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Biotechnology Industry Organization – BIO 2006

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The 14th Annual International Convention of the Biotechnology Industry Organization (BIO) was held 9–12 April 2006, in Chicago, bringing together more than 19 000 scientists, business leaders, venture capitalists, and economic development managers. BIO represents more than 1100 biotechnology companies, academic institutions, state biotechnology centres and related organisations across the US and 31 other nations. BIO members are involved in the research and development of healthcare, agricultural, industrial and environmental biotechnology products. This year's convention once again broke records for attendance, partnering meetings and exhibits.

Themes in the healthcare sector revolved around how technology is revolutionising medicine, with nanotechnology, stem cell therapy, advanced protein and antibody therapeutics, and the evolving molecular diagnostics/personalised medicine paradigm predominating the discussions in the breakout sessions.

1. Nanotechnology

Nanotechnology is a multidisciplinary field encompassing methods to control or manipulate materials on the atomic scale to create structures that have novel properties and functions because of their size, shape, or composition. In short, nanotechnology is about controlling architecture on the 1–100nm scale. The nanotechnology track at BIO 2006, sponsored by Johnson & Johnson, showcased nanotechnology applications in drug discovery and development, drug delivery, molecular imaging, and technologies specific for cancer detection and therapy.

1.1 Nanotechnology in Drug Delivery

Nanotechnology is helping to achieve the goal of providing targeted therapeutics by creating devices that by the specificity of their design can deliver drugs to the disease target while reducing toxicity. Nanotechnology also offers the possibility of a device and a drug in one, with novel and potentially synergistic effects. According to the session moderator, Youseph Yazdi, Corporate Science & Technology Director at Johnson & Johnson, drug delivery is the area in which nanotechnology will have the biggest impact but moving beyond the hype is still a major hurdle. Nonetheless, it is difficult not to get excited about the medical possibilities being enabled by nanotechnology, especially with all of the fantastically animated examples on display during the session.

1.1.1 Nanowires, Coatings, and Adhesives

Nanosys Inc. founder Larry Bock discussed nanotechnology-enabled products in medical devices, specifically detailing the production and utility of biocompatible titanium-oxide coated nanowires.

These high-performance inorganic nanostructures are synthesised atom by atom in a controlled chemical environment, creating precisely defined functional structures that, in size, are on the scale of a collagen fibril. Their flexible assembly makes them high-value nanomodules, with varying topography that determines what type of cells will grow on the surface. This offers selective adhesion (e.g. specifically allowing bone cell adhesion) and enhanced activity (e.g. catalysing growth of extracellular matrix and bone). Nanowires can be designed with extreme hydrodynamic properties such that bacteria are unable to stick to the surface, enabling their use to coat devices such as stents, catheters, embolic filters, and orthopaedic devices. Bock described the applications of a gecko-like dry adhesive in drug delivery; for example, coated microspheres could stick to the intestinal wall via atraumatic adhesion for extended delivery to the intestine.

1.1.2 Dendrimers

James Baker of the University of Michigan's Nanotechnology Institute for Medicine and Biological Sciences (NIMBS) described the potential utility of dendrimers as 'smart' cancer sensors and in cancer therapy. Dendrimer molecules are a new architectural class of macromolecules developed through nanotechnology. They are among the most versatile, compositionally, and structurally controlled nanoscale building blocks available.

A dendrimer is a tree-like, highly branched polymer molecule. A high level of synthetic control is achieved through step-wise reactions and purifications at each step to control the size, architecture, functionality, and monodispersity of the molecule. Several different kinds of dendrimers have been synthesised using different monomers, and some are commercially available.

Dendrimers are of particular interest for cancer applications because of their defined and reproducible size, but, more importantly, because it is easy to attach a variety of other molecules to the surface of a dendrimer. Such molecules could include tumour-targeting agents (including, but not restricted to, monoclonal antibodies), imaging contrast agents to pinpoint tumours, drug molecules for delivery to a tumour, and reporter molecules that might detect whether an anticancer drug is working. There have been difficulties with the synthesis and biocompatibility of dendrimers, but technology has advanced and now low-cost dendrimers can be produced.

A physical, nanoscale barrier for a targeted therapy is the need to exit from the bloodstream through pores 20–50nm in diameter. Targeted agents would need to be small enough to be acted upon by biological transporters. The G5 dendrimer is similar in size to haemoglobin, and can be internalised into tumours to allow delivery of an otherwise toxic drug. The example provided was of methotrexate given systemically to rats versus being delivered by G5 dendrimers targeted to the tumours. The tumours were necrotic but the animals were healthy in the dendrimer group, the therapeutic index having increased 500-fold with targeted delivery.

Other work from this group involves development of a multifunctional, drug-delivery system that targets subtle molecular alterations that distinguish cancer cells from healthy cells. Dendrimers conjugated to different biofunctional moieties are linked together using complementary DNA oligonucleotides to produce clustered molecules that target cancer cells that overexpress target receptors. Such a DNA-linked dendrimer nanocluster platform allows for the delivery of drugs, genetic materials, and imaging agents to cancer cells, offering the potential for developing combinatorial therapeutics. The goal is to use these nanclusters for noninvasive delivery to tumour sites and into cells, with imaging capability to document their presence at the target and to document changes, and then to select therapeutic agents based on the observed changes. This is a prime example of the coalescence of material science for the synthesis and characterisation of the agents, biological science for understanding the pathophysiology and toxicity, and the integration of imaging and bioinformatics.

