Do you think companies are being selective enough about which compounds are being screened?
No. As we learn more about structure–activity relationships, the targets we are interested in, and as we have efficient methods for supplying compound subsets to screens, we will become more selective. The days of HTS as we now know it are numbered.

Do you outsource any of your screening?
Not significantly. Screening is such a key part of our drug discovery activities that we prefer to keep most of it in-house. I think a successful outsourcing collaboration requires commitment on both sides to make it work: trust, teamwork and shared goals.

How do you think the human genome sequence information will impact on HTS?
The conventional wisdom is that it will deliver more targets. However, I think it is more likely to help us to better understand diseases and associated targets. It will probably help us to be more selective and to home in on the best targets. It might reduce the need for HTS in favour of a more considered approach.

What do you think will be the impact of informatics and computational chemistry on the direction of screening in drug discovery?
They will result in us being able to screen selected subsets of compounds against targets that we understand better using methods with a much higher degree of sophistication than is the current practice. Eventually, it will lead to the re-emergence of rational design as a viable component of *de novo* lead identification.

Advances in HTS and increased compound availability have resulted in the generation of huge amounts of data. Which data-mining methods do you think could prove to be a leader?
I don't know. Actually, I don't believe there will necessarily be one leader. We will need a range of approaches matched to the job in hand. It is similar to the mistaken belief that HTS, in isolation, would revolutionize drug discovery. We need powerful databases, query tools and visualization approaches together with people who know what data to store and what questions to ask.

Where do you think HTS will be in ten years' time?
We probably won't be doing HTS as we understand it today for the reasons discussed above.

Who do you think has the most innovative products/ideas in the HTS field?
The microplate in its various versions (starting with 96 wells) has been hugely influential and has resulted in the development of a vast range of instruments and automation. The parallel processing and the equipment developed to enable this has been crucial. However, ineffective, unreliable and inappropriate automation has probably been one of the most counter-productive developments in HTS. I anticipate that the most important developments will probably turn out to be the imaging instrumentation and the effective application of informatics, although the latter area still has some way to go.

Manfred Auer, Research Program Head of Fluorescence-Based HTS-Technology and a Novartis Leading Scientist, Novartis Research Institute, Brunnerstrasse 59, Vienna A-1235, Austria. tel: +43 1 86634 257; fax: +43 1 86634 727; e-mail: manfred.auer@pharma.Novartis.com

Has your company seen any success so far with leads generated from HTS programmes?
There has been substantial success with running HTS programmes in 96- and 384-well plates for several years now in Novartis.

Now that some drugs selected through HTS programmes have finally started to reach clinical trials, do you feel that HTS will eventually deliver all that was anticipated at the beginning?
For sure, HTS is playing and will play a major role in the current and future drug discovery process. The expectations throughput the industry are very high but, in most organizations, the screeners and the drug design people are working closely together. It is this combination between random screening and the rational approaches that are generating real added value in drug discovery.

Do you think the benefits of HTS do/will equal the level of financial input required?
The field of HTS technology is very dynamic and will not stop after a certain technology has been established. Therefore, there will always be a lot of pre-investment in screening technology. However, the strategy between different companies varies. Whereas some companies are trend-setters in establishing new screening technology in collaboration with innovative biotech firms, others wait until a certain technique has a proven track record before they get involved.

How do you see the future for miniaturization? Do you ever see

the 1536-well plate becoming the most commonly used density?

There is a common trend in the industry to move to the 1536-well plate format. Many companies, however, still believe in 384-well plate technology. In particular, the low-volume 384-well plate formats provide an appreciable cost reduction in HTS but with fewer crucial plate and instrument requirements.

Do you think companies are being selective enough about which compounds are being screened?

