The p53 transcription factor functions as one of cancer’s most potent tumor suppressors and is the most frequently mutated gene in human cancer. The majority of p53 mutations (>70%) are missense that generate a defective protein found at high levels in cells that is targetable. Restoration of wild type structure and function of mutant p53 with a small molecule (so-called reactivation) is a highly sought-after goal in anti-cancer drug development. The p53 protein requires the binding of a single zinc ion to fold properly and mutations that impair the protein’s ability to bind zinc (and cause it to misfold) are highly prevalent in cancer. We recently discovered a new class of small molecule zinc chelators named zinc metallochaperones (ZMCs) that reactivate zinc deficient mutant p53 through a novel mechanism involving both zinc ionophore activity to raise intracellular zinc concentrations and donation to restore zinc binding to mutant p53. This induces a wild type conformation change and a p53 mediated apoptotic program. The lead compound (ZMC1) displays a transient pharmacodynamics (p21 levels) in vitro. We hypothesized that the regulation of these pharmacodynamics is governed by cellular zinc homeostatic mechanisms that function to restore zinc to its physiologic picomolar levels. We examined the entire suite of zinc homeostatic genes in response to ZMC1 and manipulated several metallothionein genes by knockout and knockdown. The net effect of this was to increase the peak and duration of intracellular zinc levels that lead to a more potent and sustained duration of p21 expression. This translated to increased sensitivity to ZMC1. We further postulated that this pharmacodynamics would allow the drug to function with very minimal exposure and colony formation studies in vitro indicated that a two-hour exposure was as effective as a 72-hour exposure. We then sought to translate this mechanism in vivo using a genetically engineered murine model of KPC pancreatic cancer (Pdx-1Cre; KrasG12D) that expresses either the p53R172H (zinc deficient) allele or p53R270H (non-zinc deficient). Pharmacokinetic (PK) studies of the drug revealed a short half-life (15 minutes) indicating a minimal exposure. Despite this, daily, intermittent dosing at the maximum tolerated dose resulted in a statistically significant increase in the overall survival of the KPC-p53R172H mice while having no such effect in the KPC-p53R270H. We sought to improve the efficacy of ZMC1 by preloading it with zinc in a 2:1 molar ratio based on the crystal structure. The drug-zinc complex (Zn-1) increased the median survival of KPCp53-R172H mice from 26 days to 35 days (ZMC1 monomer versus Zn-1). These studies indicate that cellular zinc homeostatic mechanisms function as an “off” switch for ZMC’s which has important implications for the translation of ZMCs in humans. Principally, this allows the drug to function with minimal exposure which minimizes potential zinc toxicity. ZMC1 as monotherapy improves survival in an allele-specific mutant p53 manner. Furthermore, ZMC1 can be optimized by synthesizing it complexed with zinc. Overall, this “off” switch is novel for a targeted molecular therapeutic and represents a significant departure from the traditional paradigm where the goal is to develop a compound that binds the target with a PK profile that provides maximal exposure.

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Cellular zinc homeostatic mechanisms function as an off switch for zinc metallochaperone mediated reactivation of mutant p53

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