



Gentronix

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Gentronix is an innovative biotechnology company with the aim of providing cell-based assays and systems to ease or remove some of the current bottlenecks in preclinical drug discovery and development. Gentronix's first product, GreenScreen™ GC, is a combined genotoxicity and cytotoxicity test designed for high-throughput screening of new pharmaceutical lead compounds, giving drug development scientists a cost-effective preview of the battery of mandatory regulatory genotoxicity tests. Future products based on the company's extensive intellectual property rights portfolio will combine the company's expertise in cellular biosensor systems with novel optical detection technology to deliver a range of products for other applications within drug discovery, as well as industrial and environmental monitoring of toxic chemicals.

Company introduction

Gentronix Ltd was formed in July 1999 as a joint venture between the University of Manchester Institute of Science and Technology (UMIST) and Dr Richard Walmsley, a senior academic and expert in yeast molecular genetics. The company was established to exploit the novel biotechnology that forms the basis for a range of products called 'GreenScreen™' being commercialized by Gentronix.

Much effort was needed to convert the raw invention into a marketable product and to establish the relevance of the test results. A project plan was devised and initial laboratory effort focused upon optimization of the protocol steps to create a test that is simple to perform, requiring only a small quantity of test compound, and compatible with standard laboratory automated equipment. Dr Walmsley assembled a multi-disciplinary team of eight talented scientists to undertake this initial work, and the company recruited Peter McCulloch, an experienced senior executive from the biotech sector as Chief Executive Officer. Subsequently, an extensive in-house validation program was carried out by testing a large collection of known genotoxic and cytotoxic chemicals, as well as safe compounds; the results established the true commercial potential of the test and have been recently published in the journal *Mutagenesis*.

The final phase of development was to test this first product with scientists in the pharmaceutical industry with whom the group had built relationships, and a series of trials of GreenScreen GC were set up in external laboratories. These trials allowed assessment of the

reproducibility of the results, robustness of the test format, and reliability of the data handling procedure. The two initial trialists (Johnson & Johnson Pharmaceuticals and Sequani) are now purchasing GreenScreen GC under commercial contracts and further studies with other organizations have been set up, including one with GlaxoSmithKline where an extensive study of robustness and reliability has been carried out.

Yeast genotoxicity biosensor technology

Loss or damage of the coded information in DNA is dangerous for all cells. Mis-repair of damaged DNA can lead to mutations and cancers. As these are a consequence of damage to genes in the chromosomes, agents that cause such damage are termed 'genotoxic'. Agents that cause more general cellular poisoning by damaging other macromolecular components (i.e., that do not specifically target DNA) are termed 'cytotoxic'.

Genotoxicity assessment is a regulatory requirement in the development of new pharmaceuticals, and other agricultural and household chemicals where human exposure is likely to be high. Many pharmaceutical companies now prescreen drug candidates for cytotoxicity and genotoxicity before reaching the costly regulatory testing bottleneck. 'Cut-down' versions of the regulatory genotoxicity tests such as the shortened mouse lymphoma assay (MLA) and micronucleus test (MNT) are less expensive and less compound hungry than the full regulatory tests (typically using 0.15–1 g per test), but they are still technically demanding and time consuming to perform. In addition, there are

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pre-regulatory bacterial tests, including a shortened Ames test, and higher-throughput assays like Ames II and SOS umu. Though the information obtained from such bacterial genotoxicity tests is valuable, bacterial cellular architecture, chromosome structure and metabolism are significantly different to those of mammalian cells. There is also increasing use of *in silico* genotoxicity assessment using structure-activity relationship (SAR) software packages, such as DEREK and TOPKAT.

