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Elitra pharmaceuticals: new paradigms for antimicrobial drug discovery

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Antibiotic drug resistance and the limited efficacy of antifungal drugs highlight the urgent need for new antimicrobial drugs. Elitra Pharmaceuticals' original vision was to identify directly all of the essential genes in key pathogens. We have filed patents on over 4000 such targets, and aim to develop cell-based assays for all our targets. In addition, we have begun to shift the paradigm towards massively parallel screening of all possible drug targets.

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▼ Antibiotic drug resistance, limited efficacy of antifungal drugs and the recent advent of bioterrorism, all highlight the urgent need for new antimicrobial drugs [1]. In the case of antifungal drugs, the primary driver for new therapies is their limited efficacy. Despite several new antifungal drugs recently gaining approval, systemic fungal infections still remain fatal for over 50% of patients. In the US alone, figures as high as 100,000 hospital-acquired (nosocomial) deaths annually from microbial infections have been reported.

Elitra Pharmaceuticals (http://www.elitra.com) was founded in November 1997, with the goal of discovering, developing and commercializing novel antimicrobial drugs against novel targets. For the past 50 years, this area had been driven by medicinal chemists making subtle alterations to a small number of existing chemical scaffolds. Iterations of this approach identified a limited set of antibiotics and even fewer antifungal agents. Indeed, less than 30 antimicrobial targets have been exploited commercially and the available chemical space for further modification of these old drugs is becoming exhausted. More recently, selected novel antimicrobial target genes have been cloned, expressed and the corresponding proteins incorporated into *in vitro* biochemical assays for high-throughput drug screening. This approach has proved remarkably unsuccessful. Hit compounds with activity in biochemical assays are easy to find. However, there has been an almost complete failure to translate these hits into potent drugs with activity against intact cells of a pathogen.

To solve these problems, our approach has been to enable true functional genomics on a genome-wide scale. Our vision was to identify directly all of the essential genes in key pathogens. Having now identified and filed patents on over 4000 such genes [2], we are faced with the enviable challenge of prioritizing this enormous potential drug target set and then developing novel, highly sensitive screens for the best drug targets. The promise of such wholly new antimicrobials is two-fold. First, by selecting the best targets, we believe that resultant drugs will exhibit an unparalleled clinical impact. Second, the pathogens will be completely naïve and fully susceptible to the effects of these new drugs.

In addition, we have begun to shift the traditional paradigm of screening targets singly, towards massively parallel screening of all possible drug targets directly in the pathogen of choice. To exploit these technologies further, we have raised approximately US$50 million and have established collaborations with the pharmaceutical companies LG Chemical and Merck.

Elitra Pharmaceuticals – company history

Elitra was formed in 1997 based on technology developed by Judith Zyskind and Allyn Forsyth at San Diego State University (San Diego, CA, USA). In 1998, Elitra received its initial financing

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Box 1. Major investors and founders in Elitra Pharmaceuticals

Major Investors
Enterprise Partners
Mayfield Fund
Interwest Partners
Walden Group
LG Chemical
GeneChem Technologies
Merck
Cooper Hill Partners
Incyte Genomics
Pacific Venture Group

Founders
Judith Zyskind
Allyn Forsyth
Harry Hixson Jr

of US$3.0 million in a round led by Enterprise Partners and Mayfield, who also invested in its second round of financing of US$16.0 million, led by Walden Group and Interwest Partners. Elitra has raised a total of US$49 million in equity financing to date, including equity investments by its corporate partners, LG Chemical (Seoul, Korea), Merck (Rahway, NJ, USA) and Incyte Genomics (Palo Alto, CA, USA). Currently, Elitra has approximately 80 employees and occupies a total 35,000 ft^2 of facilities between its two locations in San Diego and Montreal (Canada).

Elitra's first collaboration, with LG Chemical, was signed in 2000. The company has conducted several HTS campaigns and hopes to have lead candidates ready for clinical trials in 2003. Elitra's second research collaboration, with Merck, was signed in October 2001. Elitra is providing targets and assays to Merck for HTS. Elitra's business strategy is to sign corporate partnerships to offset its R&D costs while retaining marketing rights to its products in the US and Europe whenever possible. Boxes 1 and 2 show the company's investors, founders, key executives and scientists.