Most companies intensively invest in increasingly powerful theoretical informatics tools to reduce the number of compounds to be included in their set of primary screening compounds. The trend is going in the direction of putting together so-called 'boxes' containing compounds with higher probability to deliver a hit in a screen of a certain biological class e.g. screening of kinases. Combinatorial chemistry compound collections fall into a special category as, even now, not every type of chemistry works in the solid-phase. The chemical diversity space is therefore restricted.

Do you tend to outsource your screening?

Outsourcing of HTS means to transfer compound collections to the companies that would perform the screening. In Novartis, this is currently only done in very special collaborations.

How do you think the human genome sequence information will impact HTS?

With increasing speed, novel genes are expressed and with the resulting proteins, most companies perform intensive proteomics studies. Of course, there is already a lot of pressure to integrate these potential target proteins into an HTS process before their medical target potential has been proven or even before a ligand has been identified. The resulting small-molecule high-affinity binders would then be used for functional genomics investigations. Technologies for these approaches are currently available only in limited number, and the respective groups are in process of establishing a basis of experimental experience.

What do you think will be the impact of informatics and computational chemistry on the direction of screening in drug discovery?

As mentioned earlier, informatics tools are already being used to reduce the number of compounds used in primary screening. Furthermore, sophisticated modelling tools to streamline chemistry for structure–activity relationship investigations will gain increasing importance through the next years. Combinatorial chemistry (combichem) will hardly replace historically grown compound collections, including big collections of natural products in HTS. However, it is clear now that taking the specific rules behind combichem compound collections into consideration, millions of new combichem compounds will have a strong impact on lead finding and lead optimization.

Advances in HTS and increased compound availability have resulted in the generation of huge amounts of data. Which data-mining methods do you think could prove to be a leader?

Neural networks will be necessary to interconnect the enormous quantities of data in primary screening with the information collected from hundreds of profiling assays and high-throughput biology. These specific data mining tools for the connection between the functional data and HTS are in process of being developed in specific dedicated biotech companies as well as in big pharma.

Where do you think HTS will be in ten years' time?

The HTS field is developing so rapidly that this question is difficult to answer. I think that the connection between digital electronics and interaction biology will have been broadly established. Direct reading of interaction forces will have replaced indirect detection via optical signals and screening will be performed either in dynamic flow systems or on chips containing the proteins of most of the relevant medical drug targets.

Who do you think has the most innovative products/ideas in the HTS field?

The answer to that question very much depends on where one sets the priorities. In big pharma, innovation potential is usually very strongly connected to screening costs. This means that the technology that comes out as being fairly simple yet enables HTS in the >100,000 compounds-per-day range is going to be the most innovative. In this respect, gel-based and chip-based imaging techniques like the one developed at Abbott Laboratories belong to the most innovative group of technologies. New methodologies to screen 'orphan' targets, of which no interaction partner is known or biochemically accessible, become a necessity for every company that does not want to wait until functional genomics/proteomics has defined the right assay to be set-up. Therefore, very few affinity selection procedures in solution (e.g. Neogenesis) or on the solid surface (Novartis–Evotec OAI) have been developed in the HTS field, but we expect increased efforts in chemogenomics research during the next few years.

The simplest and cheapest approach that one could take is to replace any detection and readout equipment by eye inspection, as the human eye is still the most sensitive detection instrument. From hundreds of thousands of samples, the eye would pick the brightest coloured wells to be followed up by quantitative biology. However, high-tech detection technologies such as confocal single-molecule spectroscopy pioneered at Evotec Biosystems or the fascinating

intracellular chemical and biological reporter technologies developed by Roger Tsien together with the 3456-well plate approaches developed by Aurora Biosciences will have a lot of potential. As with all high-tech approaches, though, it remains to be seen how their technologies might be able to reduce screening costs or improve screening outputs.