1.1.3 Self-Assembling Artificial Matrices

Some of the most dramatic accomplishments of nanotechnology in medicine to date employ self-assembling artificial materials to promote complex biological repair. Samuel Stupp, Director of the Institute for BioNanotechnology in Medicine, Northwestern University, presented work on regenerative therapies based on artificial matrices of bioactive nanofibres. He demonstrated how a dilute solution of molecules can be applied to a site of injury or a target tissue and with an appropriate trigger (potentially the target cell or tissue itself) initiate self-assembly of a nanofibre matrix that forms a gel. The gel can be designed to bathe the site in healing proteins.

For example, such nanofibres can be automatised for neural progenitor cell differentiation by carrying the five-amino-acid IKVAV epitope of laminin, which induces neural sprouting. Injection of a solution with this epitope into injured spinal cord forms a gel at the injured site. By mediating the bioactivity of the nanofibres, this nanotechnology can be applied for chronic wound healing, bone repair or angiogenesis for regeneration of myocardium after myocardial infarction.

Supramolecular nanofibres can also be used as carriers of bioactive signals and targeted drugs. Drug candidates can be encapsulated by a peptide amphiphile nanofibre so that the nanostructure chemically stabilises the drug. The nanofibre is endocytosed, the fibre degraded and the drug released. Stupp and coworkers are looking into using this technology in more advanced targeted therapy applications, such as multiplex therapy with multiple agents (e.g. small molecules, peptides, proteins, genes, antisense, etc.) in a single construct, and combining diagnostic and therapeutic functions. Systems biology information will be the key to the improved design of such multiplex nanofibres.

1.1.4 Targeted Nanobubbles

The use of highly selective, targeted nanobubbles in drug delivery and therapy was presented by Evan Unger, CEO of ImaRx Therapeutics Inc. Applications being pursued include delivery of targeted chemotherapy, angiogenesis therapy, oxygen delivery, and therapy of stroke using receptor-targeted nanodroplets in conjunction with ultrasound.

Oxygen Delivery

ImaRx has developed a product (MRX-850) that consists of oxygen-enriched, lipid-coated perfluorocarbon (PFC) gas nanobubbles, which are designed to deliver oxygen with potential applications in neuroprotection and cardioprotection. MRX-850 delivers about 400 times more oxygen per volume than red blood cells or PFC emulsions and is highly effective at oxygenating tissues. Patients are treated through the injection of a nanobubble emulsion into the bloodstream, where it expands into nanobubbles that circulate through the lungs. Benefits of this type of oxygenating therapy over haemoglobin-based oxygen therapeutics

include rapid clearance and a very large oxygen-carrying capacity, as well as the absence of any foreign immunogenic proteins.

ImaRx is also developing SonoLysis™ [1], a clot-dissolving technology that incorporates a perfluoropropane-filled nanobubble (MRX-815) and ultrasound to treat clots. MRX-815 nanobubbles are in a phase II clinical trial for acute ischaemic stroke and a phase I/II trial to treat peripheral arterial occlusions.

Drug Delivery by Radiation-Force Mediated Displacement

Ultrasound is exquisitely sensitive to nanobubbles. Gas bubbles reflect a very strong, scattered signal when exposed to ultrasound, making it possible to see their movement within almost any size of blood vessel. Thus, nanobubbles can be used as a contrast agent, with ultrasound imaging able to detect a single bubble. Ultrasound in concert with nanobubbles can be used in drug delivery.

Dr Unger highlighted the work of University of California, Davis, researchers Paul Dayton and Kathy Ferrara, who are investigating the strategy of coating nanobubbles with specific molecules that cause the bubbles to recognise and adhere only to blood vessels in tumours and not to those in healthy tissues. They are also designing nanobubbles within which toxic drugs such as paclitaxel can be suspended and delivered locally to tumours and then destroyed with ultrasound. Ultrasound can also be used as a force to push drug-bearing bubbles into the capillary wall of a tumour. In this scenario, the bubbles would then be destroyed and their load released into the capillary wall, and possibly to the tumour tissue on the other side. Together, the strategies have the potential to give cancer specialists new, minimally invasive and highly targeted tools to fight cancer.

1.2 Nanotechnology Applications in Cancer Detection and Therapy

Building on the previous session on nanotechnology in drug delivery, this session addressed the various ways in which nanotechnology is being used to specifically detect and treat cancer. For example, new nanoparticulate imaging agents are being developed that specifically bind to tumour cells; nanofluidic chips are being developed to manipulate and analyse tiny bits of genetic material; and nanoshells are being developed that seek and destroy tumour cells when illuminated with infrared light.

Nanotechnology could have an early, paradigm-changing impact on how clinicians detect cancer in its earliest stages. Exquisitely sensitive devices constructed of nanoscale components, such as nanocantilevers, nanowires and nanochannels, offer the potential for detecting even the rarest molecular signals associated with malignancy.

Anna Barker, Deputy Director of Advanced Technologies and Strategic Partnerships at the US National Cancer Institute (NCI), discussed the NCI Alliance for Nanotechnology in Cancer, an initiative encompassing the public and private sectors, designed to accelerate the application of the best capabilities of nanotechnology to cancer. One of the goals of the Alliance is to increase the visibility and availability of nanomaterials and nanoscale device technology within the cancer research and development community, to allow investigators the opportunity to discover and invent using new tools, just as they are doing with other disruptive technologies such as DNA microarrays and proteomic analysis. Dr Barker's view is that the best promise for applying nanotechnology in cancer is in early detection. She specifically hailed the research efforts of groups led by Chad Mirkin at Northwestern University, and Jim Heath at the California Institute of Technology.