Finding drug targets in fungal pathogens

In the case of antifungal drugs, the primary driver for the development of new therapies is limited efficacy. Despite several new antifungal drugs recently gaining approval, systemic fungal infections still remain fatal in >50% of patients.

Candida albicans is the most prevalent human fungal pathogen. However, molecular biology in this pathogen is difficult, and by the end of 2000, less than 20 genes had been shown to be essential using the 'ura-blaster' method, a technique both labor-intensive and time-consuming. To address these issues, we developed a rapid, large-scale target validation method, termed GRACE™ (gene replacement and conditional expression) [3]. In step one, we use a method based on homologous recombination to delete the first genomic copy; in step two, we introduce controllable expression of the second allelic copy, by replacing the native promoter with a tightly regulatable promoter. To date, we have identified over 900 essential genes in *C. albicans* that encode antifungal drug targets. In collaboration with Celera Genomics (Rockville, MD, USA), we completed the first 10× sequence of the second major human fungal pathogen, *Aspergillus fumigatus*, and are now extending our functional genomics platform to this pathogen.

Finding drug targets in bacteria

Elitra's proprietary solution to find essential genes in bacteria is termed 'shotgun antisense'. Now optimized, the method is capable of identifying most of the essential genes within a

Box 2. Key executives at Elitra Pharmaceuticals

Key executives	Position at Elitra	Previous positions
Harry Hixson, Jr	Chairman and Chief Financial Officer	President and Chief Operating Officer, Amgen; Vice President of Abbott
J. Gordon Foulkes	Executive Vice President, R&D	Chief Technical Officer, Aurora BioSciences; and OSI Pharmaceuticals
Gregory Tibbitts	Chief Financial Officer	Senior Manager of Audit Department, Ernst & Young
Edgardo Baracchini	Vice President, Business Development	Director, Business Development, Warner Lambert; Agouron; Isis
Phil Youngman	Vice President, Discovery Biology	Senior Director, Microbial Genetics, Millennium Pharmaceuticals
Deborah Mosca	Senior Director, Drug Development	Senior Director, Microbiology, Intrabiotics; AHP (Lederle)
Carlos Zamudio	Senior Director, Drug Discovery Informatics	Director Bioinformatics, Sequana; Perkin Elmer
Grant Carr	Director, Screening Operations	Director, Biochemistry and Screening, Axys
Terry Roemer	Director, Fungal Genomics	Director R&D, Mycota
Iraj Beheshti	General Manager	Mycota Biosciences; Abbott; Nymox

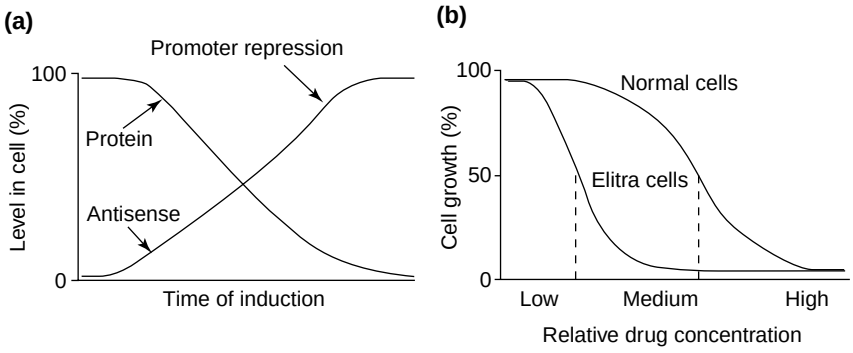

Figure 1. Elitra sensitized whole-cell assays for high-throughput screening. **(a)** As the level of antisense RNA or promoter repression is increased, the level of the cognate protein decreases. **(b)** The cells then become hypersensitive to compounds that inhibit the target protein, because the sensitized cells have reduced levels of the target protein compared to wild-type cells. In essence, cell growth is used to measure the activity of the essential protein. A compound that inhibits an unrelated essential protein exhibits equal activity on both wild-type and sensitized cells.

