The tumor suppressor protein p53 (TP53, best known as p53) is inactivated in most if not all cancers, commonly via mutation of its coding gene. This confers a selective growth advantage and resistance to conventional anti-cancer therapies. Most TP53 mutations are missense and typically occur in the central DNA binding domain, which disrupts sequence specific binding to DNA and results in loss of wild-type p53 function. Mutant p53 protein can also accumulate in cells and have gain-of-function activities that contribute to tumourigenesis.1 Given its central importance, restoration of wild-type p53 function in tumors should trigger growth inhibition and cell death. This is the rationale for the development of therapeutic small molecules such as APR-246 (also known as PRIMA-1met), a drug that is currently showing promise in early-phase clinical trials.2 Methylene quinuclidinone (MQ), the active derivative of APR-246, covalently binds to thiol groups on cysteine residues in the core domain of mutant p53 protein, driving a conformational change resulting in restoration of sequence specific DNA binding, wild-type p53 transcriptional activity and tumor suppressor function.3 These findings have been confirmed by many subsequent studies across a range of different p53 mutants and cancer types.4,5 However, a few studies have shown similar therapeutic efficacy of APR-246 in cancer cells without mutant p53 protein.6 Furthermore, we previously found that knockout of mutant p53 abrogates APR-246 induced cell cycle arrest but not apoptosis,4 suggesting that additional mechanisms are involved in the anti-tumor activity of this drug.

In a recent study7 we showed that MQ covalently binds to thiol groups on cysteine residues of glutathione (GSH), which resulted in depletion of intracellular GSH, and increased oxidative stress. This phenomenon was independent of mutant p53 reactivation, but was critical to the therapeutic activity of APR-246. The rate limiting substrate for GSH biosynthesis is intracellular cysteine, the majority of which comes from reduction of cystine, which is imported into the cell by the glutamate/cystine exchanger, system xC⁻. In keeping with these findings, ectopic expression of solute carrier family 7 member 11 (SLC7A11), the key component of system xC⁻, increased GSH biosynthesis and induced resistance to APR-246 in cancer cells with TP53 mutations. Conversely, knockdown of SLC7A11 increased sensitivity to APR-246 in p53-null cells, demonstrating that SLC7A11 expression influences APR-246 activity independent of mutant p53 protein. Consistent with this, genome-wide transcriptomic analysis demonstrated that SLC7A11 expression was the strongest predictor of sensitivity to PRIMA-1, the lead compound for APR-246, thus highlighting SLC7A11 expression as a potential predictive biomarker for response to APR-246 in addition to TP53 mutation status. Together, these findings potentially clarify seemingly conflicting reports of APR-246 sensitivity and its relationship to TP53 mutation and mutant p53 accumulation. That is, inherent defects in GSH biosynthesis, such as low expression of SLC7A11, may explain reports of APR-246 induced cell death in the absence of mutant p53 protein or the restoration of wild-type p53 transcriptional activity.

Why then, given the ubiquitousness of GSH in cells and its importance as the major intracellular antioxidant, are cancer cells with mutant p53 protein generally more sensitive to APR-246? The answer lies in the novel inverse relationship between SLC7A11 expression and mutant p53 accumulation that we.
identified in our esophageal adenocarcinoma models, and confirmed in other tumor types using The Cancer Genome Atlas database.\textsuperscript{7} Utilizing genetic approaches we established that this inverse relationship is mediated by an interaction between mutant p53 protein and nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, commonly known as NRF2), which impairs NRF2-mediated transcription of target genes involved in redox regulation, including SLC7A11. Thus, accumulation of mutant p53 protein suppresses SLC7A11 expression leading to increased basal oxidative stress and reduced cellular capacity to detoxify reactive oxygen species (Fig. 1a). As a consequence, mutant p53 effectively sensitizes cancer cells to oxidative stress resulting from further depletion of GSH by APR-246. Similarly, genetic or pharmacological inhibition of SLC7A11 creates a synthetic lethal interaction with mutant p53 accumulation,\textsuperscript{7} raising the potential for a new therapeutic paradigm to target cancers with accumulated mutant p53 that is analogous to the use of PARP inhibitors in BRCA deficient cancers. While this is a potential weakness that might be predicted to be selected against during tumor evolution, increased oxidative stress induced by mutant p53 may have pro-oncogenic effects, including increased oxidative DNA damage leading to genomic instability.\textsuperscript{8} Therefore, together with the loss of wild-type p53 tumor suppressor activity, we propose that this function of mutant p53 may instead provide a selective advantage during tumorigenesis (Fig. 1a).

