factors in enteropathogenic *Escherichia coli*. *Antimicrob Agents Chemother*, 49, 4101-9.
- [12] Pan, N. J., Brady, M. J., Leong, J. M., & Goguen, J. D. (2009). Targeting type III secretion in *Yersinia pestis*. *Antimicrob Agents Chemother*, 53, 385-92.
- [13] Hung, D. T., Shakhnovich, E. A., Pierson, E., & Mekalanos, J. J. (2005). Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization. *Science*, 310, 670-4.
- [14] Felise, H. B., Nguyen, H. V., Pfuetzner, R. A., Barry, K. C., Jackson, S. R., Blanc, M. P., Bronstein, P. A., Kline, T., & Miller, S. I. (2008). An inhibitor of gram-negative bacterial virulence protein secretion. *Cell Host Microbe*, 4, 325-36.
- [15] Lieberman, L. A., & Higgins, D. E. (2009). A small-molecule screen identifies the antipsychotic drug pimozide as an inhibitor of *Listeria monocytogenes* infection. *Antimicrob Agents Chemother*, 53, 756-64.
- [16] Carey, K. L., Westwood, N. J., Mitchison, T. J., & Ward, G. E. (2004). A small-molecule approach to studying invasive mechanisms of *Toxoplasma gondii*. *Proc Natl Acad Sci U S A*, 101, 7433-8.
- [17] Heaslip, A. T., Leung, J. M., Carey, K. L., Catti, F., Warshaw, D. M., Westwood, N. J., Ballif, B. A., & Ward, G. E. (2010). A small-molecule inhibitor of *T. gondii* motility induces the posttranslational modification of myosin light chain-1 and inhibits myosin motor activity. *PLoS Pathog*, 6, e1000720.
- [18] Lee, V. T., Pukatzki, S., Sato, H., Kikawada, E., Kazimirova, A. A., Huang, J., Li, X., Arm, J. P., Frank, D. W., & Lory, S. (2007). Pseudolipase A is a specific inhibitor for phospholipase A2 activity of *Pseudomonas aeruginosa* cytotoxin ExoU. *Infect Immun*, 75, 1089-98.
- [19] Arnoldo, A., Curak, J., Kittanakom, S., Chevelev, I., Lee, V. T., Sahebol-Amri, M., Kosciuk, B., Ljuma, L., Roy, P. J., Bedalov, A., Giaever, G., Nislow, C., Merrill, A. R., Lory,

- S., & Stagljar, I. (2008). Identification of small molecule inhibitors of *Pseudomonas aeruginosa* exoenzyme S using a yeast phenotypic screen. *PLoS Genet*, 4, e1000005.
- [20] Harmon, D. E., Davis, A. J., Castillo, C., & Mecsas, J. (2010). Identification and characterization of small-molecule inhibitors of Yop translocation in *Yersinia pseudotuberculosis*. *Antimicrob Agents Chemother*, 54, 3241-54.
- [21] Zhu, P. J., Hobson, J. P., Southall, N., Qiu, C., Thomas, C. J., Lu, J., Inglese, J., Zheng, W., Leppla, S. H., Bugge, T. H., Austin, CP, & Liu, S. (2009). Quantitative high-throughput screening identifies inhibitors of anthrax-induced cell death. *Bioorg Med Chem*, 17, 5139-45.
- [22] Lee, Y. S., Bergson, P., He, W. S., Mrksich, M., & Tang, W. J. (2004). Discovery of a small molecule that inhibits the interaction of anthrax edema factor with its cellular activator, calmodulin. *Chem Biol*, 11, 1139-46.
- [23] Schreiber, K., Ckurshumova, W., Peek, J., & Desveaux, D. (2008). A high-throughput chemical screen for resistance to *Pseudomonas syringae* in *Arabidopsis*. *Plant J*, 54, 522-31.
- [24] Schreiber, K. J., Nasmith, C. G., Allard, G., Singh, J., Subramaniam, R., & Desveaux, D. (2011). Found in translation: high-throughput chemical screening in *Arabidopsis thaliana* identifies small molecules that reduce *Fusarium* head blight disease in wheat. *Mol Plant Microbe Interact*, 24, 640-8.
- [25] Hammer, S. M., Eron, J. J. Jr, Reiss, P., Schooley, R. T., Thompson, M. A., Walmsley, S., Cahn, P., Fischl, M. A., Gatell, J. M., Hirsch, M. S., Jacobsen, D. M., Montaner, J. S., Richman, D. D., Yeni, P. G., & Volberding, P. A. (2008). Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA*, 300, 555-70.
- [26] Berger, E. A., Murphy, P. M., & Farber, J. M. (1999). Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol*, 17, 657-700.
- [27] Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., Mac Donald, ME, Stuhlmann, H., Koup, R. A., & Landau, N. R. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*, 86, 367-77.
- [28] Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., & Berger, E. A. (1996). CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science*, 272, 1955-8.
- [29] Simmons, G., Clapham, P. R., Picard, L., Offord, R. E., Rosenkilde, MM, Schwartz, T. W., Buser, R., Wells, T. N., & Proudfoot, A. E. (1997). Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science*, 276, 276-9.
- [30] Baba, M., Nishimura, O., Kanzaki, N., Okamoto, M., Sawada, H., Iizawa, Y., Shiraiishi, M., Aramaki, Y., Okonogi, K., Ogawa, Y., Meguro, K., & Fujino, M. (1999). A

small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc Natl Acad Sci U S A*, 96, 5698-703.

