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PERSPECTIVE

Looking back at multidrug resistance (MDR) research and ten mistakes to be avoided when writing about ABC transporters in MDR

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This paper presents a personal, selective, and sometimes critical retrospective of the history of ABC transporters in multidrug resistance (MDR) of cancer cells, overrepresenting discoveries of some early pioneers, long forgotten, and highlights of research in Amsterdam, mainly focussing on discoveries made with disruptions of ABC genes in mice (KO mice) and on the role of ABC transporters in causing drug resistance in a mouse model of mammary cancer. The history is complemented by a list of erroneous concepts often found in papers and grant applications submitted anno 2020.

Keywords: ABC transporters; cancer; grant application; knockout mice; multidrug resistance

I started with MDR research in 1984. Five years later, I was sitting in a panel before an audience of some 500 scientists in Bethesda. The enthusiasm was palpable. Soon, the eternal problem of drug resistance in treating disseminated cancer would be solved. The pump, P-glycoprotein (Pgp), caused the resistance, and soon, we would block the pump and incapacitate the tumor cell's defense [1]. I was not so sanguine at the time about Pgp as most of the panel members. With my medical training and from teaching chemotherapy to medical students, I knew that about half the drugs used to treat cancer patients, the alkylating agents, the platins, and the antimetabolites, were not transported by Pgp. It seemed a long shot to expect other pumps for all of these.

It helped my knowledge of chemotherapy that I had moved from the University of Amsterdam to the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital in 1983. At the time, my experience with cancer was limited. I had spent most of my thesis on rigorously demonstrating that mitochondria from cancer cells were completely normal in all properties that could be measured in 1958–1961 [2], in contrast to the concept that mitochondria in cancer cells are uncoupled, postulated with great authority, but minimal evidence, by the formidable German Nobel laureate Otto Warburg [3]. Fortunately, I discovered the malate–aspartate shuttle on the side [2,4]. Nevertheless, I left the cancer field with the impression that cancer research was one step behind basic research in biochemistry and to be avoided.

So why return to cancer in 1983 and become the director of a cancer institute? It would be nice if I could present this move here as part of a career master plan, but it was not. As I have already admitted elsewhere [5–7], my career was a haphazard affair, shaped by incidents, external circumstances, and occasional opportunities. In the eighties, conditions for basic research in the university had rapidly deteriorated and I felt increasingly stifled by irrational bureaucracy. I was fed up struggling and had no qualms to leave the university. Being director of an independent institute

Abbreviations
BBB, blood–brain barrier; KO, knockout; MDR, multidrug resistance; MVP, major vault protein; NYU, New York University; Pgp, P-glycoprotein; VRAC, volume-regulated anion channel; WT, wild-type.

would at least allow me to take rational decisions without being overruled by nonscientific managers above me.

Cancer research in 1983 was also fundamentally different from the field in 1960, when I studied tumor mitochondria. At the time, we had little idea what was really wrong in a cancer cell, but in 1983 oncogenes and tumor suppressor genes had entered the field and these genes were interesting. They steered the cell cycle, signal transduction, early development, and the cell's decision to multiply. Suddenly, cancer research was propelled to the leading edge of basic biological research. This provided not only a golden opportunity for a cancer institute, but also a risk. Up till the discovery of oncogenes, Dutch universities had been content with leaving cancer research to the Cancer Institute, as it was not interesting anyhow. With the oncogenes came an influx of academic investigators, because the processes that they were studying were governed by the genes that were best accessible through cancer research. Competition intensified, not only in research, but also for the clinical part of the Cancer Institute, as treatment of disseminated cancer, leukemias, and lymphomas with those horribly toxic drugs became more successful and, hence, more respectable. It was obvious to me that our Institute had to become a center of excellence or keel over, tough assignment for a new director who wants to spend at least 50% of his time on his personal research.

My research had to be renewed to some extent as well. The focus was on trypanosome antigenic variation, but I did not want to drop that. I like trypanosomatids; they are biochemically fascinating organisms and a fertile ground for discovery [7]. A director of a cancer institute should also do some research on cancer, however, and so I started on MDR. At the time, the genes involved in MDR had not yet been identified and we had experience in cloning genes. Moreover, there were indications that DNA rearrangements were involved in cancer and in MDR in particular and I had experience with DNA rearrangements in trypanosomes [8–10]. At the time, nobody in The Netherlands Cancer Institute (NKI) was working on drug resistance (surprisingly) and I would not be competing with members of my own staff for Dutch grants. I got a grant and started with a single student on MDR.

Getting involved with MDR

I do not want to suggest that my entry into the MDR field was easy. Investigators in the field were not so generous to the newcomer. I had already done something useful in science, but in mitochondrial biogenesis and antigenic variation in African trypanosomes, fields that did not resonate much with most of the scientists working on drug resistance and drug development, as they were nearly all chemists. My requests for cell lines made resistant *in vitro* did not get me far initially. This changed when I attended a Gordon Conference on Chemotherapy of Experimental and Clinical Cancer. Tennis proved an important pastime in this Gordon Conference and the highlight of the meeting was the doubles match Yale against NYU, usually lost by NYU. As a former postdoc of NYU, I was able to play for my old institute and we won. After that, I became an accepted member of the drug resistance community, even though I was not a card-carrying chemist. I still remember an incident when one of the major discoveries of the times was presented: The main target of doxorubicin is the plasma membrane. This was proven by an ingenious experiment in which the doxorubicin was immobilized on beads and shown to kill cells anyhow, even though it could not enter the cell. When I meekly pointed out that genetic research had shown already that the main target of doxorubicin was Topo II, a nuclear enzyme, and that cells could also become resistant to dox by overproduction of Pgp, this was dismissed as irrelevant and mere genetics and molecular biology. Yes, chemists dominated the field.

