Understanding off-target effects of cancer drugs could lead to new treatments

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These days, in the era of personalized medicine, whenever researchers discover a new cancer drug they are expected to show exactly how it works and to prove that it is exerting its therapeutic effect by hitting a

particular molecular target.

That detailed scientific understanding of a drug's molecular mechanism of action is absolutely crucial to its safe and effective use, including the selection of patients for treatment by use of biomarkers—or its combination with other treatments.

The key steps that researchers need to take in the application of biomarkers are set out in the Pharmacological Audit Trail, a gold-standard framework to guide the creation of new treatments, which was developed here at The Institute of Cancer Research, London.

But although we usually have a good understanding of how modern treatments exert their primary effects, that doesn't mean that more detailed scrutiny of a drug's molecular pharmacology can't throw up a few surprises.

A study just published in *Scientific Reports* and co-led at the ICR by Dr. Albert Antolin, Professor Bissan Al-Lazikani and myself, is a nice case in point.

Our research looked at four drugs that belong to an important family of treatments called PARP inhibitors. These are drugs that block the function of proteins known as PARPs (full name poly-ADP ribose polymerases) which carry out a key process involved in repairing DNA and so exploit deficiencies in the BRCA and other DNA damage response proteins in cancer cells—an effect known as synthetic lethality.

PARP inhibitors are among the most innovative and exciting drugs in cancer treatment today. With major input from research here at the ICR, they became the first cancer drugs targeted against inherited genetic faults, when they were approved for women with ovarian cancer who have germline BRCA mutations. Since then, PARP inhibitors have also

been licensed for women with breast cancer who have inherited BRCA mutations and are also showing benefits for some men with prostate cancer whose cancers show mutations in BRCA and other DNA repair genes.

We know that all four of the approved PARP inhibitor drugs are working well for patients, so it was perhaps a little surprising to find that the four licensed inhibitors exhibit very different levels of molecular selectivity in some of their interactions with proteins.

It was already known that, whereas all four approved drugs have the ability to inhibit key PARP proteins (most potently acting on PARP1 and PARP2), they nevertheless do exhibit some clear differences in their activity against the 17 different members within the PARP family. Also to be noted is that the licensed PARP drugs have different effects on what is known as "PARP trapping."

What our new study shows, using unbiased, large-scale computational and experimental analysis, is the extent to which the four approved drugs have additional and remarkably different activity against another group of proteins they are not designed to target—the protein kinases, which are themselves the primary targets of many other cancer drugs.

Understanding the diversity of the off-target effects of treatments at a molecular level has the potential to help uncover contributions to both the beneficial action of drugs and also the unwanted side-effects—and in the future could guide exactly how they are used in the clinic, and how they can best be combined with other treatments.

Off-target effects

There was already some information available that PARP inhibitors can bind to certain protein kinases. Our new study has provided the most

comprehensive assessment of the extent to which PARP inhibitors interact on a molecular level with the protein kinase family.

The large-scale nature of our study defines the overall landscape of the kinase off-target effects of the approved PARP-targeted drugs.

It's also the first time that PARP inhibitors have been shown to have such potent off-target effects at really low drug concentrations—below the often critical threshold concentration of one micromolar which can be readily achieved in patients.

Our study raises a number of questions. Could, for example, the effects on kinases have clinical significance or could they be exploited therapeutically in the future?

More specifically, might the kinase off-target effects help explain and potentially avoid certain toxic side-effects, or be exploited to treat additional types of cancer, or guide use of PARP inhibitors in drug combinations—such as use alongside immunotherapy?

In addition, our research raises the possibility of whether we could potentially exploit the dual activity of the drugs against both PARP and kinase targets in future drug design—a type of 'polypharmacology," defined as the design or use of pharmaceutical agents that act on multiple targets or disease pathways.

Large-scale computational prediction and experimental screening

We first took a computational screening approach to identify protein kinases that might have the ability to engage with the four approved PARP inhibitors—in a 'lock and key' type relationship.

Use of several different computational methods allowed us to predict that the clinical PARP inhibitors might indeed have the potential to bind to many protein kinases—to different extents depending on the PARP drug concerned.

Alongside, we used a large-scale, unbiased protein binding technology platform to screen experimentally for the actual ability of PARP inhibitors to bind to members of the protein kinase family (the 'kinome') – and to reveal any differences between the PARP drugs.

By taking these two approaches in parallel, we found that although the computational approach was useful in pointing to the potential of PARP drugs to engage with many different protein kinases, the precise predictive power was limited—indicating a need to further enhance the methodology for future applications.

Using the protein binding technology platform to screen for interaction with hundreds of kinases, we found that two of the four approved PARP inhibitor drugs were able to engage directly with many protein kinases—rucaparib binding to 37 and niraparib interacting with 23 protein kinases, of which only 15 were in common. In contrast, talazoparib showed only weak binding to two kinases and olaparib did not bind significantly to any of the 392 kinases tested, representing 76 percent of the entire human kinome.

We then carried out further follow-up studies using a high-throughput assay to measure the ability of PARP inhibitor drugs not only to bind to protein kinases, but also to block the functional catalytic activity of selected kinases in cell-free biochemical assays with the purified proteins. Of particular interest, rucaparib was found to potently inhibit three kinases—CDK16, PIM3 and DYRK1B—while niraparib was shown to strongly inhibit two kinases, DRK1B again and also DIRK1A.

As highlighted above, this is the first time that PARP inhibitors have been reported to have such potent off-target effects—inhibiting kinases at drug exposures below the one micromolar level. Such concentrations are commonly observed in patients, emphasising the potential clinical relevance.