- [31] Kuhmann, S. E., & Hartley, O. (2008). Targeting chemokine receptors in HIV: a status report. *Annu Rev Pharmacol Toxicol*, 48, 425-61.
- [32] Dorr, P., Westby, M., Dobbs, S., Griffin, P., Irvine, B., Macartney, M., Mori, J., Rickett, G., Smith-Burchnell, C., Napier, C., Webster, R., Armour, D., Price, D., Stammen, B., Wood, A., & Perros, M. (2005). Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother*, 49, 4721-32.
- [33] Roche, M., Jakobsen, M. R., Sterjovski, J., Ellett, A., Posta, F., Lee, B., Jubbs, B., Westby, M., Lewin, S. R., Ramsland, P. A., Churchill, MJ, & Gorry, P. R. (2011). HIV-1 escape from the CCR5 antagonist maraviroc associated with an altered and less-efficient mechanism of gp120-CCR5 engagement that attenuates macrophage tropism. *J Virol*, 85, 4330-42.
- [34] Dragic, T., Trkola, A., Thompson, D. A., Cormier, E. G., Kajumo, F. A., Maxwell, E., Lin, S. W., Ying, W., Smith, S. O., Sakmar, T. P., & Moore, J. P. (2000). A binding pocket for a small molecule inhibitor of HIV-1 entry within the transmembrane helices of CCR5. *Proc Natl Acad Sci U S A*, 97, 5639-44.
- [35] Nishikawa, M., Takashima, K., Nishi, T., Furuta, R. A., Kanzaki, N., Yamamoto, Y., & Fujisawa, J. (2005). Analysis of binding sites for the new small-molecule CCR5 antagonist TAK-220 on human CCR5. *Antimicrob Agents Chemother*, 49, 4708-15.
- [36] Maeda, K., Das, D., Ogata-Aoki, H., Nakata, H., Miyakawa, T., Tojo, Y., Norman, R., Takaoka, Y., Ding, J., Arnold, G. F., Arnold, E., & Mitsuya, H. (2006). Structural and molecular interactions of CCR5 inhibitors with CCR5. *J Biol Chem*, 281, 12688-98.
- [37] Huang, C. C., Lam, S. N., Acharya, P., Tang, M., Xiang, S. H., Hussan, S. S., Stanfield, R. L., Robinson, J., Sodroski, J., Wilson, I. A., Wyatt, R., Bewley, C. A., & Kwong, P. D. (2007). Structures of the CCR5 N terminus and of a tyrosine-sulfated antibody with HIV-1 gp120 and CD4. *Science*, 317, 1930-4.
- [38] Brower, E. T., Schon, A., Klein, J. C., & Freire, E. (2009). Binding thermodynamics of the N-terminal peptide of the CCR5 coreceptor to HIV-1 envelope glycoprotein gp120. *Biochemistry*, 48, 779-85.
- [39] Chun, T. W., Davey, R. T. Jr, Ostrowski, M., Shawn, Justement. J., Engel, D., Mullins, J. I., & Fauci, AS. (2000). Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. *Nat Med*, 6, 757-61.
- [40] Brooks, D. G., Hamer, D. H., Arlen, P. A., Gao, L., Bristol, G., Kitchen, C. M., Berger, E. A., & Zack, J. A. (2003). Molecular characterization, reactivation, and depletion of latent HIV. *Immunity*, 19, 413-23.

