HTS personal perspectives: small companies

Interviews by Rebecca Lawrence

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How would you respond to the claim that 'HTS is a waste of time as no successful leads have yet been produced'?

From my own experience when I was doing drug discovery at SmithKline Beecham (SB), a lot of the hits from screening went on to drive medicinal chemistry programs, and I believe that the same success rate has continued, if not improved, since I left. I also know from customers of Aurora that they are finding plenty of quality leads from screening that are then moving forward into chemistry. In terms of getting clinical candidates, it tends to take 2–3 years after finishing the screening to optimize drug candidates, and then an additional 1–2 years for pre-clinical safety assessment. As HTS has only been going for 5–8 years in most companies, there are clearly many leads from HTS that have only just started to make it into the clinic.

Do you think further miniaturization is the way to go in the future?

Beyond 384-well plates, definitely yes, but beyond 1–3 μl per well, probably not. There are enough problems with liquid handling with these smaller volumes that make it technically challenging to go beyond this. Also, by reducing assays volumes to 1–3 μl, you have made very large savings in reagents, which is one of the real benefits of miniaturization. Hence, miniaturization probably has a bigger impact on reducing cost than for increasing throughput.

What do you think is the main problem with HTS at the moment and how would you resolve it?

One of the greatest challenges is how to handle very large numbers of compounds and transfer them into assays reliably and relatively accurately at the sort of throughput levels at which one can run assays. Although there are plenty of solutions for compound storage, the actual transfer of compounds from storage formats into miniaturized assay formats is still a major problem. Aurora has developed a piezo system that will dispense down to about a nanoliter and will transfer 100,000–300,000 compounds a day into a miniaturized assay format, while other companies are working on this problem by running their transfer systems in parallel.

Do you think the benefits of HTS equal the level of financial input required?

I think there are a large number of targets that are relatively intractable to finding lead compounds in any way other than by using HTS. For example, there is not enough structural information or knowledge of the ligands for new drug targets that have come from the genome database [e.g. orphan G-protein-coupled receptors (GPCRs) and orphan kinases], and hence we have no rational idea of where to start with medicinal chemistry. The only way to generate leads is by using HTS to find compounds from your collection that will interact with these targets.

Do you feel HTS is essential to advance fields such as genomics?

Given the very large level of investment in genomics and the large number of targets being produced from the genome database, there is no other way of realizing your investment in genomics and target validation if you do not use HTS.

Do you think outsourcing of HTS is an essential part of pharma strategy or should it all be kept in-house?

I think you can do it either way. Outsourcing for equipment is standard. Generally, big pharma does not have the expertise or the will, nor is it really practical for them to develop their own equipment, so this will always be outsourced. As for outsourcing of screening, I think that companies will do this in a limited number of cases when the supplier companies have something special such as a large range of compounds, a specialized technology that is not worth bringing in-house, or a need for specific expertise. My general experience of big pharma companies is that they want to do it themselves in their own way, and they want to maintain a high level of control of the work. Many companies have made small initial investments in screening that are not too expensive. Hence, when it comes to outsourcing, they have already made an internal commitment that reduces the incentive to outsource. There is also the

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logistical problem of supplying compounds to external collaborators, as it is, in practice, a lot of work.

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

I think we will have probably solved the compound handling problems, but whether we will have solved it by retaining open-well microtiter formats or whether we will have gone to chips, I do not know. Chip-based systems are relatively new and, at the moment, are relatively limited in take-up. Although groups like Caliper Technologies have made big strides in this field, I cannot predict whether the closed architecture will be a common feature in ten years time. I think we will have certainly screened all the obvious families of targets (such as GPCRs, kinases and proteases), but I am not sure whether we will have leads or therapeutic utilities for all of these. We will then be looking to solve more intractable targets such as protein–protein interactions, which will offer a whole new range of pharmaceutical targets with their own problems in assay configuration and lead identification.

At your company, which well-plate size do you currently use the most?