Next we tested whether rucaparib and niraparib could maintain their potent kinase binding inside living cells by using the so-called NanoBRET assay platform. Importantly, we demonstrated binding of both CDK16 by rucaparib and DYRK1A by niraparib in living cells, with 50% inhibition in the 200-230nM range. As expected from the earlier profiling, olaparib was inactive in the live cell assay.

Potential impact of off-target kinase inhibition on clinical side-effect profiles

Our research shows that the four approved PARP inhibitor drugs, although similarly effective in the clinic, actually exhibit different molecular effects beyond their intended targets.

It is important to point out that the binding of rucaparib and niraparib to kinases is less potent than their binding to PARP1 and PARP2. Nevertheless, it is sufficiently strong to consider the possible consequences, including potential contribution to adverse effects.

As a first step and to stimulate further interest, we compiled an overview of adverse effects from the clinical trial data on the four PARP inhibitor drugs. This illustrated that although there are side effects in common, each of the drugs has a different side-effect profile.

We suggest that further studies are needed to address whether these distinct profiles might potentially be linked to differential off-target

kinase inhibition.

Future applications

We think our findings are important because they should inform future studies with PARP inhibitors in a number of possible directions, including potentially helping to guide how they are applied in the clinic—as an example, when considering drug combinations such as with immunotherapy.

We also believe that the approach we have taken here to study 'molecular promiscuity' can be applied to other drugs to better understand their molecular and clinical effects and perhaps to maximise the benefit they deliver for patients through polypharmacology.

It might be feasible to optimise any desired dual activity against PARP and kinase family targets in a 'designer polypharmacology' approach.

One idea we suggest in the publication would be to build on the potent activity of niraparib against the DIRK1A kinase that is associated with acute lymphoblastic leukaemia in children with Down Syndrome.

Drug designers might also be able to exploit the activity of PARP inhibitors against kinases to produce new drugs that act on the 'dark kinome' – that is the very large number of kinases that currently do not have inhibitors available.

Often the fact that a drug has off-target effects can be perceived as a negative—and this can be true. But in fact it is common for medicines to have multiple molecular interactions, like those we have seen here, that can contribute to the therapeutic benefit. For example, a number of anti-cancer agents hit several kinases that contribute to cancer as part of their mechanism of action.

Two recent studies from other researchers have shed light on how drugs can exert molecular interactions that are only discovered after they are approved for widespread use.

In one of these, published in *Science Translational Medicine*, the use of CRISPR gene knockout technology revealed that for several drugs undergoing clinical trial the proposed primary target is not actually essential for cancer cells to survive. Furthermore, the researchers showed that the drugs retained their activity in cancer cells in which the proposed molecular target was knocked out by CRISPR. The results demonstrated that these new developmental agents must be exerting their therapeutic effects by acting on different, hitherto unsuspected molecular targets.

The effect of knocking out the target on the response of cancer cells thought to act primarily via that target can now be added to the target validation toolbox—with an additional approach being to demonstrate resistance in cancer cells containing a mutant form of the target that no longer binds the drug.

In the second study, published on the preprint server *bioRxiv*, researchers compared the action of, on the one hand, a large collection of 397 actual drugs or prototype agents with, on the other hand, the effects of genome-wide CRISPR knockout of specific genes in a panel of 484 human cancer lines—for which extensive and systematic characterisation of their genetic features has been carried out. The results are both fascinating and informative.

Looking at the drugs for which there is information on the proposed target and for which data are available for the CRISPR knockout of that target, the researchers found a significant correlation between drug sensitivity and the nominal target for only a quarter of the agents tested. This increased to roughly half when the association with either the

nominal target itself or a functionally related protein are considered. This leaves 50% of the drugs in which there is no association between the ostensible target and cancer cell sensitivity.

Even accounting for the fact that concentration-dependent, potentially incomplete pharmacological inhibition is mechanistically different from total gene knockout by CRISPR, this leaves a very high proportion of drugs for which cancer cell sensitivity cannot be accounted for by action at a single molecular target.

It seems very likely that action on two or more drug targets—polypharmacology—is a major factor involved.

Impact for chemical probes as well as drugs

The occurrence of off-target effects is important not only for the use of drugs in patients but also for the application of drugs and other small-molecule agents as chemical probes in lab research aimed at identifying the roles of particular proteins in biological and disease mechanisms. In order to use chemical probes with confidence and avoid misleading results, it is essential that the chemical tool compound is as selective for the intended target as possible—and that any off-target effects are documented and mitigated.

The availability of large-scale screening platforms and online resources makes the ability to evaluate drugs and probes for on-target and off-target effects much more accessible. The use of these is strongly recommended.

In the case of our study on PARP inhibitors, olaparib would be preferred over the other three drugs evaluated as a chemical probe for PARP1 and 2 if avoiding off-targets effects on kinases is considered important.

So whether one is considering the development of drugs for patients or probes for biomedical research, knowing both your on-target and off-target effects is essential.

The problem is not the target effects that you think you know, but the off-target effects that you know nothing about.

More information: Albert A. Antolin et al. The kinase polypharmacology landscape of clinical PARP inhibitors, *Scientific Reports* (2020). DOI: 10.1038/s41598-020-59074-

Emanuel Gonçalves et al. Drug mechanism-of-action discovery through the integration of pharmacological and CRISPR screens, *bioRxiv* (2020). DOI: 10.1101/2020.01.14.905729

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