The interaction between NRF2 and mutant p53 has been confirmed by others\textsuperscript{9} where, remarkably, it promotes NRF2-mediated expression of proteasome machinery, leading to degradation of multiple tumor suppressors and contributing to resistance to proteasome inhibitors. Significantly, Del Sal and colleagues show that APR-246 disrupts the interaction between mutant p53 and NRF2, thereby down-regulating proteasome gene expression and restoring sensitivity to proteasome inhibitors.\textsuperscript{9} As would be predicted based on this finding, expression of SLC7A11 and other anti-oxidant gene targets of NRF2 are upregulated in cancer cells with mutant p53 protein following treatment with APR-246,\textsuperscript{10} which has the potential to negate the therapeutic activity of APR-246 mediated through GSH. This provides mechanistic rationale for combining APR-246 with inhibitors of the system xC\textsuperscript{−}/GSH axis (Fig. 1b). Indeed, antagonising SLC7A11 in combination with APR-246 selectively and synergistically induces cell death in tumors with mutant p53 accumulation.\textsuperscript{7}

Overall, our study has uncovered a potential Achilles’ heel in cancers with accumulated mutant p53 and a novel paradigm

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**Figure 1.** Accumulation of mutant tumor protein p53 (TP53) raises basal oxidative stress and induces susceptibility to glutathione depletion (a) Accumulation of mutant TP53 (shown as mutp53) in cancer cells impairs nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, best known as NRF2) function and reduces the expression of NRF2 target genes, including solute carrier family 7 member 11 (SLC7A11, also known as xCT), the cystine (Cys)/ glutamate (Glu) anti-porter. This results in reduced glutathione synthesis and higher basal oxidative stress compared with normal cells. In the absence of wild-type p53 tumor suppressor function, increased oxidative stress likely contributes to tumourigenesis via oxidative DNA damage and genomic instability. (b) As a consequence, cancer cells with accumulation of mutant p53 protein are sensitive to the glutathione depleting effects of methylene quinuclidinone (MQ, the active derivative of APR-246) or inhibition of SLC7A11. Binding of MQ also restores wild-type p53 transcriptional activity to mutant p53 and disrupts the interaction between mutant p53 and NRF2. This latter effect results in upregulation of SLC7A11 in response to oxidative stress, providing the mechanistic rationale for combining APR-246 with SLC7A11 inhibitors.

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Mutant p53 accumulation

- NRF2 target genes e.g. SLC7A11
- Oxidative Stress
- DNA damage
- Genomic instability

CANCER CELL

MQ (APR-246) + SLC7A11/XCT inhibition

- NRF2 target genes e.g. SLC7A11
- p53 target genes e.g. PUMA, NOXA
- Oxidative Stress
- DNA damage
- Genomic instability

CANCER CELL

MQ

- Glutathione
- Oxidative Stress

Cell Death
for mutant p53 directed anti-cancer therapies. Our novel insights into the mechanism of action of APR-246 and unification of our understanding of what drives APR-246 sensitivity suggest clear criteria for patient selection and rational drug combinations, with the capacity to be immediately translated into clinical trials.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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Author/s:
Clemons, NJ; Liu, DS; Duong, CP; Phillips, WA

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