Obviously we produce the 3456-well plate but we also do assays in 96- and 384-well plates for ion-channel work. However, we try to make all our assays suitable for the 3456-well plate format.

Who do you think has the most innovative products/ideas in the HTS field (other than your own company!)?

I think Scintillation Proximity Assays has changed the way a lot of radioactivity assays are being done. Also, the move to cell-based functional assays has had a large impact (e.g. FLIPR and reporter gene assays), as has the imaging of whole plates, various high-sensitivity detection systems, the use of fluorescence, and the ability to miniaturize.

Who do you think has most influenced your own career?

My post-doctoral supervisor, Ed Krebs (at the time at the University of California, CA, USA), was influential in my career as he introduced me to the areas of signal transduction and protein phosphorylation, which influenced what I did in academia and, to some extent, in industry. What probably influenced me most in terms of moving into screening was the signing of the deal between SB and Human Genome Sciences when I was at SB. This alerted SB to the need to have HTS as a centralized function rather than a small number of disparate groups working on their own separate targets. It also prompted the recognition that, with all the new targets, the only way to get leads was by having an HTS group.

Do you miss working at the bench?

I used to miss working at the bench very much as I did benchwork until my early 40s when I was in academia. Once I came into industry, I started to do less until, after three years, I did not have the time. However, I have always been very close to the science and have always looked at data and technologies and helped plan the research projects. I do not think I could go back to the bench now and run a research project as the techniques and the lab practices haved moved on too much. However, I have always tried very hard to see my job as a scientific manager rather than just a manager.

What would you like to have achieved by the end of your career?

I would like to have developed the careers of a lot of people by mentoring them and guiding them. I would also like to have contributed successfully to drug discovery, not just in screening but in general.

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How would you respond to the claim that 'HTS is a waste of time as no successful leads have yet been produced'?

I think they need to talk to people who have produced successful leads. Several talks at the industry conferences reported leads produced by HTS, in fact faster than without HTS. So I cannot imagine how it can be a waste of time when you can get your lead faster and in a more cost-efficient way.

Do you think further miniaturization is the way to go in the future?

I think that miniaturization at 3–8 μl per data point is probably a very significant enabling step and this is a step I believe will be broadly applicable – there should not be any biological limitations that will prevent you from running an assay at this level. If you go beyond that into the nanoscale, you start coming across limitations. However, that is not to say that there could not be good applications

for nanotechnology, as I believe that the fact that you can run an assay on a nanoscale could offer a significant advantage in some cases. However, so far, it is hard to see how it could be broadly applicable to all applications.

What do you think is the main problem with HTS at the moment and how would you resolve it?

I think that last year the problem was how to accelerate the throughput and how to get to the proverbial '100,000 data points per day' and run an assay in 10 µl or less. These problems have now been solved. I think the next problem is how to transfer the assays from assay development so you do not waste time waiting for assay conversion and validation, and I think this is going to become an increasing problem. One way to solve this is to push some of the screening into assay development by implementing screening workstation products that are designed to run a few, but important types of assays. Another way to solve it is to standardize throughout the enterprise, so that the technologies used in assay development are the same as those being used in HTS.

Do you think the benefits of HTS equal the level of financial input required?

I think if you are talking about a system that costs $15 million to $50 million to implement then I do not know if the benefits equal the input. However, if you are talking about a system that costs $500,000–$1,000,000 to implement, customers have reported that the savings from the reagent costs alone are enough to justify using this kind of a system.

Do you feel HTS is essential to advance fields such as genomics?

I think high-throughput is essential for genomics, and the drivers in genomics are going to be the same as in screening, i.e. that you need to get more data points at a lower cost per data point.

Do you think outsourcing of HTS is an essential part of pharma strategy or should it all be kept in-house?