Soon, this would change. Victor Ling, a card-carrying molecular biologist, had discovered Pgp [11], and several groups were already busy trying to isolate the genes involved. If I had known about that, I might have thought twice before entering the MDR field. I had 20 years of experience in molecular biology and gene isolation and I thought that I would be able to compete in this field of chemists with a single graduate student, isolating genes involved in MDR. Not a bad project, it was funded, but naive at best. Soon my enterprising student had managed to clone a set of five genes overexpressed in MDR CHO cell lines that I had obtained from Ling [12,13]. We started sequencing, but in the middle of the ABCB1 gene my poor student was overrun by 3 steamrollers: The Housman Lab in Boston with Philippe Gros as the driver [14]; the Roninson, Gottesman, and Pastan group at NIH [15]; and the collaborative groups of Victor Ling and Jack Riordan in Canada [16]. We stopped sequencing ABCB1, but continued with ABCB4, which was coamplified with the ABCB1 gene [17]. We later showed that the two genes are adjacent in the human genome, explaining their co-amplification [18]. The ABCB4 project would become one of the successes of our laboratory. The first knockout (KO) mouse made in ABC

transporters was the mouse homolog of the ABCB4 gene, Mdr2, which we showed—with the help of the group of Ronald Oude Elferink—to be a phosphatidylcholine transferase, essential for making bile [19]. This was a most unexpected discovery as the dogma of the time held that the phosphatidylcholine was passively extracted into bile by the bile salts. We searched in vain for the human inborn error due to a dysfunctional ABCB4 gene, which was later shown by a French group to cause the severe liver disease PFIC, type 3 [20].

We continued with the mouse Abcb1a and b KOs, which were also highly informative. I write 'we', but it was postdoc Alfred Schinkel in my laboratory who pushed these KOs. At the time, making KOs was still a major and risky undertaking and Alfred took that risk. We also profited, of course, from the excellent mouse house of the NKI and the development of a simplified procedure for making KOs by Te Riele and Berns [21] in the NKI.

The Abcb1a KO [22] mice entered with a splash, as often recounted [23]: When the animal caretakers sprayed the mice with ivermectin, all homozygous KOs died. This showed that Pgp in the blood–brain barrier (BBB) is essential to protect mice and us from nasty chemicals [22]. Of course, it had been shown before that there was Pgp in the BBB, but that it would protect every single capillary in the brain from nasty drugs had not really gone across. I was made aware of that when I presented the KO results at the European Blood-Brain Barrier Meeting in Amsterdam. This was before we had published anything, but my colleague Douwe Breimer, co-organizer of the Meeting, had pushed me at the last minute into the program. Before my talk, there was a long review by a physical chemist in which he presented detailed models why some large amphipathic molecules did not pass the capillary membrane. It was all explained with sophisticated chemistry: dipole moments, van der Waals forces, and the like. I then got onto the podium and explained that it was a pump. Pandemonium followed. Some members in the audience had spent part of their lives to formulate intricate chemical rules why compounds, such as taxanes or doxorubicin, would not go through the membrane and they were not pleased to see their ingenious explanations demolished by a biochemist, worse an MD, PhD. 'Did I check that every capillary in the brain contained Pgp?' No, I had not checked, but our WT mice were fully protected against ivermectin, and that could only happen if Pgp protected the entire blood–brain barrier. It was known in 1993 that Pgp was present in the BBB, but the full protective effect only became apparent in the KO mouse.

Our analysis of the function of Pgp in the gut had even more impact [24–27]. It was known that Pgp was present in the gut epithelium, but not how important it was in drug uptake. Our KO mouse became a major tool to study that in the pharma industry, after we had shown its importance in (partially) preventing uptake of taxanes [28] and other drugs. Our discovery that Pgp affected drug uptake from the gut eventually led to the development of a protocol for oral treatment with taxanes [29], which has now reached the clinic after several modifications [30].

Looking back, this was one of the more exciting periods of my scientific life. An endearing feature of KO mice is that you never know where they will lead you. For biochemists who like diversity, it is a way to visit very different areas of biochemistry. In 1992, at the time of the first two KOs, I was writing articles not only about the blood–brain barrier [26] and bile formation [19], but also about nucleotide chemistry, as we had finally established the structure of the new base that we had discovered in the DNA of trypanosomatids, base J [31], a busy time indeed, as I was also running the NKI.

Alfred Schinkel continued the study of the role of Pgp in drug pharmacokinetics with great success as an independent investigator. My group shifted to the ABCC (multidrug resistance-associated protein, MRP) family of ABC transporters. We tried to clone ABCC1 (MRP1), but Susan Cole and Roger Deeley got there first [32]. My fault, because I designed a too sophisticated and time-consuming cloning approach. In science, one should always take the high road in moral issues, but the low road usually suffices for experiments.

MRP1 spawned a rich MRP progeny, but before dealing with that a little more history. My first encounter with MRP1 was with Susan Cole in front of a poster at a meeting. She had isolated a highly drug-resistant MDR cell line and was unable to detect Pgp in it. This was the heyday of Pgp, and the investigators who passed by her poster had told her to look harder for Pgp. I found Susan's data convincing, however. It helped that I was already getting fed up with the omnipresent Pgp and open to the possibility that other pumps might exist. I knew about the work on the canalicular multispecific organic anion transporter (cMOAT) that had been studied by liver lovers (later unfortunately renamed MRP2; cMOAT was a better description of this pump than MRP2). Early vesicular transport experiments with erythrocyte membranes had shown that the transport of glutathione S-conjugates required ATP [33]. That smelled of an ABC transporter. Toshi Ishikawa [34] then pointed out in a

letter to TIBS that his experiments demonstrated that this GSH-conjugate pump could not be a product of the Mdr1 gene. This was followed by an insightful review, in which Ishikawa [35] emphasized that many drugs are conjugated to very hydrophilic ligands in our cells and that the hydrophilic conjugates formed would need organic anion transporters to get out of the cell.