bacterial genome at a rate of over 100 new targets a month [2–4]. Our shotgun antisense method uses expression plasmids to construct libraries of random genomic DNA fragments that effectively represent the entire gene complement of the organism. Introduction of these plasmid libraries into the appropriate pathogen indicates those genomic fragments whose conditional expression blocks cellular growth. We believe we have almost saturated the identification of essential genes in *Staphylococcus aureus*, and have also been successful in applying this method to a variety of other bacterial pathogens.

Target prioritization

Having identified and filed patents on over 4000 drug targets, it became crucial to develop multiple approaches to prioritize the essential genes as antimicrobial drug targets. Our platform to do this uses both bioinformatics and direct experimental approaches. In June 2000, we acquired an extensive microbial genome database business, PathoSeq™, from Incyte Genomics. Using this as our initial platform, we have extensively modified the original source code to develop a microbial bioinformatics platform that we believe is now unrivalled in the industry [3]. One can select targets based on whether the goal is to develop a broad or a narrow spectrum antimicrobial drug. In addition, we test whether a gene identified as essential in the laboratory is also essential in an established infection.

A gene-to-screen approach to HTS

The efficacy of hit compounds identified in biochemical assays can be abolished by their inability to penetrate the bacterial or fungal cell wall. This problem has been widely encountered in the drug industry, where significant time and resources have been wasted in failing to translate biochemical hits into compounds active on intact pathogens.

The principle of Elitra's assays is based on the selective decrease of the target-essential protein in cells, either by antisense expression or by direct promoter regulation (Fig. 1) [3]. The advantages of Elitra's cell-based high-throughput screens are several fold:

- The assays are cell-based and thus early hit compounds are shown to be active on live, intact pathogenic bacterial or fungal cells.
- Once a target is selected, the conditions to establish a cell-based assay for HTS can be determined within a few weeks. By contrast, the development of a conventional biochemical assay can require 6–12 months.
- Our cell-based assays exhibit up to a 100-fold increase in sensitivity compared to normal, wild-type pathogenic cells. Thus, we are able to identify novel hits to novel targets even in chemical libraries that have been tested previously for antimicrobial activity.

Parallel screening of all essential gene targets: a 'lead-to-gene' approach

A key question for any target-based discovery program is: 'is the target drugable?' That is, can one find a small molecule inhibitor of the target? Despite various opinions as to which protein targets are drugable, the only way to really address the question is to screen the targets. However, even though we have now prioritized our 4000+ essential genes to less than 300, this would still be an impractical task to screen with conventional technologies.

We therefore began to develop genome-wide drug screening approaches. A complete collection of all essential drug targets enables *en masse* parallel screening of antimicrobial compounds to identify their cognate drug target. Strains expressing less than the normal level of a particular gene product display increased sensitivities to compounds that specifically inhibit the partially depleted drug target. Consequently, the growth rate of each strain comprising the population under either control or drug-treated conditions reflects the relative drug sensitivity of each strain in the population. The strain under-expressing the target is

specifically sensitive to low concentrations of the chemical agent and is thus lost preferentially from the drug-treated population over time.

Future goals

Presently, our most advanced target array comprises almost 3000 *C. albicans* strains and a parallel system is being built for bacterial essential genes. Our primary goals for these arrays are:

(1) Rapid target identification for any antimicrobial compound of unknown mechanism.

(2) A comprehensive evaluation of compound specificity; in effect, each compound tested is screened against hundreds to thousands of individual proteins simultaneously in our cell-based assays.

We believe this represents a fundamental advance over conventional drug discovery by enabling, for the first time, a proteome-wide evaluation of a compound's specificity, as opposed to the conventional, early primary driver for chemists – that of potency. We hope to collaborate with pharmaceutical companies that have such compounds but that are unable to develop them in the absence of a known target.

References

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