Editorial

There must be another way—disulfiram and cancer treatment: editorial on "Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4"

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The cost of new anticancer therapies nowadays can only be described as astronomical. While in third world countries, even older drugs for cancer therapies are unaffordable, in countries with more developed economies prices of new drugs are prohibitive and in the western world, especially in the US, cancer has been found to be a leading cause for personal bankruptcy. Take for example the cost of Darzalex (daratumumab) for the treatment of multiple myeloma. In the US, 100 mg of Darzalex can be bought for around $3,000. When one considers that the recommended dose is 16 mg/kg per week for the initial 8 weeks of treatment, the cost of this first round of treatment for an average man of around 80–90 kg could easily reach $30,000.

One way to overcome the current astronomic drug prices is to try and repurpose a known drug for which there is ample safety information and which is not under patent. A candidate for such a repurposing effort has been for more than a decade the drug disulfiram (DSF) (1). In an article recently published in *Nature* (2), there are good indications that DSF with copper could be active in multiple myeloma resistant to bortezomib, a similar patient population as those to whom Darzalex is to be offered.

Skrott and colleagues' *Nature* article has rekindled interest in the use of the well-established alcohol abuse drug DSF for cancer treatment. In the first part of the paper, the authors used an extensive Danish data set of DSF users to obtain an epidemiological assessment of the efficacy of DSF as an anticancer drug. The second part describes a study aimed at revealing the molecular basis for the activity of DSF as an anticancer drug.

The Danish registry contains data regarding both cancer and the use of DSF as a treatment for alcohol abuse, from the general population, previous users of DSF and current users. It allowed access to data from thousands of patients and is unique in this respect. The use of this important source enabled the epidemiological study as to a possible role of DSF. The study led the authors to conclude that patients who continued with DSF therapy had a lower risk of death from cancer compared to those who stopped using the drug when diagnosed with cancer.

The table that is shown in the article combines data for all types of tumors. One of the shortcomings of this approach is that it includes data regarding tumors for which alcohol may have a cancer-inducing effect and for which alcohol does not have such an effect. This is important since the table has data from three groups. The largest was that of the general population with data on 236,950 patients, a huge number; the next data on 3,038 previous users of DSF and the third on 1,177 patients who were still being treated with DSF. When cancer-specific mortality was measured relative to that of patients who stopped using DSF, it was significantly lower in patients who continued using DSF (0.66), but also among non-users (0.68). Among patients with non-localized disease, relative mortality was 0.80 for the general population and 0.71 for those continuing to be treated with DSF. These studies are retrospective. It can be assumed on the basis of multiple studies that there is higher cancer mortality in alcoholics (who were the original users of DSF) relative to the general

population. Therefore there are two possible explanations for the results: one that DSF lowers the risk of cancer (as suggested in the article) or that current users of DSF have a lower alcohol consumption than those who stopped using the drug and that abstaining from alcohol is the also a reason for the success of DSF in lowering cancer death. The lower cancer mortality in patients with non-localized tumors who were still being treated with DSF compared to those with non-localized tumors in the general population does seem to imply that DSF has some independent anti-cancer activity. While this part of the article is really a separate study from the rest of the article, it does provide the impetus for the second part, which focuses on the mechanism of the anti-cancer effect of DSF.

The article goes on to describe *in vitro* studies. They initially compared the activity of DSF to that of DSF combined with copper, based on previous studies demonstrating increased activity of DSF when combined with copper. Indeed, in their mouse model study utilizing mice with implanted MDA-MB-231 cells, the addition of copper significantly increased the activity of DSF in this tumor model. The authors then made another assumption for the rest of their studies that a metabolite of DSF, DTT (diethyldithiocarbamate), is responsible for most of the anti-cancer activity of DSF. This metabolite can avidly bind copper. While this is a logical assumption, especially considering the anti-cancer activity of DTT, it is not conclusively supported by the literature and therefore other mechanisms of DSF activity may still be possible. The activity of the complex of DTT with copper (termed CuET in the article) was then demonstrated *in vitro* in several cell lines and in animal models. It was substantially more active than that of DSF alone. Interestingly, in most cell lines it did not cause increased apoptosis. The next step was to analyze the effect of the CuET complex on protein degradation. This step may have been influenced by the relatively weak effect on apoptosis. The initial phenotypic effect seemed similar to that of the effect of proteasome degrading inhibitors. However, through a series of experiments the effect of CuET was revealed to be distinct from that of proteasome inhibitors due to inhibition of a specific type of protein degradation pathway, termed as P97-dependent protein degradation. A complex known as p97 segregase separates polyubiquitin proteins from cellular structures such as the endoplasmic reticulum. While the CuET complex inhibited the p97-dependent protein degradation, it did not bind to p97 itself. However, the p97 complex contains other proteins, including one known as NPL4. Overexpression of GFP-NPL4 reduced the effect of CuET substantially. This protein contains two zinc fingers, structural elements that are able to bind metals. Indeed, mutations in a zinc finger domain prevented the binding of CuET to NPL4. Overexpression of these mutants was toxic to the cells, in contrast to the effect of overexpression of wild type NPL4, which prevented CuET-induced cell death. The authors therefore revealed a major target for DSF anti-cancer activity, inhibition of P97 segregase-dependent protein degradation. This is also a new target for anti-cancer therapeutics. Interestingly, in the extended data section the authors demonstrate that the CuET complex is active against multiple myeloma cell lines that are resistant to proteasome inhibitors. It is thus tempting to propose that DSF (perhaps together with copper) may turn out to be an effective drug for resistant multiple myeloma patients, hopefully offering a cheaper alternative to expensive drugs such as daratumumab. Importantly, the authors also describe the measurements of the active metabolite with copper in tumors, a finding which could pave the way for further bioassays for the clinical development of the drug.