That is one of my favorite questions and I am afraid I have a biased view on that. I remember when I was working in a large company in the early 1980s and I used to outsource my computerization needs. I could not afford my own personal computer, but the moment I could, I stopped outsourcing because it is much better to do things in-house. When companies outsource, they do it out of necessity. However, if they can apply products that enable them to do the screening in-house then they almost always can do it faster, better and cheaper themselves. It is therefore up to companies that supply technologies to make them available in a user-friendly way, to 'productize' them such that they can be routinely used by any end-users and do not require the help of the technology's inventor to run them.

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

I think the general direction of the industry, driven by the needs for time- and cost-efficiency, is that people will be looking more towards integrated screening solutions that will include the hardware, the reagents, the plates and the software, all designed from the ground up to run smoothly together. You could unpack such a product from the box, plug it in, place your compounds in it and screen. One should be able to implement these compact, cost-efficient desktop stations across many functions, such as distributing them in all therapeutic areas, creating parallel processes, similar to the way personal computers enabled distributed computing and vastly improved productivity.

At your company, which well-plate size do you currently use the most?

We make systems that use all well-plate sizes from 96 to 1536-well plates. People are generally gravitating towards the 384-well format. I think people are also moving towards the 1536-well plate format, although it is not an overwhelming format right now.

Who do you think has the most innovative products/ideas in the HTS field (other than your own company!)?

I think ABP has some good products – they always have been very creative in what they do, and also Applied Biosystems. I admire these two companies.

Who do you think has most influenced your own career?

I have been fortunate to have a number of people who became my mentors informally – there are probably a dozen people so I would be hesitant to single anyone out.

Do you miss working at the bench?

My background is engineering, and I did work at the bench as an engineer. I do not really miss working at the bench – I changed my interests over time from pure technology to more strategic interests. This is not to say that one is better than the other – strategy without technology is worth nothing – but technology applied well is a lot of fun and that is where I see myself.

What would you like to have achieved by the end of your career?

My goal is to make everybody who I associate with successful and what I would like is to have a large number of people who say that 'I worked with Lev and that was the best thing I ever did in my career'.

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How would you respond to the claim that 'HTS is a waste of time as no successful leads have yet been produced'?

I think there is some truth to this in that HTS is not producing what people might have expected – we have not seen an overall increase in products produced by the drug discovery process, and there are probably a couple of reasons for that. First, the technologies have been built for a very large industrial process to discover hits and produce lead molecules, but that is, of course, a long way from a product. Second, the processes to characterize compounds and actually turn them into products have not really been made more efficient. So you have this very high-throughput process on the front end that is being fed into an extremely slow low-throughput process, so the overall benefit from the drug discovery effort is probably not as effective as one would have liked.

Do you think further miniaturization is the way to go in the future?

There might be some short-term value in further miniaturization but in the long-term, probably not. In the long-term, I think there will be a move toward actually reducing the number of experiments. I think more will be done on the computational side upfront, and experiments will be used either to build predictive models, to generate data for validation, or as confirmatory (rather than exploratory) tests that should naturally be rapid, but not necessarily high-density HTS.

What do you think is the main problem with HTS at the moment and how would you resolve it?

State-of-the-art screening is not an area where we have a direct focus. However, what we are seeing is that people are moving away from screening huge numbers of compounds built around the same templates to using more templates and fewer numbers of compounds and being much more selective about how many compounds they screen and the nature of those compounds. They are moving away from trying to screen everything they can get hold of, as it is still cost-prohibitive, both financially and in terms of time.

Do you think the benefits of HTS equal the level of financial input required?

For some companies it probably will be equal, but for the industry as a whole, it is a tough assessment to make. I think there are two things that can come from the current investments in HTS. Obviously, one is that if you screen compounds you hopefully end up with products just because of the odds of exposing so much chemistry to so many targets, even if this process is disappointingly slow. The other is the alternative uses for the data generated from HTS. You can imagine that as you build up information, either together with the chemistry that has been used to screen compounds or independently, that information can be used to make the whole process more efficient. Ultimately, therefore, the pay-off might be that HTS can generate enough data to create truly predictive tools and therefore reduce the need to continue to try to screen ever larger numbers of compounds.

Do you feel HTS is essential to advance fields such as genomics?

