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Vaccine discovery research by reverse engineering

The field of genomics has revolutionized the way in which we conduct research today. Medical research has benefited from genomics through the identification of new disease targets, development of diagnostic kits, better understanding of genetic evolution and the discovery of novel treatments. Antimicrobial and vaccine discovery research have also benefited greatly, due to the availability of numerous complete pathogenic genome sequences. This has been supported in a statement from the World Health Organization Director General, published in the 2002 report on Genomics and World Health; together with the advances made in the deciphering of the genomes of many disease-causing microorganisms and their vectors, it is clear that the science of genomics holds tremendous potential for improving health globally [1].

To fully appreciate the advancement that genomics has made in vaccine discovery research, one must look back to the time before the first bacterial genomic sequence became available in 1995, as to how vaccine research was done. Traditionally, protein-based vaccine targets were discovered mainly as a result of brute-force experimentation, such as raising antisera of whole organisms or isolated protein fractions, testing this sera in whole-cell ELISA assays and Western blots, and then purifying and identifying the reactive band(s) by limited peptide sequence analysis. Cloning of the gene, mainly from an expression library, was tedious and it took months to obtain the complete gene and its sequence. Finally, the gene was subcloned into an expression system, the recombinant protein purified, antisera generated, and functional immunological assays and animal experiments started. This whole process, including preclinical animal studies, could take years of work for just one protein!

Today, the availability of the genomic sequence of a pathogen enables the rapid identification of genes, the analysis of the predicted protein sequences for features that might make good vaccine targets and then the design of oligonucleotide primers for cloning into an expression vector; all of which are performed before entering the laboratory [2]. This process of *in silico* mining of a genome for potential vaccine candidates has been termed 'reverse vaccinology' [3]. Since the publication of the first complete bacterial genome sequence, *Haemophilus influenzae* in 1995, there have been suggestions of using genomic sequences to identify new vaccine targets (Cambridge Healthtech Institute's Program on Infection Genomics, 1997). Pizza *et al.*, reported on the first application of reverse vaccinology - on Group B meningococcus [4]. The ~2.3 Mb genome of *Neisseria meningitidis*, an organism known to cause serious invasive disease, was sequenced and its genome mined for predicted surface or secreted proteins. Three hundred and fifty open reading frames (ORFs) were successfully expressed in *Escherichia coli* and the recombinant proteins purified and used to immunize mice. The sera were then tested in various *in vitro* immunological assays and approximately seven candidate antigens were identified that are surface exposed, conserved in sequence and induce a bactericidal antibody response – a response that is known to correlate with vaccine efficacy in humans. Pizza et al. completed this work in about 18 months; an impossible feat if one had used traditional methods and approaches.

Today, submission of genomic sequence for *in silico* mining has become standard operating procedure by many vaccine discovery research laboratories. It is no surprise, then, that there are now many published reports of applied reverse vaccinology in identifying bacterial vaccine candidates. In this issue, the article by Mora and co-workers, reviews the applications of reverse vaccinology to many different bacterial genomes.

So, is this the 'Holy Grail' for vaccine discovery research? Absolutely not. First, genomics predicts most, but not all, ORFs; the predicted start codon of the gene is far from perfect (an important feature for knowing whether a protein has a N-terminal amino acid signal sequence to be transported to the cell membrane) and; the algorithms for predicting surface or secreted proteins are, themselves, not perfect. Second, some ORFs might only express their protein during actual infection, differing significantly from laboratory-grown bacteria. Therefore, it is no surprise if the

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antisera raised against a recombinant protein show no reaction to laboratory-grown bacteria even though it might actually be a great vaccine candidate if tested in an animal model. To address some of these concerns, other complementary techniques, such as transcription profiling and proteomics, are being employed. Using transcription profiling, Grifantini *et al.* were able to identify *N. meningitidis* ORFs that were expressed only during adherence to human epithelial cells and further study of these ORFs identified potential vaccine targets, some of which were not predicted by genomic mining algorithms [5]. Several extracellular proteins have also been detected by proteomic approaches that, again, were not predicted by *in silico* mining to be surface-exposed or secreted and, hence, might be potential vaccine targets [6].