Dr Barker noted how barriers to progress in medicine can potentially be overcome by nanotechnology. These technical barriers include the need for improvements in:

- detection of very early genomic and proteomic changes;
- measuring a large number of parameters simultaneously;
- bioinformatics – algorithms to interrogate data;
- monitoring genomic and proteomic changes;
- detection of metastasis.

She highlighted the need for the development of new biomarker libraries, highly multiplexed sensors with improved sensitivity and specificity, and early detection/imaging enabled through 'smart' nanoparticles and novel contrast agents.

1.2.1 Metal Nanoshells

Jennifer West of Rice University discussed the diagnostic and therapeutic applications of carbon nanotubes, quantum dots (Q-dots), and metal colloids, nanomaterials with unique optical and/or magnetic properties that allow them to respond to the biological environment and serve as carriers with high surface area.

Nanoshells are a new type of nanoparticle, with tunable optical properties. Nanoshells for medical applications usually consist of a core nanoparticle such as silica surrounded by an ultrathin gold shell. Their absorption in tissues is wavelength dependent and noninvasive; exposure to an external laser results in localised heating to kill the target, a process termed 'photothermal ablation'. Nanoshells can be targeted to tumours by conjugating antibodies to their surface using polyethylene glycol (PEG) polymers, with a capacity of ~150 antibodies per nanoshell. For example, tumour cells can be targeted with anti-HER2-conjugated nanoshells, and

1 The use of trade names is for product identification purposes only and does not imply endorsement.

exposure to near-infrared light results in specific and localised destruction of the targeted cells, leaving normal cells unharmed.

Nanoshells can also be used as contrast agents for optical imaging in cancer, but in this application the nanoparticles would be 'tuned' to scatter light instead of absorbing it, as per therapeutic nanoparticles. A multifunctional platform could combine both diagnostic and therapeutic applications, using optical coherence tomography.

Dr West also highlighted the technology of protease-activated imaging agents based on Q-dots. These Q-dot probes are nanoparticulate luminescent probes that demonstrate inherent signal amplification upon interaction with a targeted proteolytic enzyme. Such constructs may be useful for imaging in cancer detection and diagnosis. In this system, Q-dots are bound to gold nanoparticles via a proteolytically degradable peptide sequence to suppress luminescence and are activated in the presence of a specific protease.

1.2.2 Magnetic Nanoparticles

Like West's group at Rice, Triton BioSystems, Inc. is also developing noninvasive targeted therapeutics that use heat to treat cancer. According to Triton's President, Samuel Straface, magnetic nanomaterials and magnetic field energy are being used to make antibodies tumouricidal and more therapeutically selective.

The company's Targeted Nano-Therapeutics™ (TNT™) system involves an injectable infusion of magnetic nanospheres conjugated to an anti-epithelial-cell-adhesion-molecule (EpCAM) antibody (called a 'T-probe') that targets adenocarcinoma cells. A focused external magnetic field is then applied to selectively activate the magnetic spheres in the treatment area. The infusion circulates safely because the nanospheres are inactive, and their location can be tracked in the body using standard imaging technology. Once they are bound to the tumour cells, application of a localised magnetic field generates lethal heat to kill the targeted cancer cells. Following therapy, the nanoparticles biodegrade.

1.2.3 MRI-Based Imaging and Targeted Therapy

Kereos, Inc. has also ventured into nanotechnology-enabled cancer diagnostics and therapy. CEO Robert Beardsley outlined their product pipeline, including KI-0001, a magnetic resonance imaging (MRI)-based molecular imaging agent for tumour detection, and KI-1001, a payload-carrying nanoparticle emulsion for targeted cancer therapy. Both rely on an angiogenesis targeting ligand and are being developed with the potential for use as a combined diagnostic and therapeutic agent.

Dr Beardsley wrapped up the session by commenting on the challenges for cancer nanotechnology, including the necessity for complex products in terms of manufacturing, pharmacokinetic parameters (absorption, distribution, metabolism, and excretion), and bioanalytic stability.

2. Devices and Diagnostics

The molecular diagnostics market is the fastest growing segment within the *in vitro* diagnostics industry. However, the diagnostics paradigm is rapidly changing with the advent of 'personalised medicine'. Microarrays, biomarkers for disease, and advances in molecular diagnostics are bringing profound changes to the practice of medicine and new promise for patients. The Devices and Diagnostics track was sponsored by Abbott and covered a broad range of topics, from companion diagnostics, disruptive imaging technologies, to cell and gene therapy, and included business models and economic issues in this rapidly expanding arena.

2.1 Companion Diagnostics: It's Not Just About a Better Drug

The market for companion diagnostic products has been expanding as a result of a confluence of factors, including US Food and Drug Administration (FDA) demand for better and earlier detection of potential adverse drug reactions and the desire of biotechnology companies to reduce development costs by using real-time patient response information. Clinical laboratories, physicians, payers, patients, and pharmaceutical companies all stand to benefit from the further integration of diagnostics with therapeutics, which promises to provide the most appropriate treatment for each patient while simultaneously mitigating the high costs of healthcare and drug development. A panel of experts lead by Steven Burrill, CEO of Burrill & Company, discussed the expanding market opportunity for companion diagnostic products as well as the impact of new molecular diagnostics technologies on the biopharmaceutical industry.

Historically, therapeutic drugs have been high-margin products, while diagnostics have been priced as commodity products. Although diagnostics account for only a few percent of overall healthcare spending, they have a potentially great impact, and the traditional paradigm is beginning to shift with the introduction of expensive new tests that are clearly linked to therapeutic decisions. For example, Genomic Health's Onco*type* DX™ test is priced at around $US3500, but could save $US20 000 on chemotherapy by identifying patients for whom chemotherapy is unnecessary and who would not benefit from it.