It is hard to say 'essential'. Historically, approximately 500 targets have been the genesis of all drug products to date, but now there is anticipated to be upwards of 10,000 targets. One would therefore imagine that there is going to be a fundamental change to what we have done in the past, which is to identify a target and bombard it with as much chemistry as possible. We currently do not really use the information we gain from most screening efforts other than to identify hits. Much of the negative information is thrown aside and not used in any meaningful way. As we get more targets, one can imagine repeatedly screening the same chemistry against a large number of targets and effectively using both positive and negative information associated with that chemistry to produce a large experience within the library so that we can become more efficient in predicting activity against new targets.

Do you think outsourcing of HTS is an essential part of pharma strategy or should it all be kept in-house?

Companies have an inherent desire not to send out what is going to be their core values – sending out early discovery compounds or having proprietary targets reside outside the company is an uncomfortable situation. I think screening might follow, in some ways, combinatorial chemistry, in that there was much interest in outsourcing chemistry, but then companies began to incorporate that technology internally. With screening, there will probably be certain things where it is more cost-efficient to do it outside (e.g. searching through libraries of compounds that are available externally), whereas if the screening is based on proprietary compounds, they might be more comfortable to do it internally.

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

I think there will be an immediate effort to make it faster and more miniaturized but then I think the pendulum will start to swing the other way. I think there will be many applications that will be carried out in

a lower throughput and that will be done in tandem with computational technologies. Hence, the process will be to generate some data, build a model and do a prediction to guide the experiments that need to be done, hopefully therefore becoming more time-efficient.

At your company, which well-plate size do you currently use the most?
Most of what we do is still in 96-well plates.

Who do you think has the most innovative products/ideas in the HTS field (other than your own company!)?
Independent of internal large pharma efforts, certainly Aurora and Evotec come to the top of the list when you think of companies developing an industrialized scale screening technology.

Who do you think has most influenced your own career?
Other than my parents, of course, probably my major Professor (Joseph Robinson, University of Wisconsin) while I was a graduate student had a major impact on my career, from a technical aspect as well as for the work ethic and the importance of good science and where it fits into the industry in general.

Do you miss working at the bench?
Yes, there are some days when it would be a lot simpler to be at the bench – there is a certain aspect of proposing a theory, doing a number of experiments and proving it right or wrong and getting that fairly immediate gratification – I miss that part. But that is certainly an oversimplification of what actually happens. Those series of experiments can take a very long time and can be quite repetitive and tedious and I do not miss that part at all!

What would you like to have achieved by the end of your career?
Probably two major things. I would like to have had some kind of positive impact, whether it be delivering a technology that helps the overall discovery process or whether it is involved in finding some therapeutic agent. I would also like to have some significant personal satisfaction, which would probably be in tandem with the level of contribution I had made.

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How would you respond to the claim that 'HTS is a waste of time as no successful leads have yet been produced'?
HTS is the only drug discovery technique that has stood the test of time. You can go back to Alexander Fleming and the discovery of penicillin, or even before that to the discovery of the salicylates – these drugs were discovered by trying them and seeing if they worked – and that was screening. Two-thirds of new drugs in 1999 came from screening of one form or another.

Do you think further miniaturization is the way to go in the future?
I think that until the human genome is understood or characterized better, we will not have a good idea of the number of targets, and the number of drugable targets is going to be some subset of these. It really becomes a question of how much of a trade-off [between missing a drugable target and screening against a non-drugable target] you are willing to make. Even now, miniaturization of assays from 96- to 1536-well plates, and even from 12- to 96-well plates does not work all the time. It is a trade-off between the quantity of time you want to spend doing assay development and the amount of time you want to spend doing the assay.

What do you think is the main problem with HTS at the moment and how would you resolve it?
The main bottleneck is assay development to get from genomic pseudotargets to an assay, so that you can apply each test without really limiting yourself to the easy-to-do targets such as genetic functional GPCRs or kinases.

