



Petra Ross-Macdonald
 Bristol-Myers Squibb Pharmaceutical
 Research Institute, PO Box 5400,
 Princeton NJ 08543, USA
 Tel.: +1 609 818 7118
 Fax: +1 609 818 6935
 petra.rossmacdonald@bms.com

Drug discovery without a molecular target: the road less traveled

'.....matching a patient to the most appropriate therapy depends both upon understanding the precise effects of the medication, and upon being able to diagnose specific defects and measure appropriate end points in the disease.'

Expert Rev. Mol. Diagn. 7(1), 1–4 (2007)

Why do we need to know the molecular target of small molecule therapeutics? This question is intimately entwined with the utilization of molecular diagnostics in several areas, including patient selection, and pharmacodynamic and toxicity assays. The concept of matching a patient to the most appropriate therapy depends both upon understanding the specific effects of the medication, and upon being able to diagnose precise defects and measure appropriate end points in the disease. There are many recent examples in the area of Oncology; several stories surround the molecule imatinib (Gleevec[®], Novartis), developed to specifically suppress the Abl kinase activation that underlies chronic myelogenous leukemia. While diagnostics that detect the BCR–ABL fusion event or quantify ABL expression [1] drove clinical development, knowledge of the target enabled a molecular assay of the resistant mutant forms of Abl1 that ultimately develop [2]. Such a diagnostic can determine which patients will benefit from transfer to a newer Abl inhibitor [3], and may ultimately determine the order in which available drugs are given. The knowledge that imatinib is also a potent inhibitor of Arg, Kit, and PDGFRA/B has enabled its use as therapy in several other cancer types, where diagnostic assays reveal activation of these kinases [4]. In the area of molecular assays for

pharmacodynamics, it is easier to choose relevant quantitative markers when the therapy has a known target. For example, in clinical trials of the epidermal growth factor inhibitor gefitinib (Iressa[®], Astra Zeneca), pharmacodynamics were assessed using multiple markers in skin biopsies [5]. Knowledge of the molecular target also enables compounds to be counter-screened against related proteins; where cross-reactivity cannot be eliminated it allows clinical development to incorporate appropriate diagnostics for resulting toxicity. For example, development of effective gamma-secretase inhibitors must balance their toxicity due to Notch inhibition [6]. Assays for Notch-related events could provide closely linked biomarkers for patient monitoring [7].

Biology-based discovery: the old route to drugs

Given such advantage lies in knowing the target, can a drug be developed without a molecular target in the modern era? An excellent case history of ezetimibe [8], a cholesterol-lowering agent with a novel mechanism approved in 2004, indicates that the answer is yes... and no. The compound that initiated the story was an unwanted byproduct from a synthetic reaction, and was evaluated in a cholesterol-fed hamster model as part of scientific diligence. Unexpectedly, this compound had a

therapeutic effect on liver cholesterol ester and serum cholesterol levels, despite poor activity against the original target of the program. A decision was made to focus on optimizing the *in vivo* activity of derivative compounds, guided solely by activity in the cholesterol-fed hamster. Clader notes that the process, from initiation of the discovery program to clinical candidate, was completed in a similar timeframe to traditional biochemistry-driven programs. Discovery and clinical development ran concurrently with an intense, decade-long effort to identify a molecular target [9]. Had this effort to clarify the mechanism not succeeded, it could have had unwanted consequences for approval and labeling.

Should more drugs be developed like ezetimibe, relying on relevant and accepted diagnostics such as serum cholesterol? It could be argued that molecular diagnostics in themselves could drive development of appropriate therapies without knowledge of the target. For example, with a blood pressure cuff as an assay, the first drugs to combat hypertension were centrally acting agents with additional unwanted effects [10]. If plasma angiotensin II level was the measured criterion, direct or indirect inhibitors of angiotensin converting enzyme could be discovered without knowledge of the molecular target [11].

A real or perceived decline in research and development productivity in this era of target-based molecular screening has led some to urge a return to such physiological or cellular screening. It has even been proposed that if pharmaceutical discovery effort was to use such recognized disease end points, bypass the target identification and validation steps, and screen pre-selected drug-like compounds in low-throughput assays, it could produce savings of cost, time, and quality that more than offset additional effort in optimization [12]. Appealing as this is to anyone who has sat through meetings about exquisitely potent inhibitors that have the solubility of brick dust, is it a realistic perspective of a better road forward?

Same destination, different roadblocks

I would argue that pharmaceutical discovery will not largely abandon target-based development in the near term for one reason: risk. Pharmaceutical development is already hard enough, without adding a step that often requires heroic measures. The uneasy marriage between single-minded business goals and the unfortunate complexity of biology would be further strained if the open-ended process of retrospective target identification joined the ménage. A chemistry team can estimate the odds and timeframe for improving the solubility of brick dust, but who would comfortably volunteer to estimate the time needed to find a compound's target? It would be, of necessity, an ill-informed estimate, since history tends to remember only successes of target identification. Meanwhile,

the story of ezetimibe indicates that lack of a molecular mechanism of action is neither optional for regulatory approval, nor optimal for development. Ezetimibe entered the clinic without the knowledge that there were polymorphisms in NPC1L1 that significantly affect the clinical response [13]. A molecular assay for responders would likely have cut clinical costs significantly.