The trend towards personalised medicine will clearly accelerate, said Burrill, but the harsh economic reality is that the driver of change will be the payer community (Medicare, employers, managed care, etc.), which clearly has an economic incentive to

keep healthcare spending in check. This trend should not, however, be viewed by pharma as necessarily shrinking the market share for drugs by limiting the patient pool; in fact, some markets will grow by identifying more patients who could potentially benefit from certain treatments.

Dr Jorge Leon, President of Leomics Consulting, furthered the concept that companion diagnostic testing is the doorway to the practice of personalised medicine, but indicated that we have a long way to go before the market, and the practice, has matured. There are currently around 20 tests on the market, with ~1 million tests performed in 2005 at an average price of ~$US600. Growth and success of new tests will depend heavily on continuing technological advances, clean clinical data and therapeutic guidelines, reimbursement, and endorsement of the FDA. Dr Leon predicted that, within 3–5 years, the CYP450 theranostic market will grow to a $US1 billion market and the market for epidermal growth factor receptor (EGFR)-targeted drug selection tests will be $US0.5 billion. But to get there (and beyond), education of patients, physicians and payers is fundamental. When doctors are educated about a drug, it will also be necessary to inform them of an accompanying test that identifies those patients who will respond to it without experiencing adverse effects. Differentiation of a drug from its competitors will be based on individual efficacy, 'ethnic efficacy' and reduced adverse drug reactions. In Dr Leon's words, "Pharma needs to reload their marketing weapons with new ammunition."

2.1.1 DNA Methylation Biomarkers

Several examples were presented of the technological advances that are fueling the market expansion in companion diagnostics. Nathan Lakey, President and CEO of Orion Genomics, discussed the company's development of DNA methylation biomarkers and methylation-based molecular diagnostic tests.

Changes in methylation lead to incorrect silencing or activation of genes. Incorrect methylation is linked to cancer, as a result of altered expression of a variety of genes involved in apoptosis and tumour growth suppression, and methylation changes are found in every tumour – not just every tumour type. Incorrect methylation is also linked to aging.

Orion uses an array technology called MethylScope® whole genome scanning, a chip that reads the 'second code' of methylation patterns to profile patients who respond versus those who do not respond to a drug. In theory, a single MethylScope® microarray can quantitatively detect the methylation status of every gene in the human genome, and thus can be used to discover novel biomarkers at >1000 times the capacity of other platforms, which scan a selected set of genes.

Orion has initiated a collaboration with researchers at the University of Glasgow to discover novel epigenetic biomarkers for the development of tests that screen for cancer at an early stage and provide personalised information about how tumours are most effectively treated. Since drugs such as 5-azacytidine can reverse methylation of human *MLH1* and other genes, its use may be a means of sensitising tumours to anticancer drugs. Orion's microarray and polymerase chain reaction (PCR) technologies for methylation analysis have potential both for marker discovery and for applying methylation diagnostics in a clinical setting.

2.1.2 Advanced Diagnostics

Elaine Weidenhammer of Nanogen, Inc. discussed technology platforms for advanced diagnostics – improved methods and tools to predict, diagnose and ultimately help treat disease. Currently, Nanogen's molecular diagnostic technologies include such tools as immunoassays, real-time PCR, and molecular tests including multiplex tests; these are moving toward therapy selection and monitoring, and in the future may be used for predictive testing. Nanogen's platforms include the NanoChip® electronic microarray, which can be used in multiplex genetic assays, and the MGB Eclipse® real-time PCR system for gene expression analysis and pathogen detection. In partnership with Jurilab, Nanogen offers the DrugMEt™ pharmacogenetic test for genotyping cytochrome P450 and phase II enzymes involved in drug metabolism. This test is currently approved for research use only, although Weidenhammer touched on the expansion of NGEN™ reagents for clinical use, with the company's first focus on products for CYP450 genes.

Also in collaboration with Jurilab, Nanogen is involved in a genome-wide scanning programme for type 2 diabetes mellitus markers in a homogeneous Finnish population, searching for unique markers that could be used as prognostic indicators for future predictive tests.

2.1.3 Chromogenic In Situ *Hybridisation*

According to Dr William Sweet, Invitrogen's Director of Clinical and Research Affairs, the company known primarily for providing research reagents and analytical equipment is now looking at combining its cutting-edge technologies to provide diagnostic solutions. Multiplexed antibody cocktails, enzyme-linked immunosorbent assays (ELISAs) for drug therapy selection (e.g. β-amyloid for Alzheimer's disease) or early cancer detection (prolactin receptor binding protein [PRL-BP] for breast cancer) are among the research reagents of potential value in the clinic.

Dr Sweet highlighted Invitrogen's new chromogenic *in situ* hybridisation (CISH) technology, which integrates cellular morphology with genetic information (gene amplification or deletion) and is being developed for use with such DNA probes as *HER2* (*ERBB2*), *EGFR* and topoisomerase II-α (TOPO-II; *TOP2A*).

The company has submitted a premarket application to the FDA for their HER2-CISH assay as a companion diagnostic for trastuzumab (Herceptin®) therapy selection. Dr Sweet predicted HER2-CISH would become a first-line test in the near future, followed by TOPO-II-CISH for anthracycline therapy in breast cancer. EGFR-CISH for use in conjunction with erlotinib (Tarceva®), cetuximab (Erbitux®) and gefitinib (Iressa®) is also in the pipeline for submission to the FDA. Although the technology has advantages in terms of the breadth and accuracy of information possibly derived from a single test, what was not clear was how easy it would be to adapt to a clinical laboratory setting. Nonetheless, the company is clearly embracing the future of molecular diagnostic tests that are companions to cutting-edge therapies.

