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Extended Abstract

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Leveraging NQO1 bioactivatable drugs for tumor-selective use of poly (ADP-ribose) polymerase inhibitors

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Therapeutic drugs that block DNA repair, including poly (ADP-ribose) polymerase (PARP) inhibitors fail because of a lack of tumor-selectivity. When PARP inhibitors and NQO1 bioactivatable drugs (ß-lapachone or isobutyldeoxynyboquinone (IB-DNQ)) are combined, synergistic antitumor activity results from sustained NAD(P)H levels that refuel NQO1-dependent futile redox drug recycling. Significant oxygen-consumption-rate/reactive oxygen species cause dramatic DNA lesion increases that are not repaired due to PARP inhibition. In NQO1+ cancers, such as non-small-cell lung (NSCLC), pancreatic or breast cancers, the cell death mechanism switches from PARP1 hyperactivation-mediated programmed necrosis with NQO1 bioactivatable monotherapy to synergistic tumor-selective, caspase-dependent apoptosis with PARP inhibitors and NQO1 bioactivatable drugs. Synergistic antitumor efficacy and prolonged survival were noted in human orthotopic pancreatic and non-small-cell lung xenograft models, expanding use and efficacy of PARP inhibitors for human cancer therapy. Poly(ADP-ribose) polymerase-1 (PARP1) is crucial to multiple DNA repair pathways, including DNA base excision (BER), single strand (SSB) and double strand break (DSB) repair. Once bound to DNA lesions, PARP1 consumes NAD^+ and PARylates nearby proteins, with activation and deactivation consequences. Self-PARylation (PAR-PARP1) is a post-translational modification that enzymatically inactivates the protein, rendering it unable to bind DNA and function in DNA repair. DNA repair defects in breast cancer associated genes 1 and 2 (BRCA1/2) yielded hypersensitivity to PARP inhibition and caused a rush to develop new PARP inhibitors for targeted therapy in these rare (~5%) hereditary breast and ovarian cancers. This subtype of cancers exhibits defective homologous recombination (HR) repair and reliance on PARP-dependent alternative non-homologous end joining (Alt-NHEJ) for survival. Exposing HR-defective BRCA1/2 cells to PARP inhibitors results in synthetic lethality that stimulated great interest in PARP inhibitors. Attempts to broaden their clinical application, including combined approaches using DNA damaging agents (e.g., ionizing radiation (IR), temozolomide, or gemcitabine limited tumor-selective rationale and increased normal tissue toxicities. NAD(P)H:quinone oxidoreductase 1 (*NQO1*) bioactivatable drugs have the potential to deliver tumor-selective DNA damage and cell death. They are a unique class of rare quinones that include β-lapachone (β-lap, ARQ761 in clinical form) and deoxynyboquinone. NQO1 catalyzes the two-electron oxidoreduction of β-lap to generate an unstable hydroquinone that spontaneously reacts in a two-step back-reaction with oxygen to regenerate the original compound. NQO1-dependent futile redox cycling oxidizes ~60 moles of NAD(P)H to create ~120 moles of reactive oxygen species (ROS) in ~2 min. High levels of superoxide dismutase (SOD) in cancers generate long-lived and cell membrane-permeable hydrogen peroxide (H_2O_2) that diffuses into nuclei to induce massive oxidative base and SSB DNA lesions. A significant bystander effect, blocked by Catalase (*CAT*), occurs from $NQO1^+$ cancer cells affecting neighboring $NQO1^-$ cancer cells. Rapid accumulation of DNA lesions overwhelms DNA repair capacity and causes 'hyperactivation' of PARP. Rapid protein PARylation, including PAR-PARP1, severe NAD^+/ATP depletion, massive DNA lesions and repair inhibition follows. ROS (H_2O_2) formation only occurs while pools of NAD(P)H are available for NQO1-driven futile redox cycling. A lethal β-lap dose induces caspase-independent programmed necrosis (i.e., NAD^+-Keresis). β-Lap-induced cell death is specific for cancers over-expressing NQO1 and suppresses GAPDH/glycolysis, OXPHOS, triggering μ-calpain-directed programmed necrosis. Although β-lap shows evidence of single-agent activity in phase 1 clinical trials, strategies to enhance its efficacy without augmenting toxicity are needed (Gerber et al., 2007). We hypothesized that inhibiting PARP activity prior to β-lap exposure would enhance both agents, extending NQO1-mediated ROS production and inhibiting PARP-driven DNA repair in a tumor-selective manner. Examination of *NQO1* and *CAT* mRNA expression in matched NSCLC tumor tissue showed relatively elevated *NQO1* expression, with concomitant lowered *CAT* levels vs associated normal tissue. As reported, a significant ($p \leq 2.2 \times 10^{-38}$) elevation in *NQO1* mRNA levels in a larger dataset (n=432) of NSCLC patient tumor vs associated normal lung tissue by gene expression microarray analyses was noted. In contrast, *CAT* mRNA expression was significantly lower ($p \leq 5.6 \times 10^{-48}$) in tumor vs normal lung tissue. Concomitant high *NQO1* and low *CAT* mRNA levels (high *NQO1:CAT* ratios ($p \leq 1.1 \times 10^{-88}$), in NSCLC tumor tissue offer an ideal target for NQO1 bioactivatable drugs. Fresh, snapfrozen pathology-assisted dissection of tumor vs associated normal tissue from NSCLC patients confirmed elevated NQO1 enzyme levels in tumor vs normal tissue. Western analyses confirmed lowered *CAT* levels in NSCLC tumors, with high levels in associated normal lung tissue. Immunohistochemical (IHC) analyses confirmed NQO1 elevations in NSCLC, PDA and high grade cancers, including triple-negative breast cancers (TNBCs). Enzyme assays confirmed elevated NQO1 levels in cancer vs associated normal tissue, even when protein was not noted by Westerns (patient 2823, . Advanced and treatment-resistant NSCLC cases also exhibit *NQO1* over-expression, with increased levels in progressive disease (PD) vs patients who exhibited clinical responses (CR). Elevated *NQO1* levels were greater in high vs low grade PDAs.

Bottom Note: *This work is partly presented at EuroScicon congress on Biochemistry, Molecular Biology & Allergy* October 11 - 12, 2018 Amsterdam, Netherlands