My own professional experience in this area has led to a healthy skepticism about our ability to reliably match compound to target. This may seem surprising, since I am a senior author on two publications describing success in the field [14,15], and have a manuscript in preparation on a third. These successes were generated by teams of scientists, using the diverse approaches of *Caenorhabditis elegans* and yeast genetics, biochemical assays, high-content analysis of cells, expression profiling and RNA interference (i). Yet we also spent countless hours trying to understand and usefully extend the data generated by applying these techniques, and several more, to over a

‘Should more drugs be developed like ezetimibe, relying on relevant and accepted diagnostics such as serum cholesterol? It could be argued that molecular diagnostics in themselves could drive development of appropriate therapies without knowledge of the target.’

dozen other compounds. Only results that furthered the aim of understanding therapeutic effect are published. In the ezetimibe story, we are fortunate that there are good publications documenting some of the enormous effort, including science that does not fit into a neat view of NPC1L1 as the sole molecular target [16,17].

It is true that there is a gamut of technologies for matching compound to target, and there is a

recent excellent review of such possible approaches [18]. However, this degree of choice is the opposite of reassuring – if a scientific goal is reasonably easy to achieve, it does not support development of a dozen different approaches! If we accept that an attack on multiple fronts is required to be certain of success, a novel multi-disciplinary scientific ‘special operations’ team would have to be assigned to every non-target based discovery program. An allowance of US\$10 million for this step [12] represents full-time support for approximately eight scientists and their supplies for 4 years. This may be an underestimate, since the paper describing NPC1L1 as the target of ezetimibe has 24 authors [9]. A US\$10 million price tag is not outlandish when compared to clinical development costs, but the perspective could be different from a discovery organization.

Proceeding with caution

For these reasons, pharmaceutical discovery is not close to abandoning target-based programs. Yet, drug discovery scientists are not blind to the advantages of cellular screening, targeting relevant pathways in appropriate disease models. How are these divergent points of view being reconciled? One emerging answer is to use a discovery approach that maximizes the chance for connecting compound-to-target, but is tolerant of a low success rate. Run a cell-based screen of compounds, but measure as

many parameters as possible in the treated cells, and run the screen in parallel with a well-characterized compound library, and with gene-based reagents. Such deeper investigation of the cellular pathway will enable connection of compound to target for some percentage of the hits. The rest can be deprioritized.

What are the gene-based reagents mentioned above? They are primarily RNAi-based knockdown, using small-interfering (si)RNA oligonucleotides or, more recently, hairpin siRNA constructs (shRNAs) carried on lentiviral vectors. Lentivirus can infect a broad range of host cells, and reagents are now available for most mouse and human genes [19]. Gene-by-gene knockdown and compound libraries are now viable concurrent screens that could be run in many cell-based models. A recent example is a parallel screen that identified small-molecule inhibitors of the Aurora B pathway in cytokinesis [20]. Findings in low- to medium-throughput screens could be supplemented by data from systems with higher throughput, such as microarrays of shRNA-infected cells [21]. Testing over-expression of defined genes in the same assay is another way to characterize the players in a cellular system. Gene expression analysis chips are a third useful gene-based reagent. Although cost and labor currently prevent their concurrent use in screening, the effect of a compound of interest on gene expression can be matched with the effect of other compounds or gene knockdowns to provide a link to a target [22–24] or even directly identify a target (D. Jackson & P. R-M, in preparation). A collection of gene expression profiles for mammalian cells treated with compounds is now publicly accessible [25]. The ability to compare profiles from new compounds to this dataset, as envisaged in a large project using yeast cells some years ago [26] will greatly increase the value of the expression profiling approach.

Comparing compound phenotypes to gene knockdown and gene-expression profiling are unlikely to be the optimum approaches for any given target identification problem. However, they are a reasonable approach for the majority. It is no accident that they are the first two target-identification partners of choice for cell-based screening in the pharmaceutical industry. They are predictable, scalable technologies with good throughput, and the organization and expertise to run them already exists in all large companies. Minimizing risk and maximizing return may seem like the triumph of the business over science, but in practice there is plenty of interesting biology in the subset of compounds that are selected by this approach. In my own experience, a compound was matched to a second target based on similarity to effect of a gene knockdown, and on knowledge of homology to the original target protein. Proving the activity on the second target, and its unexpected relevance to oncology, was a typical, interesting scientific project [14].

Final thoughts

These baby steps down the road of ‘compound before target’ are a new direction for pharmaceutical discovery. We don’t know how they will play out – will compounds with easily assigned targets always trump, or will a few ‘ezetimibe stories’ play out? Will increased demand for a reliable, broadly applicable way to connect compounds to a target spur a technological breakthrough? For the near term at least, an increased interest in cellular and disease model screening means that molecular diagnostics have a role in a new area – not just identifying disease or quantifying the effects of therapy, but perhaps also being the assays that identify therapeutic compounds.

