CHEMOPREVENTION

A cellular chill-out pill?

Short interfering RNA (siRNA) is an effective and highly specific strategy for 'knocking down' gene expression in animal models. This method of molecular control might now become more clinically relevant, as Tim Devling and colleagues report that siRNA against *KEAP1* could represent a unique class of cancer chemopreventive agent.

Reducing neoplastic disease through chemoprevention is a highly desirable goal and several strategies have been considered. Chemopreventive blocking agents, for example, increase the expression of cytoprotective genes in human cells. These include drug-metabolizing enzymes such as NAD(P)H:quinone oxidoreductase 1 (*NQO1*) and glutathione (GSH) transferases, as well as antioxidant genes such as glutamate cysteine ligase catalytic (*GCLC*) and modifier (*GCLM*) subunits, which help synthesize GSH. However, to be chemoprotective, such chemicals must induce reduction–oxidation (redox) stress in the cells, which can be potentially damaging.

Genes that are regulated in this manner contain antioxidant response elements (AREs) in their promoters, and their transcription is stimulated in response to the ARE-mediated recruitment of a complex that contains nuclear factor erythroid 2 p45-related factor 2 (NRF2). NRF2 accumulates in the nucleus in response to redox stress and after treatment with chemopreventive blocking agents.

The redox-sensitive kelch-like ECH-associated protein 1 (KEAP1) is an NRF2-specific adaptor protein for the CULLIN3–ROC1 ubiquitin ligase. Under homeostatic conditions it promotes the proteasome-mediated degradation of NRF2 and thereby keeps ARE-containing genes switched off. Under conditions of natural redox stress, or after treatment with chemopreventive blocking agents, KEAP1 no longer fulfills this role, NRF2 is stabilized and the ARE-containing genes are switched on. The authors therefore wanted to test whether specifically reducing KEAP1 levels using siRNA would activate transcription of the ARE-containing genes in the absence of stress.

A duplex 21-nucleotide siRNA was designed that successfully knocked down *KEAP1* mRNA to <30% of normal levels after transfection into human keratinocytes. This upregulated NRF2 and increased the levels of NQO1, GCLC and GCLM as well as aldo-keto reductase 1C1/2 and GSH in the cells.

Therefore, the authors suggest that siRNA against KEAP1 is a potentially valuable method for inducing the pre-adaptation of human cells to oxidative stress. This could be useful for treating degenerative disease without exposing cells to potentially harmful chemicals.

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References and links

ORIGINAL RESEARCH PAPER Devling, T. W. P. *et al*. Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. *Proc. Natl Acad. Sci. USA* **102**, 7280–7285 (2005)

TRIAL WATCH

Successful discontinuation

Cytostatic drugs are not expected to cause tumour shrinkage, so they are usually assessed in Phase II trials using duration of stable disease as the end point. The CALGB 69901 trial is the first to use a new design — the randomized discontinuation trial design — to test the clinically relevant effect of a cytostatic agent in a cancer that has a variable natural history, a property which makes the 'no treatment effect' control hard to ascertain.

In a randomized discontinuation trial all patients receive the investigational drug for an initial period. Patients who show tumour shrinkage then continue on therapy, patients who progress or have unacceptable toxicity discontinue the investigational therapy, and those with stable disease are randomized to continue or discontinue therapy. In this way the trial is enriched for patients with stable disease, so increasing the power of the trial for detecting a clinically relevant difference.

The CALGB 69901 trial recruited 368 patients with renal-cell carcinoma (RCC) to receive the anti-angiogenic cytostatic agent carboxyaminoimidazole (CAI). RCC usually has a poor prognosis, but up to 10% can spontaneously regress, making the 'no treatment effect' by which to judge the effect of a cytostatic agent difficult. CAI has shown activity in xenograft models and toxicity seen in Phase I trials was acceptable. In the intial part of the trial serious toxicity was seen in 34% of patients — mostly asthenia and neuropsychiatric problems. After the initial therapy 51% patients had progressed, 30% withdrew, 1% had a partial response, and 17% had stable disease and were randomly assigned. The 49 patients with stable disease received 16 weeks of blinded post-randomization therapy — over half in each group progressed during this time. Using a Bayesian futility analysis it was concluded that the probability of patients in the CAI and placebo groups progressing was very similar, so the trial was stopped early. The observed stable disease rate was probably due to the slow progression of some metastatic RCCs and not due to the effects of CAI.

The trial gave clearer and more robust results than a previous, more traditional Phase II trial of CAI in a similar patient population — 25% were progression free at 6 months and CAI was deemed worthy of further investigation. The CALGB 69901 investigators now suggest that this was a false-positive result. They suggest that the randomized discontinuation trial design should now be tested for evaluating other cytostatic drugs.

ORIGINAL RESEARCH PAPER Stadler, W. M. *et al*. Successful implementation of the randomized discontinuation trial design: an application to the study of the putative antiangiogenic agent carboxyaminoimidazole in renal cell carcinoma — CALGB 69901 *J. Clin. Oncol.* **23**, 3726–3732 (2005)

30 years on

Cure rates for Hodgkin's lymphoma (HL) are high, but what are the long-term effects of having had HL on mortality? Graca Dores and colleagues analysed the follow-up information of 41,146 HL survivors held by cancer registries in North America and Europe for the cause of death. Most of the survivors had been followed for 10 years, with just over 1,000 having 30-year follow up. Significant excess deaths were due to second cancers (SCs; absolute excess risk (AER) = 63 excess deaths per 10,000 patients per year) and non-cancer (NC; includes cardiovascular, respiratory, infection and haematological; AER = 49). These risks remained for more than 30 years following HL diagnosis. The risks of SC and NC were highest in patients initially treated with both radiation and chemotherapy. The authors conclude that HL patients should therefore be targeted for preventive measures to attempt to reduce excess mortality.

ORIGINAL RESEARCH PAPER Dores, G. *et al*. Long-term cause-specific mortality among 41,146 one-year survivors of Hodgkin lymphoma (HL). *J. Clin. Oncol. (Suppl.)* **23**, A6511 (2005)