2.1.4 Challenges for the Future

Wrapping up the session by highlighting future challenges for the companion diagnostics industry was Glenn Miller, Vice President and General Manager of Genzyme Analytical Services, a division of Genzyme Genetics that provides molecular pathology-based preclinical and clinical trial services to the pharmaceutical and biotechnology community worldwide. Genzyme Genetics occupies a leading market position in its focus on complex diagnostic testing.

Dr Miller stressed that not every new gene that is identified as being associated with a disease or condition automatically translates into a new test. In fact, the time required to demonstrate utility can range from a few months to many years. It also takes time to change physician practices. The establishment of a market for a new test must progress through the time-intensive steps of sample collection, marker discovery, research-test development, clinical studies, laboratory test development, and, finally, the development of a test kit. The clinical lab can be a key component of the process, offering development expertise, clinical trial services, commercial clinical lab services, and marketing and reimbursement advice. Dr Miller reiterated the sentiments of many speakers, saying that a fundamental base for establishing a new diagnostic test in the market is to create a sustainable competitive advantage by changing physicians' attitudes.

2.1.5 Who is the Customer?

A panel discussion followed the session, and focused on the question of identifying the 'customer' for companion diagnostics. The answer from Nathan Lakey of Orion Genomics was that, for a screening test, the customer is the reference lab, which pushes the test to doctors. Jorge Leon countered that the main customer is the medical organisation (e.g. the American Society of Clinical Oncology [ASCO]), which, through education, directs its members to order the test. The second customer is the patient, via patient advocacy groups, which champion new technologies to advance disease prevention and therapy. The third customer is the payers, who are selling to the demand curve.

The question of the customer also hinges on the capacity of the system to adopt a large number of new tests. According to the panel, a lack of capital for investment will not be the issue; innovation will drive venture capitalists to diagnostics, although they are somewhat nervous about the 'home brew' concept for developing a test and will push for an approved product.

2.2 Targeted Medicine: Biomarkers in the Discovery and Development Process toward Value-Added Diagnostics and Therapeutics

This session highlighted a new trend in the use of the term 'targeted medicine', that being to use the term to refer to medicine targeted at a specific and identifiable patient population, rather than therapy aimed at a molecular target. Under this definition, biomarkers based on expressed proteins, genes, single-nucleotide polymorphisms and metabolites have emerged as a powerful tool for designing and executing 'targeted' clinical trials for diagnostic and therapeutic development, enabling the faster marketing of new therapeutics with clear advantages over existing treatments, and improving the risk-benefit ratio facing pharmaceutical developers. The panelists were in agreements that the use of biomarkers in discovery and development has the potential to expand, rather than shrink, effective market sizes for drugs and diagnostics.

2.2.1 A Trend in Diagnostic Test Development

Eric Shulse, Senior Director of Business Development for Celera Genomics, provided the examples of HIV therapy and the similar evolving paradigm for cancer therapy. Resistance to antiretroviral drugs is a common problem in the treatment of HIV-infected individuals, but specific tests have been developed based on HIV biomarkers reflecting both patient response and the potential for viral resistance to drug therapy, such that the use of viral load and genotype testing is now considered standard-of-care. Similarly, treatment decisions for hepatitis C virus infection can now be based on tests for viral load and genotype, allowing patient stratification for therapy. Cancer therapy is mimicking this trend, for example, with the development of resistance to the drug imatinib in chronic myeloid leukemia. A genotype test now has the potential to identify mutations that predict such resistance and suggest that alternate drugs should be used.

2.2.2 Biomarkers Increase the Success Rate in Drug Development

Both Nicholas Dracopoli of Bristol-Myers Squibb and Steven Seelig of Abbott emphasised the role of diagnostic biomarkers in driving the practice of targeted medicine, as well as the clear value of patient selection for drug development. Patient selection

for trastuzumab therapy is a shining example, wherein trial cost savings have been estimated at $US35 million based on preselecting patients likely to respond to the drug. A further consequence was the shortening of clinical trial time, resulting in a $US1.7 billion revenue acceleration based on early entry to the market.

2.3 Cell and Gene Therapy for Chronic Diseases

Device therapy is currently one of the cornerstones for the treatment of chronic diseases such as cardiac arrhythmias and diabetes. The treatment options include electronic pacemakers, implantable defibrillators, external and implantable drug pumps, and other devices. It is predicted that scientific and technological advances in the field of stem cell biology and gene therapy could complement or even replace traditional device-based therapies. In a divergence from the diagnostics talks predominating the Devices and Diagnostics track at BIO 2006, this session emphasised the promise, but also the challenge, of commercialising cell and gene therapies to supplement or replace traditional devices for cardiac and noncardiac disease.

2.3.1 The Promise of Biological Rhythm Management

Professor Douglas Zipes of the Indiana University School of Medicine provided an overview of research that represents the convergence of medical device technology and biotechnology to improve patient management in reviewing cell and gene therapy for biological rhythm management. The key to advancement in this area is understanding what triggers sudden cardiac arrest. Challenges include both a better method for identifying patients at risk for sudden cardiac death, atrial fibrillation and heart failure, as well as a greater understanding of the complexity of arrhythmia initiation.

The current status of treatment for cardiac arrhythmias is the implantable defibrillator (electronic pacemaker), which is better than pharmacotherapy but has shortcomings including a limited battery life, the need for lead implantation into heart, and a lack of response of the device to the autonomic and physiological demands on the heart. The development of new devices demonstrates both increasing simplicity, for example, with a subcutaneous device, and increasing complexity, with the potential of a future device for comorbidity management and remote patient monitoring and management. It is clear, however, that one device does not fit all, and Professor Zipes predicts that the next 20 years will see a furthering of the quest for a biological pacemaker.