Gentronix has developed GreenScreen GC, a patented pre-regulatory eukaryotic cell screen for genotoxicity and cytotoxicity, for use at the 'hits to leads' and lead candidate selection stages of the drug development process. The assay is designed to provide a cost-effective preview of the late-stage regulatory tests (e.g., Ames, MLA, MNT) and to give an overall indication of the more general cytotoxicity of the compound in question. The core technology underlying GreenScreen GC is a yeast strain (*Saccharomyces cerevisiae*) that has been genetically modified to produce green fluorescent protein (GFP) when its DNA damage repair system (in this case, the *RAD54* gene promoter) is upregulated. As the damage occurs, the yeast cells become increasingly fluorescent, hence acting as indicators for the presence of genotoxic agents. The GFP used in the assay is a derivative of a non-toxic protein from the jellyfish *Aequorea victoria*. Cellular production of GFP in this system is easily monitored by non-invasive, fluorescence measurements. There is no need for the addition of substrates or co-factors, and there is negligible biosynthetic load on the cell. The test system is designed to be compatible with standard off-the-shelf laboratory automation; its microplate format allows a much higher throughput than existing technologies used for eukaryotic genotoxicity testing. The test requires the use of a fluorescence plate reader capable of performing both fluorescence and absorbance measurements on 96-well microplates (e.g., commonly used instruments such as the Tecan Ultra 384, BMG POLARstar Galaxy, and PerkinElmer Wallac VICTOR).

Yeast cells have a number of inherent advantages for use in toxicity and genotoxicity testing over bacteria, including typically eukaryotic chromosome structure, DNA repair mechanisms, and a metabolism which more closely represents that of mammalian cells. Extensive validation studies performed with GreenScreen GC [1] reveal that a positive result with the assay

provides a reliable predictor of positive results in the regulatory battery of genotoxicity tests.

GreenScreen GC provides complementary data to bacterial tests and SAR software: each providing significantly non-overlapping end points. The current GreenScreen assay is performed without S9, the liver extracts included in other tests to mimic processing by the human liver. Many of the compounds missed by GreenScreen GC (such as aromatic amines and amides) are picked up by bacterial tests with S9 and contain motifs that are well recognized by SAR programs. Importantly, GreenScreen GC is particularly effective in identifying clastogens missed by bacterial tests.

The synergy from combining SAR systems and high-throughput *in vitro* toxicity tests like GreenScreen GC offers the potential of dramatically improving the management of current drug discovery programs; it enables the culling of lead candidates with harmful properties much earlier than has hitherto been possible, thus targeting resources more effectively on the lead compounds that are most likely to make it to the clinic.

Enhancements of GreenScreen technology

Gentronix is keenly aware that there are several enhancements to the core yeast genotoxicity biosensor technology that will result in three further products within the GreenScreen family, each of which will offer additional valuable information to a drug candidate's information profile (often referred to as the 'drug's CV'). The company has development resources committed to each of these enhancements, which will be discussed in the following sections.

Metabolic activation by S9

Bacterial genotoxicity assessment was conceived as an alternative to animal testing by Dr Bruce Ames in 1973. Results showed that there are certain chemicals that cause cancer in animals but have no effect in bacterial tests. This often reflects the ability of mammalian cells, particularly liver cells, to convert benign substances (pro-mutagens) into genotoxins. Many types of 'metabolic activation' do not happen in bacterial cells, so it is now routine to 'mimic' the process in such tests by adding the S9 liver extracts of drug-treated rats to the test compounds. As a result of the differences between mammalian cell types, S9 is also used in the mammalian cell assays. A modified,

S9-supplemented version of GreenScreen GC is being designed with encouragement and financial support from Unilever.

Metabolic activation by cytochrome P450

Yeast has some of the enzymes responsible for metabolic activation. In humans, the cytochrome P450 (CYP) family of enzymes is crucial in the conversion of many pro-mutagens. Yeast has native CYPs and validation studies have demonstrated that they can convert some of the known pro-mutagens. However, the new product under development, GreenScreen MT, builds upon the successful demonstration that genetically modified yeast strains expressing human CYPs, can identify still more pro-mutagens without the need for liver extracts. GreenScreen MT strains will therefore give an even wider preview of regulatory mammalian cell tests.