With hindsight, MRP1 was not the first MRP to be cloned. In an off-shoot of my trypanosome project, we had been studying methotrexate and arsenite resistance in Leishmania [36–38] and found it to be associated with a plasmid [39]. Postdoc Marc Ouellette then did an audacious experiment: He isolated the plasmid and hybridized it with a mammalian Pgp cDNA probe. I had advised against this experiment for good reasons. Leishmania is very far from us in evolution, much further than yeast or even plasmodium. The odds were near zero that homology would be detected between the Pgp genes of Leishmania and mammals. Hence, Marc was delighted when he showed me the blot with a weak signal. The gene was sequenced and called Pgp-A [40]. Only when Susan Cole noted in her Science paper that Leishmania Pgp-A looked like a typical MRP [32] did we realize that Pgp-A was not an authentic Pgp. Leishmania Pgp-A was duly renamed MRP-A and, thanks to the persistence of Marc as an independent investigator in Canada, became fully characterized. It is actually a transporter of metal-glutathione-like conjugates, which it transports into the Leishmania vacuole, resulting in modest resistance [41]. Interestingly, MRP-A has been shown to cause resistance against antimony, used to treat Leishmaniasis in patients [42]. This may still be the only MRP that has been proven to regularly cause resistance in patients, but I hope that other MRPs may eventually follow, as MRPs are certainly protective in mice. For instance, Mrp1 does help in protecting mice against drug-induced damage [43,44].

After our failure to clone MRP1, we went on to look for other MRPs and soon there was an MRP family [45–47]. We contributed to the cloning and characterization of ABCC2-5 and made the corresponding KOs. I have often been ridiculed for investing time in the unimaginative experiment of making KO mice, double KOs, triple KOs, but looking back I still think that it was a good investment. The KO mice have yielded more useful information about MDR and drug pharmacokinetics than many more sophisticated approaches. The decision to invest in KO mice was also inspired by the institute I worked in, which had an excellent mouse house and a highly experienced and competent pharmacology group, always willing to do the necessary pharmacokinetic studies. The alternatives looked less attractive: I was skeptical of the mechanistic studies that occupied most of my colleagues, as I expected these soon to be overtaken by the detailed structures of transporter proteins being generated by structural biologists.

The work on MRPs in my laboratory received a boost when postdoc Koen van de Wetering started to use the KOs as a tool to find new endogenous substrates by metabolomics [48,49]. This worked like a charm. Let me just mention the two most recent highlights: Koen and postdoc Robert Jansen found a range of new substrates for ABCC5 [50], including a class of lactoyl-amino acids, not seen before in mammals [51]. The second highlight was the discovery of the function of ABCC6 by Robert and Koen. This project started with my hypothesis about the nature of the substrate of ABCC6 [52] that was soon proven to be completely wrong [53], after which Robert and Koen (without further interference from me) showed that ABCC6 mediates the excretion of ATP from the liver [54,55]. This discovery not only solved how the absence of ABCC6 causes the enigmatic inborn error of metabolism, Pseudoxanthoma elasticum (PXE), but also pointed the way to new therapies for this slowly developing, but eventually debilitating disease [56]. That was a rather satisfying end to my 30-year stint in the ABC transporter field.

In the end, I think our research on MDR led to some useful results in the elucidation of the biochemical basis of inborn errors and in improving our insight into the role of ABC transporters in drug pharmacokinetics. I have to admit, however, that my long-term aims to solve mechanisms of drug resistance and to develop predictive markers to guide the application of drugs in cancer chemotherapy have not been reached [57]. We still do not know what determines whether a tumor responds to taxanes and why it becomes resistant during treatment [58]. Notwithstanding the incredible armamentarium developed in molecular biology in the past 35 years, such elementary problems have still not been solved and not for lack of trying. It would therefore be fair to conclude that my MDR efforts have failed to reach their major goals. There were many useful spin-offs, but the main goals were not reached. This holds, of course, for the entire field: that pumps would fully explain drug resistance has proven an illusion, but not everybody subscribes my sour conclusion. See Ref. [59] and the other reviews in this volume for a more optimistic picture and a more balanced historic overview of the ABC transporter field. Enough about my mistakes and failures of the past and on to the present.

Mistakes to be avoided now

The opinions expressed in this section reflect my personal views and not those of any organization I may belong to, or of any of my expert colleagues (including reviewers of this paper).

1 Please, do not start your papers or grant applications with the claim that MDR caused by ABC transporters is important in human cancer. It is not. This unfortunate finding has made life hard for scientists who invested their efforts in Pgp, and it remains one of the most remarkable results of MDR research. Remarkable, because Pgp provides an excellent mechanism for resistance against the drugs that Pgp transports. This was already shown by early work with rodent cell lines, and it was confirmed by Rottenberg and coworkers [60] in a mouse model of breast cancer that resembles human breast cancer [61]. In fact, these tumors upregulate Pgp so readily that it is impossible to get other mechanisms of drug resistance against drugs transported by Pgp without inactivating Abcb1a and Abcb1b genes [62]. Why then is MDR in human tumors rare? Two factors probably contribute, multidrug treatment and low Pgp expression in most tumors. Multidrug treatment is the basis of cancer therapy in human patients, and these drug cocktails always contain drugs not transported by Pgp, such as platins, other alkylating agents, and antimetabolites.
The low expression of Pgp in most human tissues relative to rodent tissues should be another factor. Gene expression is known to fluctuate in mammalian cells, and if there is some Pgp produced, the cells with the highest levels may withstand the first attack by drug and be selected out. Repeated selection will then lead to transcriptional activation, as seen in the mouse mammary tumors. In contrast, the ABCB1 gene appears to be tightly shut down in most human tissues, no expression fluctuation, nothing to select from. Only a drastic DNA rearrangement, hooking the silent ABCB1 gene up to an active promoter, is able to activate expression [63]. Of course, there are exceptions of rigorously documented patients in whom Pgp plays a role in resistance [59], but unfortunately these are rare. Moreover, the extensive work on Pgp has alerted the pharma industry to the transporters that new drugs may encounter. Developing drugs that avoid being transported by Pgp or ABCG2 has become a priority. A good example is the introduction of the third-generation ALK inhibitor alectinib in the clinic. Patients who receive the older ALK inhibitors eventually develop drug resistance; a subset of these patients were shown to have high levels of Pgp in their tumors [64]. Alectinib, which is not a Pgp substrate, was more effective in these patients [65], also against brain metastases.