- [41] Richman, D. D., Margolis, D. M., Delaney, M., Greene, W. C., Hazuda, D., & Pomerantz, R. J. (2009). The challenge of finding a cure for HIV infection. *Science*, 323, 1304-7.
- [42] Lehrman, G., Hogue, I. B., Palmer, S., Jennings, C., Spina, CA, Wiegand, A., Landay, A. L., Coombs, R. W., Richman, D. D., Mellors, J. W., Coffin, J. M., Bosch, R. J., & Margolis, D. M. (2005). Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. *Lancet*, 366, 549-55.
- [43] Siliciano, J. D., Lai, J., Callender, M., Pitt, E., Zhang, H., Margolick, J. B., Gallant, J. E., Cofrancesco, J. Jr, Moore, R. D., Gange, S. J., & Siliciano, R. F. (2007). Stability of the latent reservoir for HIV-1 in patients receiving valproic acid. *J Infect Dis*, 195, 833-6.
- [44] Edelstein, L. C., Micheva-Viteva, S., Phelan, B. D., & Dougherty, J. P. (2009). Short communication: activation of latent HIV type 1 gene expression by suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor approved for use to treat cutaneous T cell lymphoma. *AIDS Res Hum Retroviruses*, 25, 883-7.
- [45] Archin, N. M., Espeseth, A., Parker, D., Cheema, M., Hazuda, D., & Margolis, D. M. (2009). Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. *AIDS Res Hum Retroviruses*, 25, 207-12.
- [46] De Christopher, B. A., Loy, B., Marsden, M., Schrier, A. J., Zack, J. A., & Wender, P. A. (2012). Designed, synthetically accessible bryostatin analogues potently induce activation of latent HIV reservoirs in vitro. *Nat Chem; In Press*.
- [47] Williams, S. A., Chen, L. F., Kwon, H., Fenard, D., Bisgrove, D., Verdin, E., & Greene, W. C. (2004). Prostratin antagonizes HIV latency by activating NF-kappaB. *J Biol Chem*, 279, 42008-17.
- [48] Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E., & Ben-Neriah, Y. (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature*, 431, 461-6.
- [49] Micheva-Viteva, S., Kobayashi, Y., Edelstein, L. C., Pacchia, A. L., Lee, H. L., Graci, J. D., Breslin, J., Phelan, B. D., Miller, L. K., Colacino, J. M., Gu, Z., Ron, Y., Peltz, S. W., & Dougherty, J. P. (2011). High-throughput screening uncovers a compound that activates latent HIV-1 and acts cooperatively with a histone deacetylase (HDAC) inhibitor. *J Biol Chem*, 286, 21083-91.
- [50] Micheva-Viteva, S., Pacchia, A. L., Ron, Y., Peltz, S. W., & Dougherty, J. P. (2005). Human immunodeficiency virus type 1 latency model for high-throughput screening. *Antimicrob Agents Chemother*, 49, 5185-8.
- [51] Moy, T. I., Ball, A. R., Anklesaria, Z., Casadei, G., Lewis, K., & Ausubel, F. M. (2006). Identification of novel antimicrobials using a live-animal infection model. *Proc Natl Acad Sci U S A*, 103, 10414-9.

- [52] Moy, T. I., Conery, A. L., Larkins-Ford, J., Wu, G., Mazitschek, R., Casadei, G., Lewis, K., Carpenter, A. E., & Ausubel, F. M. (2009). High-throughput screen for novel antimicrobials using a whole animal infection model. *ACS Chem Biol*, 4, 527-33.
- [53] Junker, L. M., & Clardy, J. (2007). High-throughput screens for small-molecule inhibitors of *Pseudomonas aeruginosa* biofilm development. *Antimicrob Agents Chemother*, 51, 3582-90.
- [54] Zhou, Y. M., Shao, L., Li, J. A., Han, L. Z., Cai, W. J., Zhu, C. B., & Chen, D. J. (2011). An efficient and novel screening model for assessing the bioactivity of extracts against multidrug-resistant *Pseudomonas aeruginosa* using *Caenorhabditis elegans*. *Biosci Biotechnol Biochem*, 75, 1746-51.
- [55] Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M., & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog*, 3, e18.
- [56] Watabe, M., Hishikawa, K., Takayanagi, A., Shimizu, N., & Nakaki, T. (2004). Caffeic acid phenethyl ester induces apoptosis by inhibition of NFkappaB and activation of Fas in human breast cancer MCF-7 cells. *J Biol Chem*, 279, 6017-26.
- [57] Park, J. H., Lee, J. K., Kim, H. S., Chung, S. T., Eom, J. H., Kim, K. A., Chung, S. J., Paik, S. Y., & Oh, H. Y. (2004). Immunomodulatory effect of caffeic acid phenethyl ester in Balb/c mice. *Int Immunopharmacol*, 4, 429-36.
- [58] Okoli, I., Coleman, J. J., Tampakakis, E., An, W. F., Holson, E., Wagner, F., Conery, A. L., Larkins-Ford, J., Wu, G., Stern, A., Ausubel, F. M., & Mylonakis, E. (2009). Identification of antifungal compounds active against *Candida albicans* using an improved high-throughput *Caenorhabditis elegans* assay. *PLoS One*, 4, e7025.
- [59] Fox, D. S., Cruz, M. C., Sia, R. A., Ke, H., Cox, G. M., Cardenas, M. E., & Heitman, J. (2001). Calcineurin regulatory subunit is essential for virulence and mediates interactions with FKBP12-FK506 in *Cryptococcus neoformans*. *Mol Microbiol*, 39, 835-49.
- [60] Steinbach, W. J., Schell, W. A., Blankenship, J. R., Onyewu, C., Heitman, J., & Perfect, J. R. (2004). In vitro interactions between antifungals and immunosuppressants against *Aspergillus fumigatus*. *Antimicrob Agents Chemother*, 48, 1664-9.