Genetically engineered pacemakers could be a possible alternative to implantable electronic devices for the treatment of bradyarrhythmias. Biological pacemaker science is currently proceeding in three parallel directions:

- gene therapy – genetic modification of existing cells;
- gene plus cell therapy – implantation of genetically modified cells;
- cell therapy – implantation of derived or unmodified cells.

Strategies involving genetic manipulation include upregulation of β_2-adrenergic receptors, downregulation of K^+ current, I_{K1}, and over-expression of HCN2 (hyperpolarisation activated cyclic nucleotide gated) channels, the molecular correlate of the endogenous cardiac pacemaker current, I_f.

Human embryonic stem cells could be used to create pacemakers, or adult mesenchymal stem cells may be used as platforms for delivery of pacemaker genes to myocardium. Human embryonic stem cells can differentiate into cardiac stem cells and then into cardiomyocytes with contractile activity. But gene and cell therapy continue to face a number of critical issues, not the least of which is that most diseases are polygenic, and delivery remains a challenge. It is critical that stem cell differentiation is directed into, and maintained as, the desired cell type, and not as unwanted lineages. Long-term reliability, the potential for inflammation and infection, and response to normal control systems remain concerns. Nonetheless, according to Professor Zipes, the goal of biological repair of cardiac arrhythmias will not remain elusive forever.

2.3.2 The Gene/Cell Therapy Approach

Yair Feld, CEO of GeneGrafts, Ltd, discussed the approach of introducing cells expressing specific ion channels to correct molecular defects in atrial fibrillation and Parkinson's disease. Inwardly rectifying K^+ channels (Kir) are responsible for stabilising resting membrane potential. For example, Kir2.1 (KCNJ2; potassium inwardly-rectifying channel, subfamily J, member 2) can be downregulated to decrease I_{K1} current density. This removes an important determinant of repolarisation, leading to prolonged repolarisation in cells lacking this current. GeneGrafts' scientists are also collaborating with Jeffrey Olgin at the University of California, San Francisco on animal studies with Kv1.3 (KCNA3; potassium voltage-gated channel, shaker-related subfamily, member 3).

For atrial fibrillation, the most common cardiac arrhythmia, gene-modified cardiac fibroblast tissue is transplanted, whereas for Parkinson's disease, neural tissue is modified and transplanted. A distinct advantage for patients with atrial fibrillation is the potential for a single, local treatment to restore normal cardiac activity, rather than relying on continual artificial stimulation, with the goal of offering permanent maintenance of sinus rhythm. Challenges include weighing the advantages and disadvantages of autologous versus allogeneic transplantation, issues of graft regulation, and long-term survival of the transgene.

2.3.3 Pluripotent Stem Cell Therapy of Ischaemic Cardiovascular Disease

The goal of cell therapy for ischaemic cardiovascular disease, according to Robert Deans of Athersys, Inc., is to limit tissue damage in the acute phase and to stimulate recovery via trophic pathways (e.g. angiogenesis, cell homing, etc.). Describing the work of Dr Catherine Verfaillie and her colleagues at the University of Minnesota, Deans outlined Athersys' nonembryonic stem cell platform, MultiStem™, which is based on the multipotent adult progenitor cell (MAPC) technology.

MultiStem™ cells have the capacity to grow and develop into multiple cell or tissue types, regenerate tissue in a developing or mature organism, and be expanded at scale in an *ex vivo* setting (i.e. outside the body). In addition, MultiStem™ cells can be isolated from a variety of nonembryonic tissue sources, such as bone marrow and other tissues, and have been established from a variety of species including rodents, pigs, nonhuman primates, and humans. The MultiStem™ product concept is based on a cell banking approach using cryopreserved cells, with allogeneic utility allowing for an 'off the shelf' product.

Athersys' clinical development approach has been to show that the cells are safe, demonstrate their potency, and demonstrate production capability. MultiStem™ has been tested for *in vitro* immunogenicity by mixed lymphocyte reaction, and in a rat, acute myocardial infarction, dose-ranging study, the allogeneic stem cells persisted throughout the 6-week study. The product is currently in phase I development under the direction of Dr Marc Penn at the Cleveland Clinic for optimisation of gene delivery to infarcted myocardium. Clinical considerations currently centre around delivery – and whether it should be catheter-based, intramyocardial (during intervention reperfusion), or intracoronary (during inflammatory phase). This study is in progress, and the earliest expected Investigational New Drug (IND) submission is quarter 1, 2007.

3. Drug Discovery and Development

3.1 Molecular Design of Protein Therapeutics: Considerations from Discovery through Development

A successful protein therapeutic must meet many criteria throughout research and development: achieving the desired biological effect, ability to manufacture needed quantity and quality, suitability for intended use in the clinic, etc. Alteration of the primary amino acid sequence as well as post-translational and/or chemical modifications are common strategies employed to improve the characteristics of the therapeutic candidate. Because of the important position of antibodies in the category of protein therapeutic, this session overlapped with another in the Drug Discovery and Development track, *Antibodies Revisited*, which focused on the future direction of antibody technology. The chairperson of this session, Kendall Mohler, Senior Vice President of R&D at Trubion Pharmaceuticals, Inc., reviewed the history of protein therapeutics (with a focus on antibodies) that has led to greater understanding of the many considerations that impact on the success of this broad therapeutic class.