In the preface of the article the authors list some of the possible reasons for the failure to clinically develop DSF as a cheap, well-tolerated anti-cancer drug. These include a lack of knowledge regarding the toxicity of DSF metabolites, the way to measure them and of course the specific mechanism of activity of DSF.

There have been several small phase 2 trials incorporating DSF into clinical trials (3,4). We are the only group to publish a randomized phase 2 trial of DSF in cancer therapy (4). Our perspective of the development of DSF as a therapeutic agent seems to be significantly different to that of Skrott and colleagues. When we began our study, there were already studies described in the literature demonstrating some effects of DSF on cancer. Most notable for us at the time were articles published by a group at the Weizmann Institute that demonstrated an effect of DSF on angiogenesis (5). These studies were the basis for our decision to use a lower dose of DSF than the maximum tolerated dose. Our trial was possible due to the patent of the Weizmann Institute on the anti-cancer effects of DSF. In spite of the mechanistic basis for the use of DSF, there were no further developments due most probably to patent issues. We demonstrated the superiority of combining DSF with chemotherapy over chemotherapy alone for the treatment of patients with metastatic lung cancer. The difference was statistically significant (4). There were two long-term survivors in this phase 2 trial, which was an unexpected result in this kind of

disease, but two patients is indeed a very small number that could certainly be a chance result.

Over the last decade, there has been a number of articles published relating to possible effects of DSF on cancer cells. One of the important suggestions is that DSF causes the accumulation of several chemotherapeutics in cancer cells and that DSF is more effective when used in combination with several chemotherapies (6). Another important mechanism of action, which seems very attractive to those trying to use DSF in clinical trials, is the possible effects of DSF on cancer stem cells due to its well-known activity as an inhibitor of aldehyde dehydrogenase (7-9). This activity is especially appealing considering the proposed importance of cancer stem cells (or as others prefer, "tumor initiating cells") in the resistance to known anti-cancer agents.

Although our article was published several years ago, and previous data regarding DSF was published decades ago, the drug has not been developed as an anti-cancer drug. It seems to us that this is mainly due to the general problem of repurposing a drug for cancer therapy-the huge price of clinical trials. Perhaps the only example of a successful repurposing of a drug for cancer therapy is that of thalidomide. Indications that this drug, which had such terrible teratogenic effects, had anti-cancer properties were available years before it was repurposed as an anti-myeloma drug. Indeed, it could be bought very cheaply for non-approved indications, such as mucositis, for years. However, its successful repurposing was achieved by a pharmaceutical company who managed to patent it again, and then the drug was sold for thousands of dollars a month as an anti-myeloma agent (10), until it was succeeded by a derivative, another very expensive drug. Interestingly the exact mechanisms of its anti-cancer activity are not very clear even today. Thus, repurposing did not lead at all to reduce prices and was dependent upon the establishment of a renewed patent for the drug. In the past there have been collaborative efforts for clinical trials without the help of commercial companies. A notable example of such a trial is the adjuvant quasar trial, which used 5-FU as adjuvant chemotherapy in colon cancer (11) and was based on an idea for a trial that would be quick and simple. However, most clinical trials are now run by pharmaceutical companies and are far from simple. In fact, they are becoming more and more complex in their demands from the treating physicians. Without the prospects of a patented drug by a commercial company, it is nowadays exceedingly difficult to perform a meaningful phase 3 trial. Any such a drug will again be incredibly expensive in order to recover the developmental costs before a similar derivative will replace it. We have tried in vain to obtain public funds for a larger phase 3 study of DSF. Just obtaining enough patients for such studies is very difficult nowadays, when immunological combinations are in advanced clinical trials that pay substantial funds to the principal investigators of such trials.

The article by Skrott and colleagues is important in this regard not only because it proposes a new mechanism of action, but also because it again throws light on the possible importance of DSF as an anti-cancer drug. It seems that further development of DSF as an affordable drug requires the recruitment of public money. This might be achieved in non-western countries where the drug companies are not so dominant and the important immunotherapies do not attract all the attention, such as Thailand or China where there might still be a great interest in a cheap drug for use as an anti-cancer agent. There must be another way and that probably involves collaborations with countries with lower health care budgets.

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Footnote

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