Shifting the bottlenecks: HTS matures

Dirk Ullmann, Drug Discovery Services, Screening Operations, Evotec OAI, Schnackenburgallee 114, D-22525 Hamburg, Germany; tel: +49 40 5608 1247; fax: +49 40 5608 1222; e-mail: dirk.ullmann@evotecoai.com

The tremendous volume of data generated by the Human Genome Project has resulted in an unprecedented level of opportunity for the pharmaceutical industry. As expectations turn to the HTS to fill the gaps in the drug discovery pipeline, the investment in this sector has increased. We are now approaching the post-genomic era, and with it a new set of threats and opportunities. The HTS benchmarks are moving steadily, as highlighted at the recent *Screentech 2001* conference in San Diego, CA, USA (13–16 March 2001). The meeting attracted a blend of industrial and academic technology developers along with end-users, to address the application of HTS to different targets classes. In addition, the neighbouring processes, such as assay development and miniaturization, compound logistics and data mining were also highlighted, indicating where the bottlenecks will shift as HTS principles change.

Although many themes were discussed, prominence was given to readout and screening technology development studies and examples of its application in case studies. These applications are increasingly shifting away from soluble systems and towards cellular assay systems. This was highlighted in the opening keynote presentation given by Roger Tsien (Howard Hughes Medical Institute, University of California, San Diego, CA, USA). Tsien set the scene for many of the presentations as he discussed the scope of applying labelling techniques and natural probe sources to investigation of biochemical interactions at the cellular level. Green fluorescent protein (GFP)-based fluorescent indicators are applicable to almost all organisms and can be targeted to specific tissues, cells, organelles or proteins. The comparison of fluorescence resonance energy transfer (FRET) between GFP mutants offers a general mechanism to build genetically encoded indicators and monitor dynamic protein–protein interactions. The potential of this method was demonstrated for the Fas/TNFR receptor superfamily as an example. Tsien also discussed the application of FlAsH (fluorescein-based arsenical hairpin binder) reagents for intracellular labelling of CCXXCC sequences for use in fluorescence anisotropy to monitor protein orientation. In the future, FlAsH analogs might be used to label proteins that are altered by GFP fusions (for protein expression and enhanced purification), which would be a key step in making reporter gene assays universally applicable.

Target validation – challenging assays

Potentially, efficiency within the gene-to-lead process is, in the most part, determined by target validation. Michael Kuranda (Millennium Pharmaceticals, Cambridge, MA, USA) reminded the audience that parallel target validation is thought to shorten the target validation process from 3–7 years to 2–3 years. To achieve this goal, Millennium has set up an Integrated Target Discovery Platform (involving 200 people) with competitive strengths in process optimization using SHERPA, a knowledge management tool, to assess expression and/or activity space, and predict assay configuration success. This platform enables screening to be performed in parallel to target validation, and enzyme targets have already been used to test this paradigm.

In two presentations, Jeremy S. Caldwell and Daniel Sipes (The Genomics Institute of the Novartis Research Foundation, San Diego, CA, USA) underlined the importance of an appropriate robotics infrastructure for cellular screening. Caldwell presented chemical and biological strategies for high-throughput gene functionalization using pathway-based screens. The company has built an impressive 1536-well-plate-compatible screening platform for cellular assays, enabling a production throughput of >125,000 compounds per day. The platform fluidics is composed of the Robbins Hydra (Robbins Scientific, Sunnyvale, CA, USA) for compound pipetting, and the synQUAD™ (Cartesian Technologies, Irvine, CA, USA) for cell dispensing.

In addition, they introduced a new concept to whole-genome functional analysis based on cDNA-HTS. Former methods have relied on the generation of a cDNA library from the cells of choice and subsequent cloning of each cDNA into a retroviral expression vector, which typically needs a very high signal-to-noise ratio (S/N) and limits the investigation to dominant effectors only. The new approach involves cDNA expression arrays and a 384-well-plate screening system that can be used in low S/N situations, providing access to both dominant and recessive effectors. Sipes outlined the principal screening concept at GNF, which enables a cell-based screening capacity of >60 screens per year. An interesting technical solution to compound

1359-6446/01/$ – see front matter PII: S1471-1931(01)00154-9