2 Please, do not start the history of ABC transporters in cancer with the discovery of Pgp, undoubtedly a landmark discovery, but not the start. MDR started with Kessel [66] and Biedler and Riehm [67] describing the phenomenon. Then came the outstanding work of Keld Dano. With elegant experiments, he showed that MDR was due to an energy-requiring process lowering intracellular drug concentration by export of the drug from the cell, presumably caused by a promiscuous drug transporter [68]. He actually wrote the word 'daunomycin-pump', not bad for a medical student, working in a Department of Internal Medicine. Unfortunately, Dano's brilliant work was long overlooked and was rarely quoted, also by me. I only found out about it in 1991. An obvious reason for this lack of appreciation is that Dano's work was not quoted in the paper of Juliano and Ling [11] describing the discovery of Pgp. Had Ling not missed Dano's paper, he would not have coined the misnomer permeability glycoprotein for Pgp. Juliano and Ling [11] write in their paper that it seemed unlikely that a single glycoprotein would be able to bind structurally unrelated different drugs. They therefore opted for the explanation that Pgp modulated the fluidity of the plasma membrane reducing the entry of drugs, hence permeability glycoprotein. How a tiny amount of membrane protein would be able to alter the fluidity of the entire membrane is not explained.

3 Please, do not forget about Philippe Gros, if you write about the early history of MDR. Philippe was the first to demonstrate that MDR can be caused by a single protein. He isolated the cDNA of the mouse Mdr1a gene, sequenced it, and then did the crucial transfection experiment to demonstrate that the overexpression of this cDNA caused MDR [14]. If I had to choose 3 landmark papers from the early MDR literature, the Nature letter with the experiments of Gros would be one of them, the other two being the Dano paper [68] and the Juliano and Ling [11] paper with the discovery of Pgp.

4 Please, PLEASE, do not ask your student to develop a new inhibitor of Pgp for clinical application. The ones we have are fine. Also do not write that we do not know whether the available inhibitors actually work in real tumors. Rottenberg and coworkers [60] tested that in the murine breast cancer model and doxorubicin resistance, caused by Pgp upregulation, is completely reversed by the third-generation Pgp inhibitor tariquidar.

5 Please, do not use extracted mRNA or total tissue protein to determine whether there is an ABC transporter in a tumor. Tumors are full of stroma, and stromal cells may contain the transporter. A good example is breast cancer. There are numerous reports about the presence of Pgp in breast tumors, based on total tissue extracts. The one paper in which the Pgp in individual cells was painstakingly analyzed by microscopy with MABs and adequate controls saw no Pgp whatsoever in the tumor cells. All Pgp was in stroma, especially the macrophages invading the tumor were rich in Pgp [69]. The best test would be to measure the drug levels directly in the tumor cells. That approach was never pushed, because of the difficulty of determining drug concentrations in individual cells of a heterogeneous tumor. Studies that do exist suggest that drug levels might vary as much as ten-fold.

6 Please, do not claim that a transporter is fulfilling an important function, because it gets upregulated. This is one of the most frequent mistakes in papers about drug resistance in cancer. Transporters are usually part of a network of proteins that are controlled by the same set of transcriptional activators. The selection may operate on any member of the network, if the network gets upregulated, not necessarily a transporter. A nice historic example is the upregulation of the lung resistance protein, later known as the major vault protein (MVP), in many tumors. The level of MVP correlated very well with drug resistance, and Scheper and coworkers [70] postulated that the MVP was a new protein involved in drug resistance. I was not convinced, as MVP did not look like it had any business with drugs and upregulation by itself means little. What is required to prove involvement in drug resistance is transfection experiments. These were finally done by a former postdoc of Scheper with unambiguously negative results [71].

If you want to know whether upregulation of a transporter is contributing to resistance in the tumor of your patient, DNA sequencing may help. If the upregulation is in cis, that is, only in one of the alleles encoding the gene and associated with a gene rearrangement, there are good reasons to assume that the upregulation was selected for during the growth of the tumor. This principle was first formulated by Tito Fojo *et al.* and used to demonstrate upregulation of ABCB1 in some human leukemia samples [63]. More recently, upregulation, associated with DNA rearrangement, was found in 8% of the tumor samples of high-grade serous ovarian cancer patients, who relapsed on chemotherapy including a taxane [72]. Even upregulation in cis is not unambiguous without additional evidence. A case in point is the adriamycin resistance-associated (ARA) gene found upregulated in doxorubicin-resistant cell lines. This turned out to be an authentic red herring. Marcel Kool showed in our laboratory that the ARA gene was a truncated (inactive) version of ABCC6 [73]. As we found the ABCC6 gene located next to the ABCC1 gene [73], the resistance attributed to ARA was caused by co-amplification of ABCC1. ABCC6 does not transport MDR drugs. Hence, even if upregulation occurs in cis, it is necessary to verify that resistance is not due to co-amplification of an adjacent gene.

7 Please, do not write that ABCB4 does not transport drugs and is only a phosphatidylcholine translocator involved in lipid transport. Kazu Ueda and coworkers [74] discovered that ABCB4 can transport some drugs and that finding was extended in my laboratory [75]. In fact, ABCB4 transports quite a few anticancer drugs and its substrate specificity is reminiscent of ABCB1, albeit that B4 is far less proficient as a drug transporter than B1 [75]. Since ABCB4 came late in ABC transporter evolution and is adjacent to ABCB1 in the genome, it may have arisen by gene duplication and the drug transport could be an echo of its origin. Whether this drug transport function plays any physiological role remains to be sorted out. ABCB4 has been linked to drug resistance in rare cases, but the evidence is not compelling in my opinion [76].