Increased sensitivity

Genetic manipulation of the yeast cell wall permeability will improve the transfer of certain classes of test compounds from the extracellular environment into the cells. The GreenScreen PC product will share the same formats as the GC and MT products, and will also be targeted at the pharmaceutical industry where it will provide the additional sensitivity required for certain classes of compound.

Multichannel flow cytometer technology

In addition to the core yeast genotoxicity biosensor technology, Gentronix has exclusively licensed further intellectual property rights (IPR) for exciting applications within drug discovery. In particular, the company is developing a multichannel flow cytometer based on proprietary optical technology to provide additional ADMET information from a variety of cell types.

Flow cytometry is a powerful analytical tool that is beginning to be used in various applications within drug discovery. These include genotoxicity, the isolation of bioactive molecules from synthetic combinatorial libraries, and other cell-based assays (e.g., flow cytometry is being investigated as means to speed up the MNT, which is one of the battery of regulated genotoxicity tests). However, conventional flow cytometers are currently expensive pieces of equipment (£50k to £150k per unit) that require highly trained staff to operate and maintain them, and analyze samples just one at a time.

Gentronix has already successfully demonstrated 'proof of principle' of this novel system. This technology offers an attractive and unique alternative to existing flow cytometry instruments on the market by providing parallel testing of multiple samples, proprietary disposable flow cells, and a low cost instrument, which is simple to use.

Environmental monitoring with GreenScreen and YETI

In addition to early genotoxicity screening in drug discovery, toxicity monitoring of environmental samples will be possible using the same yeast strain as used in GreenScreen GC. The presentation of the product GreenScreen EM will be optimized for convenient and rapid use in the field with a portable toxicity monitor for manual 'one-off' sample analysis rather than the multiple samples per batch automated analysis with GreenScreen GC. GreenScreen EM will be presented in ready-to-use individual cuvettes rather than the microplate reagent kit format of GreenScreen GC.

A lightweight and robust instrument, code-named Yeast Environmental Testing Instrument (YETI), has been developed to a production prototype stage. YETI combines fluorescence spectrometry and nephelometry measurements, and supports the use of GreenScreen EM reagent kits. Samples can be collected and tested in individual tubes and results determined without the need to return to a laboratory.

Outlook – what the future holds for Gentronix

The company's primary goal is to establish GreenScreen technology as a valuable tool within the drug discovery community. To accomplish this goal, the company must convince the pharmaceutical industry that there are significant financial and/or efficiency benefits in identifying genotoxic compounds much earlier in the discovery process. The efficiency argument is convincing – a more productive development program will result from either rejecting compounds that have a very high probability of failing the regulatory battery of genotoxicity tests, or in bringing forward the chemistry development of otherwise good candidates. The economic argument is self-evident – as drug compounds progress along the development pathway, costs rise inexorably. Therefore, to be able to reject problem compounds at the 'hits-to-leads' stage

COMPANY PROFILE

Highlights

- Significant financial and efficiency benefits of using GreenScreen to identify genotoxic compounds much earlier in the discovery process than is presently possible.
- GreenScreen provides a cost-effective and reliable preview of positive results from the battery of regulatory tests.
- The assay offers analytical advantages including: a robust protocol; reproducible results; runs on standard laboratory equipment; and the requirement of very little test compound (< 0.5 mg).
- Extensive internal validation exercise recently published in *Mutagenesis*.
- Rigorous and successful customer validation studies by Johnson & Johnson and GlaxoSmithKline.
- GreenScreen technology protected by extensive patent portfolio.
- Future products under development which offer valuable tools for drug discovery.

will lead to considerable savings in the later, more expensive development stages.

Once the primary goal is achieved, the company's medium-term future should be secure, and the company can focus on introducing more cell-based assays and systems designed to ease or remove some of the remaining bottlenecks in preclinical drug discovery.

Bibliography

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