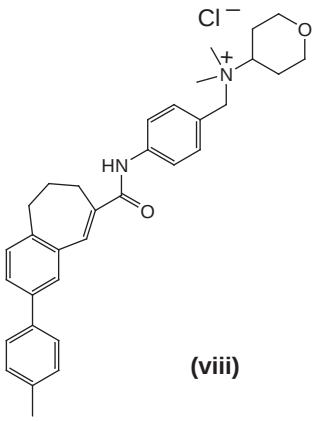
chemical library held by Takeda Chemical Industries Ltd (Osaka, Japan) using Chinese hamster ovary cells stably expressing CCR5 (Ref. 5). Lead optimization identified *N*,*N*-dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5*H*-benzocyclohepten-8-yl]carbonyl]amino]benzyl]-tetrahydro-2*H*-pyran-4-aminium chloride (**viii**) as a potent and selective CCR5 antagonist (IC_{50} = 1.4 nM). Further studies demonstrated that this compound also inhibited the replication of macrophage M-tropic HIV-1 (Ba-L strain) in both MAGI–CCR5 cells and peripheral blood mononuclear cells with EC_{50}s of 1.2 and 3.7 nM, respectively. This compound has subsequently been selected for clinical evaluation.



(viii)

5 Shiraishi, M. *et al.* (2000) Discovery of novel, potent, and selective small-molecule CCR5 antagonists as anti-HIV-1 agents: synthesis and biological evaluation of anilide derivatives with a quaternary ammonium moiety. *J. Med. Chem.* 43, 2049–2063

Andrew Lloyd

Pharmacogenomics

Qualitative gene profiling using differential analysis of transcripts with alternative splicing

RNA splicing is a tightly regulated process essential for gene expression. The process is cell-type specific and enables different cells to be characterized by their splicing potential or spliceome. The splicing profile might be modulated by both mutations and modifications of signaling pathways. As the deletion of exons or retention of introns within the coding sequence will modify the functional domains of proteins, the identification of changes in the spliceome provides a means of identifying functional domains that are specifically regulated at the level of RNA splicing. Comparisons of spliceomes from diseased cells could enable the identification of novel therapeutic targets. Similarly, spliceome profiling could provide an alternative means of assessing toxicity and efficacy of candidate drugs and the identification of information that will enable differentiation between responders and non-responders.

As part of a review of this field, Schweighhoffer, F. and coworkers have described a novel approach to systematically characterize RNA splicing alterations using differential analysis of transcripts with alternative splicing (DATAS)[1]. DATAS enables the systematic identification of spliced sequences that are differentially expressed in mRNA populations. Samples for comparison can be obtained from human or animal biopsies as well as cell culture. Cytosolic polyadenylated RNAs and their corresponding cDNAs are prepared using standard techniques from the samples under comparison. Heteroduplexes of RNA and DNA resulting from cross-hybridization of the different samples are then prepared and RNaseH used to release non-hybridized sequences (Fig. 1). These sequences represent splicing differences, translocations and deletions within the genes and are subsequently isolated, reverse transcribed and cloned to give libraries that can be examined using polyacrylamide gel electrophoresis (PAGE).

This alternative pharmacogenomic approach provides new tools for identifying potential therapeutic targets in