8 Please, do not write that ABCG2 is a major determinant of human MDR, because all evidence for that comes from *in vitro* experiments and there is no proof yet that it plays any role in MDR in human tumors, although I should add that the clinical issue has still not been rigorously scrutinized. There is no doubt, however, that ABCG2 plays a major role in protecting our body against toxins/drugs, often in close collaboration with Pgp, for instance in the BBB [77,78]. It is a major pharmacokinetic player [79], but not an MDR player.

9 Please, do not claim that the members of the ABCC family are important determinants of MDR in human tumors. There is a large body of evidence that these MRPs can cause resistance to various drugs in cell line in the test tubes and even in mice, and my laboratory has amply contributed papers to the topic [45,80]. Proof that these transporters contribute to drug resistance in human tumors is sorely lacking, however. MRP8 could be an exception, as it is a really good taxane transporter [81] and it has not been as intensely studied as some of the other members of the ABCC family. It causes taxane resistance in tumors in mice

[81], but I have not seen conclusive evidence that human tumors make use of it.

10 Please, do not write that Pgp is a dual function protein, pump and chloride channel. Although this mistake has virtually disappeared from the literature, it is worth recounting. In 1992, the group of Chris Higgins published a spectacular finding: Pgp could also function as a chloride channel. The discovery was published in Nature [82] and Cell [83], and these papers soon were among the ones most quoted in the field, not surprisingly, as Higgins was known as one of the undisputed leaders in the field. He even coined the name ABC transporters. Higgins had used one of our cell lines and we repeated the experiments, helped by Kees Jalink, a postdoc from another group in the Cancer Institute, who knew how to do the electrophysiology required for looking at channels. The results were completely negative. I know from experience that experiments can be hard to repeat. There is even a repeatability crisis in science [84], but this is usually not caused by sloppy experimentation, or occasional fraud. Biochemical experiments are intrinsically messy; animals are never identical, and procedures may be difficult to exactly duplicate; and we often do not know all parameters that are critical. It is therefore not useful to sulk if you cannot repeat an experiment, but find out why the experiment does not reproduce. This is what we did. I contacted Chris Higgins and I went with Jalink to Cambridge to compare experiments. We spent a whole day with postdocs combing through experimental details, and in the course of the day, it became clear that experimental results in Cambridge varied. Rather, often the same negative result was obtained as we had seen in Amsterdam, but this was booked as a failed experiment. We went home confident that the channel function of Pgp would meet its Brexit and that is what happened [85]. Science is self-correcting, politics unfortunately not, at least in the case of the real Brexit. The channel function became firmly established in the medical community, however. Whenever I would lecture to a medical audience, I would get questions about it. Why did I not talk about this interesting channel function of Pgp? I felt guilty toward Jalink, but he did not suffer. He became staff member in the Cancer Institute, professor, and he is now Head of the Division of the Cancer Institute in which I work, that is, my boss.

All these stories illustrate what counts in science: evidence, not authority. Always keep in mind that complex problems have simple, appealing, INCORRECT solutions, to paraphrase Mencken.

There are more mistakes than 10 and many more beyond the MDR field. Let me just add one from the platin field. Do not write that cisplatin resistance can be caused by pumps extruding platins from cells. The membrane glycoprotein overexpressed in cisplatin-resistant cells described by Ling's Lab [86] should not be quoted, as it has not survived more detailed scrutiny, Ling has told me. MRP2 has been linked to cisplatin extrusion from cells, but it has never been shown to be a contributor to resistance in human tumors. The same holds for some non-ABC transporters [87]. A possible role in resistance might be played by VRAC, an organic anion channel that can promote entry of platins into cells [88]. There is suggestive evidence that the loss of this channel might contribute to cisplatin resistance [88], but this needs confirmation with more clinical samples.

How about the future?

I have no idea, and I have learnt to avoid speculating. Predictions about the future of biology are either trivial extrapolations of existing trends, or just wrong. Let me remind you of the famous paper by molecular biologist Gunther Stent: 'That was the molecular biology that was' [89]. In 1968, Science published his prediction that molecular biology was nearly done [89]. We knew how nature basically worked, only some mopping up was required. Indeed, some pretty famous molecular biologists at the time switched to neurobiology and came to regret it. Neurobiology proved more intractable than they had anticipated and molecular biology thrived. Since 1970, we have seen a spectacular series of unexpected discoveries that have changed our views of biology.

It is not hard to see why profound predictions in biology are always wrong. We live in a Darwinian world shaped by mutation selection, and mutation is random. Nature cannot make big jumps and can only make new organisms by tinkering, as Jacob [90] has called it. The direction that mutations and tinkering happen to take is intrinsically unpredictable.

How about the trends? I can only offer my extrapolations of current trends. The last ABC transporter meeting in early March 2020 in Innsbruck was dominated by structure of ABC transporters. That trend may still accelerate with cryo-EM, which has simplified the analysis of complex structures, but it will not last forever. In the end structure is only really interesting in the context of function and the underlying biology. I think other trends will soon prevail: the analysis of the role of ABC transporters (variations) in drug pharmacokinetics and in disease, inborn errors,

personalized medicine and even cancer. Yes, cancer, but not in the naive fashion in which we started. There will come a time that cancer chemotherapy will be strictly based on tumor properties, DNA, RNA protein analyses, and precision medicine will prevail. Then, the inhibitors of ABC transporters may make a modest comeback as part of a sophisticated highly targeted therapy cocktail.

