BC Cancer Agency
CARE & RESEARCH
An agency of the Provincial Health Services Authority

Alexia-Ileana Zaromytidou, PhD
Chief Editor, Nature Cancer
Nature Research
Friday, May 31, 2019

Dear Alexia,
As discussed at AACR, we are submitting the manuscript titled "Targeting of DNA damage response-associated ubiquitination sensitizes cancer cells to G-quadruplex stabilizers" for consideration at Nature Cancer.

The significance of our work is that it identifies the requirement of the regulatory ubiquitin signaling pathway for the sensing/repairing of G-quadruplex (G4)-associated DNA damage. This finding is pertinent to the clinical phase 1 compound CX-5461 (and a tool compound, pyridostatin, of a different structural class). We previously showed in 2017 that CX-5461 binds and stabilizes DNA G4-structures resulting in replication-associated DNA damage at G4 sites; defective DNA repair via homologous recombination (HR) sensitizes cancer cells to CX-5461. CX-5461 is currently in phase 1 clinical trial to determine the therapeutic effect in patients with HR repair defects (patients with BRCA1/2 mutated tumors). Our new findings have a potential translational significance of further expanding the range of solid tumors responsive to CX-5461.

The novelty of the current manuscript is that we are the first to demonstrate that loss of function of the DNA damage response (DDR)-associated chromatin ubiquitination pathway results in increased sensitivity of cancer cells to G4-stabilizing drugs. We arrive at this conclusion by performing a sub-genomic depletion screen with CRISPR-Cas9-mediated targeting of 480 genes, using multiple G4-binding drugs including CX-5461. We identified, in addition to the expected members of the HR pathway, novel gene-drug interactions with factors responsible for DDR-associated ubiquitin signaling. We re-validated these findings in additional cell lines using multiple methods. In addition, we showed that CX-54651 treatment induces ubiquitin signaling in nuclei; conjugated ubiquitin co-localises with DNA G4 structures and G4-associated DNA damage. Moreover, inhibitors of the relevant E2-conjugating enzyme act synergistically with CX-5461-induced G4-stabilization suggesting the potential for combination therapy with CX-5461. In short, to our knowledge, G4-associated DNA damage has not been linked previously to the DDR-ubiquitin pathway.

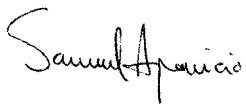
We suggest the following as potential reviewers based on their expertise in the field and no conflicts as far as we know:

Laurence Hurley (expert on G4 chemistry), hurley@pharmacy.arizona.edu
Jeffrey Trent (expert on translational biomarkers), TGen, Phoenix, Arizona, jtrent@tgen.org
Daniel Von Hoff (clinical expert on G4 clinical trials), dvh@tgen.org
Simon Powell (radiation biologist, expert on DDR), powells@mskcc.org

We ask that you kindly don't send the manuscript to the following individuals or their groups as we believe there are severe differences of opinion or overlapping interests that might preclude a fair review:

Denis Drygin; Ross Hannan (Peter MacCallum Cancer Centre); Madalena Tarsounas (University of Oxford)
Sven Rottenberg (University of Bern)

Kind regards



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