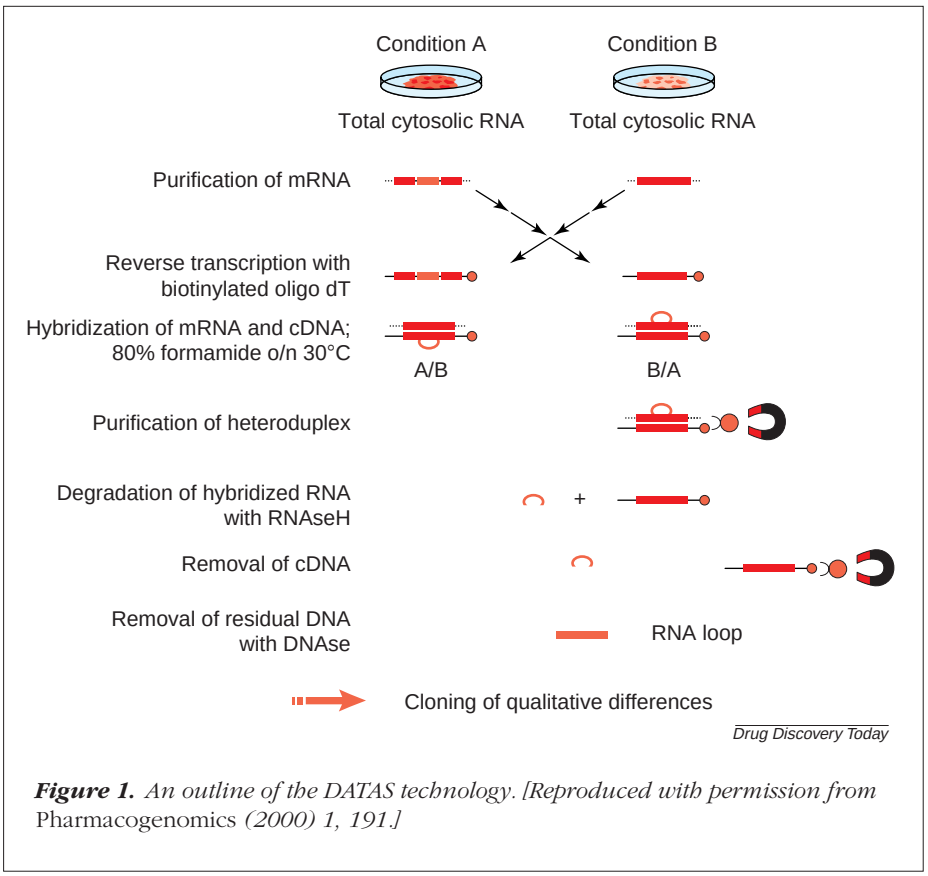


Figure 1. *An outline of the DATAS technology. [Reproduced with permission from* Pharmacogenomics *(2000) 1, 191.]*