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References

1 Gottesman MM and Pastan I (1989) Clinical trials of agents that reverse multidrug-resistance. *J Clin Oncol* **7**, 409–411.
2 Borst P (1962) The aerobic oxidation of reduced diphosphopyridine nucleotide formed by glycolysis in Ehrlich ascites tumour cells. *Biochem Biophys Acta* **57**, 270–282.
3 Warburg O (1956) On the origin of cancer cells. *Science* **123**, 309–314.
4 Borst P (1963) Hydrogen transport and transport metabolites. In Funktionelle und Morphologische Organisation der Zelle (Karlson P, ed.), pp. 137–162. Springer, Berlin, Heidelberg.
5 Borst P (2006) From cancer cells to trypanosomes and back again. *Cell Mol Life Sci* **63**, 745–754.
6 Borst P (2006) How I became a biochemist. *IUBMB Life* **58**, 177–182.
7 Borst P (2016) Maxi-circles, glycosomes, gene transposition, expression sites, transsplicing, transferrin receptors and base. *J Mol Biochem Parasitol* **205**, 39–52.
8 Borst P and Cross GA (1982) Molecular basis for trypanosome antigenic variation. *Cell* **29**, 291–303.
9 Borst P and Greaves DR (1987) Programmed gene rearrangements altering gene expression. *Science* **235**, 658–667.
10 Hoeijmakers JH, Frasch AC, Bernards A, Borst P and Cross GA (1980) Novel expression-linked copies of the genes for variant surface antigens in trypanosomes. *Nature* **284**, 78–80.
11 Juliano RL and Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* **455**, 152–162.
12 de Bruijn MH, Van der Bliek AM, Biedler JL and Borst P (1986) Differential amplification and disproportionate expression of five genes in three multidrug-resistant Chinese hamster lung cell lines. *Mol Cell Biol* **6**, 4717–4722.
13 Van der Bliek AM, Van der Velde-Koerts T, Ling V and Borst P (1986) Overexpression and amplification of five genes in a multidrug-resistant Chinese hamster ovary cell line. *Mol Cell Biol* **6**, 1671–1678.
14 Gros P, Croop J, Roninson I, Varshavsky A and Housman DE (1986) Isolation and characterization of DNA sequences amplified in multidrug-resistant hamster cells. *Proc Natl Acad Sci USA* **83**, 337–341.
15 Roninson IB, Chin JE, Choi KG, Gros P, Housman DE, Fojo A, Shen DW, Gottesman MM and Pastan I (1986) Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc Natl Acad Sci USA* **83**, 4538–4542.
16 Riordan JR, Deuchars K, Kartner N, Alon N, Trent J and Ling V (1985) Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature* **316**, 817–819.
17 Van der Bliek AM, Baas F, Houte T, de Lange T, Kooiman PM, Van der Velde-Koerts T and Borst P (1987) The human mdr3 gene encodes a novel P-glycoprotein homologue and gives rise to alternatively spliced mRNAs in liver. *EMBO J* **6**, 3325–3331.
18 Lincke CR, Smit JJ, van der Velde-Koerts T and Borst P (1991) Structure of the human MDR3 gene and physical mapping of the human MDR locus. *J Biol Chem* **266**, 5303–5310.
19 Smit JJM, Schinkel AH, Elferink RPJO, Groen AK, Wagenaar E, van Deemter L, Mol C, Ottenhoff R, van der Lugt NMT, van Roon MA *et al.* (1993) Homozygous disruption of the murine MDR2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* **75**, 451–462.
20 Deleuze JF, Jacquemin E, Dubuisson C, Cresteil D, Dumont M, Erlinger S, Bernard O and Hadchouel M (1996) Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. *Hepatology* **23**, 904–908.
21 te Riele H, Maandag ER and Berns A (1992) Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. *Proc Natl Acad Sci USA* **89**, 5128–5132.
22 Schinkel AH, Smit JJM, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol C, van der Valk MA, Robanus-Maandag EC, te Riele HPJ *et al.* (1994) Disruption of the mouse mdr1a P-glycoprotein gene

leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**, 491–502.
23 Borst P and Schinkel AH (2013) P-glycoprotein ABCB1: a major player in drug handling by mammals. *J Clin Invest* **123**, 4131–4133.
24 Borst P and Schinkel AH (1997) Genetic dissection of the function of mammalian P-glycoproteins. *Trends Genet* **13**, 217–222.
25 Mayer U, Wagenaar E, Beijnen JH, Smit JW, Meijer DK, van Asperen J, Borst P and Schinkel AH (1996) Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr 1a P-glycoprotein. *Br J Pharmacol* **119**, 1038–1044.
26 Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, van der Valk MA, Voordouw AC, Spits H, van Tellingen O *et al*. (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci USA* **94**, 4028–4033.
27 Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH and van Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci USA* **94**, 2031–2035.
28 van Asperen J, van Tellingen O, Sparreboom A, Schinkel AH, Borst P, Nooijen WJ and Beijnen JH (1997) Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. *Br J Cancer* **76**, 1181–1183.
29 Meerum Terwogt JM, Malingré MM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Koopman FJ, van Tellingen O, Swart M and Schellens JH (1999) Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res* **5**, 3379–3384.
30 Yu H, Janssen JM, Sawicki E, van Hasselt JGC, de Weger VA, Nuijen B, Schellens JHM, Beijnen JH and Huitema ADR (2020) A population pharmacokinetic model of oral docetaxel coadministered with ritonavir to support early clinical development. *J Clin Pharmacol* **60**, 340–350.
31 Gommers-Ampt JH, Van Leeuwen F, de Beer AL, Vliegenthart JF, Dizdaroglu M, Kowalak JA, Crain PF and Borst P (1993) beta-D-glucosyl-hydroxymethyluracil: a novel modified base present in the DNA of the parasitic protozoan *T. brucei*. *Cell* **75**, 1129–1136.
32 Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM and Deeley RG (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**, 1650–1654.
33 Kondo T, Murao M and Taniguchi N (1982) Glutathione S-conjugate transport using inside-out vesicles from human erythrocytes. *Eur J Biochem* **125**, 551–554.
34 Ishikawa T (1990) Is the glutathione S-conjugate carrier an mdr1 gene product? *Trends Biochem Sci* **15**, 219–220.
35 Ishikawa T (1992) The ATP-dependent glutathione S-conjugate export pump. *Trends Biochem Sci* **17**, 463–468.
36 Borst P and Ouellette M (1995) New mechanisms of drug resistance in parasitic protozoa. *Annu Rev Microbiol* **49**, 427–460.
37 Ouellette M and Borst P (1991) Drug resistance and P-glycoprotein gene amplification in the protozoan parasite *Leishmania*. *Res Microbiol* **142**, 737–746.
38 Ouellette M, Hettema E, Wüst D, Fase-Fowler F and Borst P (1991) Direct and inverted DNA repeats associated with P-glycoprotein gene amplification in drug resistant *Leishmania*. *EMBO J* **10**, 1009–1016.
39 White TC, Fase-Fowler F, van Luenen H, Calafat J and Borst P (1988) The H circles of *Leishmania tarentolae* are a unique amplifiable system of oligomeric DNAs associated with drug resistance. *J Biol Chem* **263**, 16977–16983.
40 Ouellette M, Fase-Fowler F and Borst P (1990) The amplified H circle of methotrexate-resistant Leishmania tarentolae contains a novel P-glycoprotein gene. *EMBO J* **9**, 1027–1033.
41 Leprohon P, Légaré D and Ouellette M (2009) Intracellular localization of the ABCC proteins of *Leishmania* and their role in resistance to antimonials. *Antimicrob Agents Chemother* **53**, 2646–2649.
42 do Monte-Neto RL, Coelho AC, Raymond F, Légaré D, Corbeil J, Melo MN, Frézard F and Ouellette M (2011) Gene expression profiling and molecular characterization of antimony resistance in *Leishmania amazonensis*. *PLoS Negl Trop Dis* **5**, e1167.
43 Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P and Borst P (1997) Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nat Med* **3**, 1275–1279.
44 Wijnholds J, Scheffer GL, van der Valk M, van der Valk P, Beijnen JH, Scheper RJ and Borst P (1998) Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the testicular tubules against drug-induced damage. *J Exp Med* **188**, 797–808.
45 Borst P, Evers R, Kool M and Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* **92**, 1295–1302.
46 Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F and Borst P (1997) Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-