In the 1980s, mouse monoclonal antibodies came to the forefront of biomedical research, but they suffered from problems of immunogenicity and were functionally restricted by some of the inherent properties of antibodies. In the 1990s, chimeric antibodies were being developed with a focus on humanisation and increased target affinity. Since the turn of the century, advances in understanding the factors affecting antibody functionality in patients have offered the possibility of improved functionality. Modification of effector functions of antibodies (e.g. antibody-dependent cell-mediated cytotoxicity [ADCC] and complement-dependent cytotoxicity [CDC]) is now being addressed both by amino acid changes in the Fc region (e.g. by Xencor, Inc.) and glycosylation changes to lower the content of fucose in the carbohydrate chains of an antibody (e.g. by BioWa, Inc.).

3.1.1 Improving Antibody Efficacy: The Rituximab Example

Henry Lowman, Director of Antibody Engineering at Genentech, Inc., provided an excellent example of how the design of therapeutic antibodies has become more precise as a result of our increased knowledge of molecular mechanisms.

The anti-CD20 antibody rituximab, the first molecularly targeted therapy in oncology, was approved for therapeutic use by the FDA in 1997. Although it is clinically effective in non-Hodgkin lymphoma (NHL), the molecular mechanisms of its activity have only recently been elucidated. *In vitro*, rituximab induces complement- and antibody-dependent cytotoxicity and apoptosis, and may also have a vaccine-like effect. Rituximab also appears to be involved in modulation of Ca^{2+} flux and macrophage phagocytosis.

Rituximab targets CD20, a multi-transmembrane protein expressed on most human B lymphocytes, but not on plasma cells or B-lymphoid stem cells. It is expressed on both normal and malignant cells. CD20 does not display the typical properties of a receptor, and no CD20 ligand has been identified, thus, although CD20 appears to play a central role in regulating cell cycle progression in normal B cells, the exact *in vivo* function of the molecule remains unknown.

According to Lowman, Genentech's goal was to increase the efficacy of rituximab for therapy of B-cell malignancies and to decrease the need for combined chemotherapy. Understanding the mechanisms of B-cell depletion and antilymphoma activity was key to achieving increased binding affinity and enhanced

molecular interactions of the antibody. The rationale for the company's approach to improving the functionality of the antibody was based on the finding that response to rituximab is influenced by allelotype at the FcγRIII gene (*FCGR3A*). Binding of antibodies to FcγRIII regulates ADCC and CDC activity, which appears to translate to *in vivo* efficacy.

Working with Xencor scientists, Genentech's 'super anti-CD20' strategy was to combine modular Fc mutations with variable domain changes such that binding to both CD20 and FcγRIII was significantly improved. The second-generation anti-CD20 agent, known as ocrelizumab, was derived from Xencor's 'variant 16' and showed improved CDC activity, dependent on the type of target cell. The third-generation antibodies, still in development, are fully humanised and show a higher rate of target cell killing compared with ocrelizumab.

3.1.2 Antibodies Revisited

Focus on Fc

In the session on the future direction of antibody technology, Bassil Dahiyat, CEO and President of Xencor, elaborated on the company's proprietary protein design technology that focuses on the Fc region of antibodies in order to modify effector functions. Xencor has engineered a series of Fc variants with optimised Fcγ receptor affinity and specificity, using computational design algorithms and high-throughput screening. This approach to antibody engineering allows for better binding of Fc receptors, enhanced immune cell activation, increased target cell killing, modulation of complement activity, and the ability to 'tune' the half-life of the antibody for specific therapeutic purposes. Because the Fc region is constant for all antibodies, this approach allows for the 'design once, use multiple times' concept. Xencor's suite of XmAb™ engineered Fc domains encompasses a wide range of affinities, enabling improved antibody design via selection from pre-existing variants.

The company's own pipeline of therapeutic antibodies includes XmAb2513, an anti-CD30 antibody in development for the treatment of NHL and T-cell lymphomas, and six other oncology products, while also collaborating with leaders in antibody development to improve the potency of their products, including Genentech (rituximab and trastuzumab), Roche, Centocor, Inc., and Medimmune, Inc.

V_H and V_L Domain Antibodies

Focusing on the opposite end of the antibody molecule, the variable region, Robert Connelly, CEO of Domantis, Inc., introduced the company's strategy for the discovery of novel, fully human antibody therapeutics. Human domain antibodies (dAbs) use the smallest functional binding domain of either the variable heavy (V_H) or light (V_L) chains of human antibodies. These antibody fragments have a molecular weight of approximately 13 kDa, or less than one-tenth the size of a full antibody, allowing easy expression in bacterial, yeast and mammalian cell systems. Libraries of V_H and V_L dAbs are used to select dAbs that are specific to therapeutic targets. Their valency can be modified and their half-life tailored via PEGylation.

Because of their small size, dAbs can be used to block receptors directly, rather than interfering with receptor ligands. For example, the receptor for tumour necrosis factor (TNF)α can be blocked directly for therapy of rheumatoid arthritis, as opposed to approaches designed to block the activity of the cytokine at the receptor (either by antibody interference or by saturating TNFα using a soluble receptor). The small size, solubility and stability of dAbs also make them amenable to noninjected delivery systems such as pulmonary delivery. Domantis is partnering with Argenta Discovery for pulmonary proof-of-concept studies with dAbs for respiratory disorders.

Domantis is also working on engineering dual-targeting formats by creating bispecific antibodies. An example in development is DOM1112 (anti-CD38/CD138) for multiple myeloma, which is designed to kill tumour cells selectively, and spare healthy cells, by binding only when both targets are present.