Genomics and the application of reverse vaccinology have changed the way vaccine discovery research is conducted today. The early-phase timeline to identify vaccine targets has been dramatically reduced but, consequently, many more targets must now be tested. Therefore, rate-limitation in preclinical vaccine discovery research now lies in the immunological screening and animal model testing processes. Although it is too early to be certain, it is probable that a vaccine discovered by genomic methods will make it into the market in the future. Only time will tell.

References

1 Director–General, WHO (2002) *Genomics and World Health: Report of the Advisory Committee on Health Research*, World Health Organization (http://www.who.int/gb/EB_WHA/PDF/EB112/eeb1124.pdf)

2 Zagursky, R.J. *et al.* (2003) Bioinformatics: how it is being used to identify bacterial vaccine candidates. *Expert Rev. Vaccines* 2, 417–436

3 Rappuoli, R. (2001) Reverse vaccinology, a genome-based approach to vaccine development. *Vaccine* 19, 2688–2691

4 Pizza, M. *et al.* (2000) Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* 287, 1816–1820

5 Grifantini, R. *et al.* (2002) Previously unrecognized vaccine candidates against group B meningococcus identified by DNA microarrays. *Nat. Biotechnol.* 20, 914–921

6 Lei, B. *et al.* (2000) Identification and immunogenicity of group A Streptococcus culture supernatant proteins. *Infect Immun.* 68, 6807–6818

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Biotechnology discovery productivity: a note of caution

In a recent issue of *Drug Discovery Today* [1], Jurgen Drews makes a case for the future productivity of drug discovery to fall increasingly to the star performers of the biotechnology sector. Drews is a well-known commentator on the strategy of pharmaceutical R&D, and is justified in his focus on the importance of drug discovery output. But while the leading companies have succeeded laudably, the record of the biotechnology sector as a whole is patchy.

First, what is the 'biotechnology' sector? The term arose from the early focus of the first generation 'alternative' pharmaceutical companies on biologically produced therapeutics, rather than chemically manufactured ones. Current trends indicate that the proportion of new products of this kind is increasing (albeit slowly and inconsistently) each year. One of the attributed benefits of this kind of development is the lower attrition rate of biological research in comparison with medicinal chemical approaches. But pharmaceutical R&D on biologicals is not confined to what most people understand to be the 'biotechnology' sector (for example, drotrecogin alfa (Xigris™), a recombinant protein for sepsis from Lilly). Nor is this sector confined to R&D on biotechnological approaches to new drugs. Drews, in his comment that the 'first tier of biotechnology companies is likely to become the most effective segment of the drug industry', comments on a set of now large companies that were established to challenge the status quo. Many such companies, such as Biogen, have a mixed portfolio of developments, some biological and some small molecules. In a sense, Drews' focus on large biotechnology selects for the brightest and the best, whereas, the significant bulk of the biotechnology sector is composed of small innovative pharmaceutical R&D companies, with a mixed set of aims and strategies.

This leads to the second point, concerning whether or not small companies really have been more successful in R&D, and/or are more likely to be so in the future. Here, the evidence is mixed at best. While 'Pharmaprojects' records that the number of compounds in early phases of drug development has increased over the past seven years [2], this increase is concentrated only in Phases I and II. Over a similar period, there has been a tremendous increase in the number of biotechnology sector companies and their R&D expenditure. To date, there has not been a commensurate increase in Phase III investigational compounds. Indeed, evidence from the Centre for Medicines Research suggests that there has been a 71% increase in the time taken in Phase II between 1997 and 2001 (Society for Medicines Research Symposium: Is there a best strategy for drug discovery? 13 March 2003; http://www.prous.com/drugdiscovery). The success rate for compounds in Phases I, II and III have declined over the same period. A more detailed analysis of the time taken for various phases of development by the different sizes of company shows a