associated protein gene (MRP1), in human cancer cell lines. *Cancer Res* **57**, 3537–3547.
47 Sharom FJ, Slot AJ, Molinski SV and Cole SPC (2011) Mammalian multidrug-resistance proteins (MRPs). *Essays Biochem* **50**, 179–207.
48 Krumpochova P, Sapthu S, Brouwers JF, de Haas M, de Vos R, Borst P and van de Wetering K (2012) Transportomics: screening for substrates of ABC transporters in body fluids using vesicular transport assays. *FASEB J* **26**, 738–747.
49 van de Wetering K, Feddema W, Helms JB, Brouwers JF and Borst P (2009) Targeted metabolomics identifies glucuronides of dietary phytoestrogens as a major class of MRP3 substrates in vivo. *Gastroenterology* **137**, 1725–1735.
50 Jansen RS, Mahakena S, de Haas M, Borst P and van de Wetering K (2015) ATP-binding cassette subfamily C member 5 (ABCC5) functions as an efflux transporter of glutamate conjugates and analogs. *J Biol Chem* **290**, 30429–30440.
51 Jansen RS, Addie R, Merkx R, Fish A, Mahakena S, Bleijerveld OB, Altelaar M, IJlst L, Wanders RJ, Borst P *et al*. (2015) N-lactoyl-amino acids are ubiquitous metabolites that originate from CNDP2-mediated reverse proteolysis of lactate and amino acids. *Proc Natl Acad Sci USA* **112**, 6601–6606.
52 Borst P, van de Wetering K and Schlingemann R (2008) Does the absence of ABCC6 (multidrug resistance protein 6) in patients with Pseudoxanthoma elasticum prevent the liver from providing sufficient vitamin K to the periphery? *Cell Cycle* **7**, 1575–1579.
53 Fülöp K, Jiang Q, Wetering KVD, Pomozi V, Szabó PT, Arányi T, Sarkadi B, Borst P, Uitto J and Váradi A (2011) ABCC6 does not transport vitamin K3-glutathione conjugate from the liver: relevance to pathomechanisms of Pseudoxanthoma elasticum. *Biochem Biophys Res Commun* **415**, 468–471.
54 Jansen C, Wang H, Kooistra AJ, de Graaf C, Orrling KM, Tenor H, Seebeck T, Bailey D, de Esch IJP, Ke H *et al*. (2013) Discovery of novel *Trypanosoma brucei* phosphodiesterase B1 inhibitors by virtual screening against the unliganded TbrPDEB1 crystal structure. *J Med Chem* **56**, 2087–2096.
55 Jansen RS, Duijst S, Mahakena S, Sommer D, Szeri F, Váradi A, Plomp A, Bergen AA, Oude Elferink RPJ, Borst P *et al*. (2014) ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report. *Arterioscler Thromb Vasc Biol* **34**, 1985–1989.
56 Borst P, Váradi A and van de Wetering K (2019) PXE, a mysterious inborn error clarified. *Trends Biochem Sci* **44**, 125–140.
57 Borst P (2012) Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? *Open Biol* **2**, 120066.
58 Rottenberg S, Vollebergh MA, de Hoon B, de Ronde J, Schouten PC, Kersbergen A, Zander SAL, Pajic M, Jaspers JE, Jonkers M *et al*. (2012) Impact of intertumoral heterogeneity on predicting chemotherapy response of BRCA1-deficient mammary tumors. *Cancer Res* **72**, 2350–2361.
59 Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE and Gottesman MM (2018) Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* **18**, 452–464.
60 Pajic M, Iyer JK, Kersbergen A, van der Burg E, Nygren AOH, Jonkers J, Borst P and Rottenberg S (2009) Moderate increase in Mdr1a/1b expression causes in vivo resistance to doxorubicin in a mouse model for hereditary breast cancer. *Cancer Res* **69**, 6396–6404.
61 Rottenberg S, Nygren AOH, Pajic M, van Leeuwen FWB, van der Heijden I, van de Wetering K, Liu X, de Visser KE, Gilhuijs KG, van Tellingen O *et al*. (2007) Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer. *Proc Natl Acad Sci USA* **104**, 12117–12122.
62 Rottenberg S and Borst P (2012) Drug resistance in the mouse cancer clinic. *Drug Resist Updates* **15**, 81–89.
63 Huff LM, Lee J-S, Robey RW and Fojo T (2006) Characterization of gene rearrangements leading to activation of MDR-1. *J Biol Chem* **281**, 36501–36509.
64 Katayama R, Sakashita T, Yanagitani N, Ninomiya H, Horiike A, Friboulet L, Gainor JF, Motoi N, Dobashi A, Sakata S *et al*. (2016) P-glycoprotein mediates ceritinib resistance in anaplastic lymphoma kinase-rearranged non-small cell lung cancer. *EBioMedicine* **3**, 54–66.
65 Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, Kim D-W, Ou S-HI, Pérol M, Dziadziuszko R, Rosell R *et al*. (2017) Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med* **377**, 829–838.
66 Kessel D (1986) Circumvention of resistance to anthracyclines by calcium antagonists and other membrane-perturbing agents. *Cancer Surv* **5**, 109–127.
67 Biedler JL and Riehm H (1970) Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. *Cancer Res* **30**, 1174–1184.
68 Dano K (1973) Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim Biophys Acta* **323**, 466–483.
69 Faneyte IF, Kristel PM and van de Vijver MJ (2001) Determining MDR1/P-glycoprotein expression in breast cancer. *Int J Cancer* **93**, 114–122.