Antibody-Drug Conjugates

Clay Seigall, President and CEO of Seattle Genetics, discussed the concept of empowering antibody therapeutics by using a conditionally stable (enzyme-cleavable) linker to arm the antibodies with a synthetic drug payload. The enzyme-cleavable linkers are substrates for human lysosomal cathepsin B. Once internalised in the target cell, interaction with cathepsin B allows release of the fully active, unmodified cytotoxic drug. Auristatins, highly potent inhibitors of tubulin polymerisation, have been the company's drug class of choice for its antibody-drug conjugate technology platform, but other drug classes are now also being evaluated. Seattle Genetics continues to advance genetic engineering programme to improve the stability of the linkage system, and to allow more precise attachment and optimisation of drug loading, in addition to researching the conjugation of drugs with antibody fragments. Technology partners include Genetech, Medimmune, PDL BioPharma, CuraGen Corporation, Bayer, UCB, and PSMA Development Company.

3.1.3 Carbohydrates and PEGylation

Moving away from antibodies, Dr Tara Chapman-Arvedson of Amgen discussed the effects of carbohydrates and PEGylation on the biological activity of recombinant human protein therapeutics. Glycoengineering has shown the capacity to enhance *in vivo* activity, extend serum half-life, and increase the solubility and stability of protein-based drugs.

For example, serum clearance is the primary determinant of the *in vivo* activity of recombinant human epoetin alfa (EPO). Increasing the sialic acid content significantly increases the half-life of the drug. The carbohydrate on EPO is heterogeneous in structure; discovery of new glycosylation analogues, including the glycosylated form darbepoetin alfa (Aranesp®), showed that, although such molecules may display decreased *in vitro* activity, their *in vivo* activity is profoundly enhanced as a result of prolonged residence time in serum. Therefore, fewer doses of darbepoetin alfa are required for the same therapeutic effect as the original molecule.

PEGylation can have similar dramatic effects on protein stability and therefore on activity. The recombinant granulocyte colony-stimulating factor (G-CSF) agent pegfilgrastim (PEG-G-CSF, Neulasta®) is a sustained-duration formulation of filgrastim (G-CSF, Neupogen®) that maintains the biological properties of the parent molecule but with increased half-life and activity.

3.2 RNAi Inferference: Advancing Therapeutic Development

RNA interference (RNAi) came to light approximately 8 years ago and has been one of those highly hyped technologies that is watched with great anticipation. Several companies have emerged in recent years attempting to capitalise on RNAi for therapeutic applications. This session by some of the industry leaders displayed the current state of the RNAi, which continues to advances amid the hype. The ongoing research into RNAi has offered extensive knowledge of its molecular mechanisms, although there remains a need to identify the best candidate (i.e. non-redundant) target sequences, since therapeutic concerns relating to off-target effects continue to linger.

Edward Tenthoff, Biotechnology Research Analyst at Piper Jaffray, summed up the session in his introductory talk. RNAi essentially hijacks a natural cellular mechanism that evolved to prevent viral infection. This approach to turning off target genes has the potential for enhanced potency over traditional drug approaches, and, overall, the initial safety signs are encouraging. RNAi can address traditionally nondruggable targets, as can its predecessor technology, antisense, and in a much more potent manner.

Although RNAi is still an early and unproven technology in the clinic, clinical progress will drive the industry value, with new IND filings, efficacy trials commencing and safety data, new collaborations and partnerships emerging. In addition to the smaller biotechs Acuity Pharmaceuticals, Sirna Therapeutics, Alnylam Pharmaceuticals and Benitec, large pharmaceutical companies are also getting involved, as seen in Alnylam's collaborations with Merck & Co. on ophthalmic diseases and Novartis on pandemic influenza, and Sirna's collaborations with Allergan on ophthalmic diseases and GlaxoSmithKline on respiratory disorders. Interested parties will continue to watch this space for developments in this exciting therapeutic area.

4. Recognising Achievements

BIO presented three outstanding biotechnology companies with the prestigious James D. Watson Helix Award for Biotechnology Industry Leadership at BIO 2006. Recipients of this award for outstanding corporate achievement are nominated and voted on by peers in the industry. Performance is assessed in three distinct areas: scientific innovation, company growth, and corporate citizenship.

4.1 Large-Capitalisation Winner: Genentech, Inc.

Genentech, considered the founder company of the biotechnology industry, has over 25 years' experience delivering on the promise of biotechnology. It has one of the leading product portfolios in the industry, commercialising and manufacturing 12 protein-based biotherapeutics for serious or life-threatening medical conditions. 2005 was an unprecedented year in product development for Genentech, with positive phase III data from eight clinical trials on four products.

4.2 Emerging Company/Mid-Capitalisation Winner: Alnylam Pharmaceuticals

Alnylam's goal is to build a leading product company based on novel RNAi therapeutics, a recent breakthrough in biology. It is believed that RNAi has the potential to generate an entirely new class of innovative medicines. 2005 saw Alnylam make significant scientific and clinical progress, and it executed important business strategies to help it continue to grow and build its business. The company's achievements last year included initiating two phase I trials of a candidate drug called ALN-RSV01 for the treatment of respiratory syncytial virus infection. Alnylam also formed a major alliance with Novartis, one of the most significant innovation-based drug discovery alliances in the biotech industry.

4.3 International Winner: Novo Nordisk, Inc.

Novo Nordisk is a leader in diabetes care. In 2005, the company won FDA approval of Levemir® (insulin detemir [rDNA origin] injection) for the treatment of type 1 and 2 diabetes. It also received FDA clearance for the product to be use in the treatment of diabetes in children. In September 2005, the company had a Supplemental New Drug Application approved for NovoLog® (insulin aspart [rDNA origin] injection) for the treatment of diabetes in children, a new indication for the product.