Do you think the benefits of HTS equal the level of financial input required?
I think the benefits are greater – you are talking about experimentation done more efficiently. I think that if you agree that the experiments have to be done, then obviously it is going to be cheapest to do it in the most efficient way possible.

Do you feel HTS is essential to advance fields such as genomics?
Yes, absolutely.

Do you think outsourcing of HTS is an essential part of pharma strategy or should it all be kept in-house?
I think there is still a level of skepticism that outsourcing can be used to hedge your bets or bring in more diversity. I think the fundamental problem with outsourcing is that

if you can do something yourself, why let someone do it for you? The key thing is developing a trust and an understanding between organizations of the value that each one brings to the table. Obviously, right now, there are more targets and more leads to be developed than ever before, but we are still not making drugs faster, so I think outsourcing is a crucial approach. If you look at the auto industry now, they outsource everything (parts, manufacture, etc.) – the only thing they do is design. If pharma is going to go along the same path, they will have to outsource everything, and they are going to have to develop a level of trust with their suppliers.

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

With the accelerating pace that we are seeing, I think that HTS will be in the same boat that sequencing is today: 'What do you do with the information?' I think the function of HTS is going to ultimately change from lead identification to identifying tools for research or being able to characterize how small molecules interact with biological molecules.

At your company, which well-plate size do you currently use the most?

We mostly use 384-well plates.

Who do you think has the most innovative products/ideas in the HTS field (other than your own company!)?

Probably Applied Biosystems – they have some bright engineers, they have the reagents and where they have identified weakness, they have gone out and bought the technologies to fill those gaps so I think they are putting together quite a powerhouse.

Who do you think has most influenced your own career?

My PhD supervisor (Jeremy Knowles, Harvard) – he has always had an interest in science and how science (in particular chemistry), medicine and biochemistry interact. He also has the ability to take ideas from different areas and to synthesize completely new fields of endeavor, and that is something I have learnt from him and which has been essential in HTS.

Do you miss working at the bench?

Yes, every 3rd or 4th day. I miss the short length of time between an idea and action – in the lab, if you have an idea, you try it and sometimes it works and sometimes it does not. It is probably the instant gratification that I miss.

What would you like to have achieved by the end of your career?

I would like to see that an innovation, big or small, that I have had a part in actually changes the way people live.

See the next HTS supplement in June 2001 for the HTS personal perspectives from big pharma companies.

Pseudomonas gene chips – a new research tool for cystic fibrosis

Sharon Dorrell, freelance writer

Gene chips based on the newly sequenced *Pseudomonas aeruginosa* genome[1] will enable researchers to identify ways of fighting this highly antibiotic-resistant pathogen. The gene chips will be developed by Affymetrix (Santa Clara, CA, USA) in collaboration with the Cystic Fibrosis Foundation (CFF; Bethesda, MD, USA) and will be available to cystic fibrosis (CF) researchers via the CFF.

While *P. aeruginosa* rarely causes problems in healthy people, it has dire consequences for people with CF, whose mucus-filled lungs provide an ideal breeding ground for the bacterium (see Box 1). Once established in the lungs, the organism causes progressive tissue damage that eventually leads to death. These problems are compounded by the pathogen's resistance to treatment with antibiotics.

Genome sequence

The *P. aeruginosa* genome was recently sequenced by Stover and colleagues in close collaboration with the CFF (Ref. 1; http://www.pseudomonas.com). They discovered that the genome, at 6.3 million base pairs, is remarkably large and bigger than any of the bacterial genomes sequenced so far. Moreover, with 5570 predicted open reading frames (ORFs), *P. aeruginosa* is as genetically diverse as the simple eukaryote, *Saccharomyces cerevisiae*, whose genome encodes 6200 proteins, and has over one-third as many genes as the fruitfly *Drosophila melanogaster*.

Stover and colleagues used bioinformatics techniques to compare the *P. aeruginosa* genome with those of other bacteria

1359-6446/00/$ – see front matter PII: S1471-1931(00)00113-0