70 Scheffer GL, Wijngaard PL, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, Meijer CJ, Clevers HC and Scheper RJ (1995) The drug resistance-related protein LRP is the human major vault protein. *Nat Med* **1**, 578–582.
71 van Zon A, Mossink MH, Schoester M, Scheper RJ, Sonneveld P and Wiemer EAC (2004) Efflux kinetics and intracellular distribution of daunorubicin are not affected by major vault protein/lung resistance-related protein (vault) expression. *Cancer Res* **64**, 4887–4892.
72 Patch A-M, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, Nones K, Cowin P, Alsop K, Bailey PJ *et al*. (2015) Whole-genome characterization of chemoresistant ovarian cancer. *Nature* **521**, 489–494.
73 Kool M, van der Linden M, de Haas M, Baas F and Borst P (1999) Expression of human MRP6, a homologue of the multidrug resistance protein gene MRP1, in tissues and cancer cells. *Cancer Res* **59**, 175–182.
74 Kino K, Taguchi Y, Yamada K, Komano T and Ueda K (1996) Aureobasidin A, an antifungal cyclic depsipeptide antibiotic, is a substrate for both human MDR1 and MDR2/P-glycoproteins. *FEBS Lett* **399**, 29–32.
75 Smith AJ, van Helvoort A, van Meer G, Szabo K, Welker E, Szakacs G, Varadi A, Sarkadi B and Borst P (2000) MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. *J Biol Chem* **275**, 23530–23539.
76 Borst P, Van Meer G and Oude Elferink R (2003) Lipid transport by ABC-transporters. In ABC Proteins (Holland IB, Cole SPC, Kuchler K and Higgins CF, eds), pp. 461–478. Academic Press, London.
77 van Hoppe S, Jamalpoor A, Rood JJM, Wagenaar E, Sparidans RW, Beijnen JH and Schinkel AH (2019) Brain accumulation of osimertinib and its active metabolite AZ5104 is restricted by ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein). *Pharmacol Res* **146**, 104297.
78 Vlaming MLH, Lagas JS and Schinkel AH (2009) Physiological and pharmacological roles of ABCG2 (BCRP): recent findings in Abcg2 knockout mice. *Adv Drug Deliv Rev* **61**, 14–25.
79 Sarkadi B, Homolya L, Szakács G and Váradi A (2006) Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system. *Physiol Rev* **86**, 1179–1236.
80 Borst P and Elferink RO (2002) Mammalian ABC transporters in health and disease. *Annu Rev Biochem* **71**, 537–592.
81 Domanitskaya N, Wangari-Talbot J, Jacobs J, Peiffer E, Mahdaviyeh Y, Paulose C, Malofeeva E, Foster K, Cai KQ, Zhou Y *et al*. (2014) Abcc10 status affects mammary tumour growth, metastasis, and docetaxel treatment response. *Br J Cancer* **111**, 696–707.
82 Valverde MA, Díaz M, Sepúlveda FV, Gill DR, Hyde SC and Higgins CF (1992) Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature* **355**, 830–833.
83 Gill DR, Hyde SC, Higgins CF, Valverde MA, Mintenig GM and Sepúlveda FV (1992) Separation of drug transport and chloride channel functions of the human multidrug resistance P-glycoprotein. *Cell* **71**, 23–32.
84 Harris R (2017) Rigor Mortis: How Sloppy Science Creates Worthless Cures, Crushes Hope, and Wastes Billions, 1st edn. Basic Books, New York, NY.
85 Valverde MA, Bond TD, Hardy SP, Taylor JC, Higgins CF, Altamirano J and Alvarez-Leefmans FJ (1996) The multidrug resistance P-glycoprotein modulates cell regulatory volume decrease. *EMBO J* **15**, 4460–4468.
86 Kawai K, Kamatani N, Georges E and Ling V (1990) Identification of a membrane glycoprotein overexpressed in murine lymphoma sublines resistant to cis-diamminedichloroplatinum(II). *J Biol Chem* **265**, 13137–13142.
87 Borst P, Rottenberg S and Jonkers J (2008) How do real tumors become resistant to cisplatin? *Cell Cycle* **7**, 1353–1359.
88 Planells-Cases R, Lutter D, Guyader C, Gerhards NM, Ullrich F, Elger DA, Kucukosmanoglu A, Xu G, Voss FK, Reincke SM *et al*. (2015) Subunit composition of VRAC channels determines substrate specificity and cellular resistance to Pt-based anti-cancer drugs. *EMBO J* **34**, 2993–3008.
89 Stent GS (1968) That was the molecular biology that was. *Science* **160**, 390–395.
90 Jacob F (1977) Evolution and tinkering. *Science* **196**, 1161–1166.