 1359-6446/00/$ – see front matter PII: S1359-6446(00)01552-X

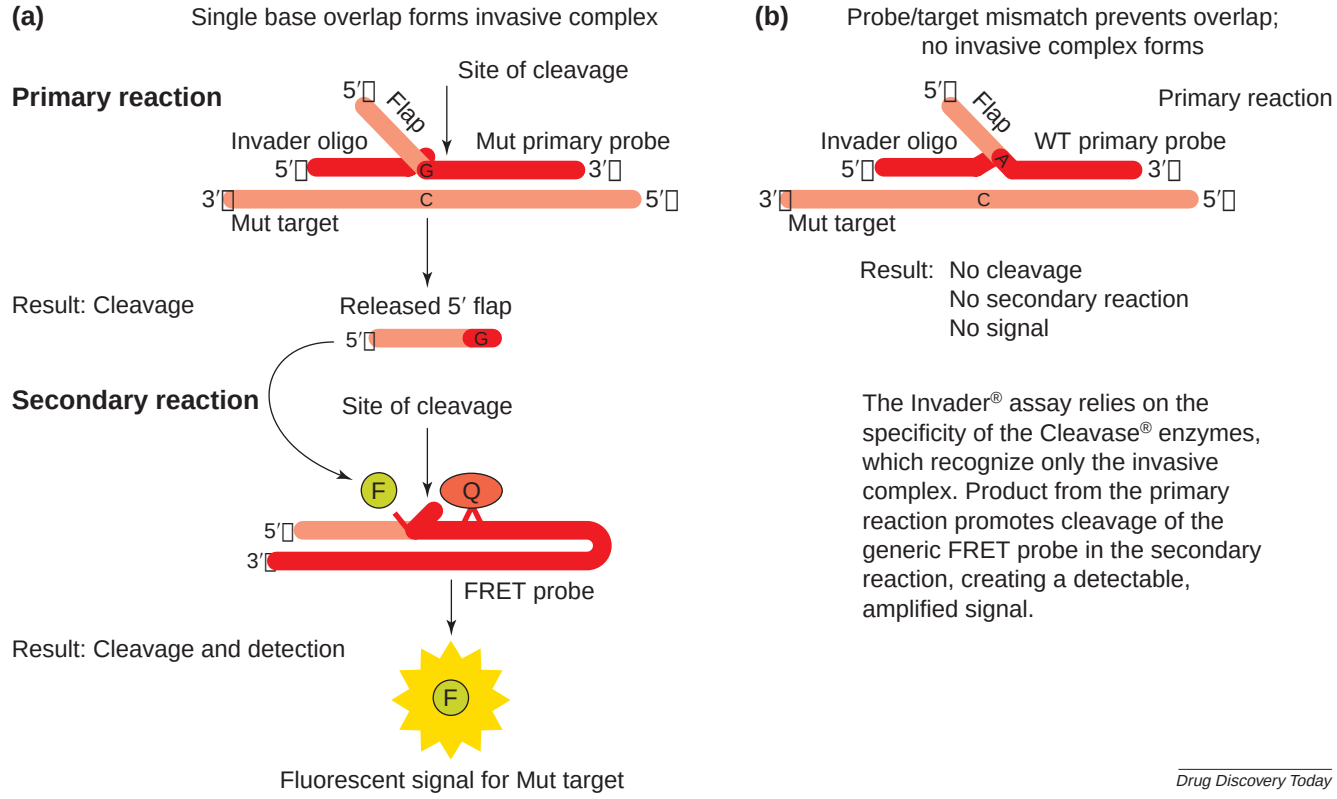


Figure 2. *Schematic of the Invader assay. [Reproduced with permission from* Pharmacogenomics *(2000) 1, 224.]*

diseased tissue, assessment of drug toxicity and screening of patients to select the most appropriate treatment.

1 Schweighhoffer, F. *et al.* (2000) Qualitative gene profiling: a novel tool in genomics and pharmacogenomics that deciphers messenger RNA isoforms diversity. *Pharmacogenomics* 1, 187–197

High-throughput SNP scoring

The correlation of single nucleotide polymorphisms (SNPs) to human disease will increase the demand for rapid genetic screening using high-throughput technologies. Such technologies must be inexpensive and amenable to automation. Although many existing technologies have been developed, these routinely use PCR-based assays the accepted 'gold standard' for gene amplification. However, this technology has several limitations including the problems of false-positive and false-negative results arising from misincorporations and cross-contamination. Assays that use unamplified DNA might therefore offer an advantage if the necessary levels of detection are achieveable.

Fors, L., Lieder, K.W., Vavra, S.H. and Kwiatkowski, R.W. have recently described the use of the Invader® Third Wave Technologies (Madison, WI, USA) assay that enables large-scale SNP scoring from unamplified genomic DNA (Ref. 2).

The assay utilizes a structure-specific 5′ nuclease (or flap endonuclease) to cleave a 5′ flap sequence when two synthetic oligonucleotide probes hybridize in tandem to the target sequence. The flap-containing probe cycles on and off the target sequence and is only cleaved when the appropriate structure forms, resulting in the presence of the sequence being amplified by the release of flap sequences (Fig. 2). The flap sequences participate in a second amplifying reaction involving a dye-labelled fluorescence resonance energy transfer probe. Cleavage of this probe leads to a fluorescent signal that can be detected using a fluorescent microtitre plate reader. To positively identify any given allele, the assay requires only three specific components, the Invader oligonucleotide and two primary probe oligonucleotides (mutant and wide-type). As the oligo-nucleotide sequences involved in the second reaction are independent of the target sequence, this secondary assay might be useful as a generic reaction for the identification of any target. This is a rapid alternative to PCR-based assays, and might be of use for the high-throughput routine screening of SNPs associates with particular diseases.

2 Fors, L. *et al.* (2000) Large-scale SNP scoring from unamplified genomic DNA. *Pharmacogenomics* 1, 219–229

Andrew Lloyd