References

- Nashed AL, Rao KW, Gulley ML. Clinical Applications of BCR-ABL Molecular Testing in Acute Leukemia. *J. Mol. Diagn.* 5(2), 63–72 (2003).
- Shah NP, Nicoll JM, Nagar B *et al.* Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2(2), 117–125 (2002).
- Hochhaus A, Kantarjian HM, Baccarani M *et al.* Dasatinib induces notable hematologic and cytogenetic responses in chronic phase chronic myeloid leukemia after failure of imatinib therapy. *Blood* (2006) (E-pub ahead of print).
- Sawyers C. Targeted cancer therapy. *432(7015)*, 294–297 (2004).
- Baselga J, Rischin D, Ranson M *et al.* Phase I Safety, Pharmacokinetic, and Pharmacodynamic Trial of ZD1839, a Selective Oral Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, in Patients With Five Selected Solid Tumor Types. *J. Clin. Oncol.* 20(21), 4292–4302 (2002).
- Barten DM, Meredith JE Jr, Zaczek R, Houston JG, Albright CF. Gamma-secretase inhibitors for Alzheimer’s disease: balancing efficacy and toxicity. *Drugs R D* 7(2), 87–97 (2006).
- Searfoss GH, Jordan WH, Calligaro DO *et al.* Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional {gamma}-secretase inhibitor. *J. Biol. Chem.* 278(46), 46107–46116 (2003).
- Clader JW. The discovery of ezetimibe: a view from outside the receptor. *J. Med. Chem.* 47(1), 1–9 (2004).
- Garcia-Calvo M, Lisnock J, Bull HG *et al.* The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *PNAS* 102(23), 8132–8137 (2005).
- Moser M. Historical perspective on the management of hypertension. *Am. J. Med.* 80(5B), 1–11 (1986).
- Nussberger J, Waeber B, Brunner HR. Angiotensin converting enzyme inhibition and renin inhibition. *J. Hypertens. Suppl.* 7(2), S75–S79 (1989).
- Butcher EC. Can cell systems biology rescue drug discovery? *Nat. Rev. Drug Discov.* 4(6), 461–467 (2005).
- Simon JS, Karnoub MC, Devlin DJ *et al.* Sequence variation in NPC1L1 and association with improved LDL-cholesterol lowering in response to ezetimibe treatment. *Genomics* 86(6), 648–656 (2005).
- Lackner MR, Kindt RM, Carroll PM *et al.* Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors. *Cancer Cell* 7(4), 325–336 (2005).
- Fitzgerald K, Tertyshnikova S, Moore L *et al.* Chemical genetics reveals an RGS/G-protein role in the action of a compound. *PLoS Genet.* 2(4), E57 (2006).

- 16 Smart EJ, De Rose RA, Farber SA. Annexin 2-caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport. *Proc. Natl Acad. Sci. USA* 101(10), 3450–3455 (2004).
- 17 Kramer W, Girbig F, Corsiero D *et al.* Aminopeptidase N (CD13) Is a molecular target of the cholesterol absorption inhibitor ezetimibe in the enterocyte brush border membrane. *J. Biol. Chem.* 280(2), 1306–1320 (2005).
- 18 Hart CP. Finding the target after screening the phenotype. *Drug Discov. Today* 10(7), 513–519 (2005).
- 19 Moffat J, Grueneberg DA, Yang X *et al.* A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell* 124(6), 1283–1298 (2006).
- 20 Eggert US, Kiger AA, Richter C *et al.* Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. *PLoS Biol.* 2(12), e379 (2004).
- 21 Silva JM, Mizuno H, Brady A, Lucito R, Hannon GJ. RNA interference microarrays: High-throughput loss-of-function genetics in mammalian cells. *PNAS* 101(17), 6548–6552 (2004).
- 22 Parker RA, Flint OP, Mulvey R *et al.* Endoplasmic reticulum stress links dyslipidemia to inhibition of proteasome activity and glucose transport by HIV protease inhibitors. *Mol. Pharmacol.* 67(6), 1909–1919 (2005).
- 23 Wei G, Twomey D, Lamb J *et al.* Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. *Cancer Cell* 10(4), 331–342 (2006).
- 24 Hieronymus H, Lamb J, Ross KN *et al.* Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. *Cancer Cell* 10(4), 321–330 (2006).
- 25 Lamb J, Crawford ED, Peck D *et al.* The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313(5795), 1929–1935 (2006).
- 26 Hughes TR, Marton MJ, Jones AR *et al.* Functional discovery via a compendium of expression profiles. *Cell* 102(1), 109–126 (2000).

Affiliation

- Petra Ross-Macdonald
Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5400, Princeton NJ 08543, USA
Tél.: +1 609 818 7118
Fax: +1 609 818 6935
petra.rossmacdonald@bms.com