



## Mini-review

## Novel treatments for hepatocellular cancer

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## ABSTRACT

Hepatocellular cancer (HCC) has always been considered a therapeutic challenge, given the cytotoxic drug resistant nature of the cancer and associated disorder in liver function, reducing the safety of many conventional chemotherapy agents. The Multikinase inhibitor sorafenib has been found to prolong survival in patients with advanced HCC, by around 3 months compared to placebo, but novel treatments need to be explored. Current experimental therapeutic approaches encompass a broad range of science, ranging from intrahepatic irradiation to virus directed immunotherapy. This chapter presents a horizon scan of novel treatments which are currently at early stages of trial development.

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## 1. Introduction

Hepatocellular cancer (HCC) has a low survival rate [1] as the majority of patients present with advanced and late stage disease. Coupled to the fact that its aetiology is driven by serious underlying liver disease, HCC patients often have poor performance status and significantly impaired general health, making them less suitable candidates for conventional cancer treatment. There are three therapeutic mainstays used in the multidisciplinary management of cancer – surgery, radiotherapy and chemotherapy.

The key to successful oncological surgery is careful patient selection. Improved imaging techniques, including CT, MRI and PET-CT scanning have made an important contribution to the anatomic localisation of the cancer, and there are several algorithms that have been developed to assess relative hepatic function e.g., child's staging. There is an old adage that tumour biology will always outweigh surgical technique, and this is particularly so of HCC. All patients must be assessed by the multidisciplinary team and a case for resection would be made for patients with good performance status, child's A and B classification,

adequate liver reserve, suitable tumour location, no portal hypertension and a suitable liver remnant following resection. There is controversy about the role of liver transplantation, but this has been proposed for patients with tumour <5 cm in diameter, or 2–3 tumours <3 cm each with no evidence of macrovascular involvement or extrahepatic disease and whose liver function or remnant cannot support life following resection [2,3].

Inoperable lesions 3 cm or less may be worth ablating with radio frequency techniques, but if they are multiple or more than 5 cm in diameter, then transarterial chemoembolization (TACE) is often used.

Conventional chemotherapy has no role to play in the management of advanced HCC. Despite multiple, phase II trials, there are no, well powered, definitive studies which show a survival advantage for intravenous chemotherapy.

Transarterial chemoembolization (TACE) has become more widely used in the treatment of unresectable HCC. This treatment combines antineoplastic agents mixed with iodised oil administered via the hepatic artery in an attempt to reduce the rate of tumour growth. Chern et al. completed a small study of 26 people using a combination of cisplatin, doxorubicin and mitomycin C mixed with Lipiodol and Ivalon. All patients with residual tumours received a repeat TACE 6–8 weeks after previous treatment. The study followed up patients until their death or until

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the study ended (over a 10 year period). Twelve of the twenty six patients in the study did not respond, and had a median survival rate 3.3 months. The 14 patients who responded to the treatment had a median survival rate of 13.5 months. Even though it was a small study it suggests that if the patients respond to TACE it, increases their life expectancy [4].

External beam radiation therapy is not effective on its own as a treatment for HCC but can be used in conjunction with TACE to increase the rate of tumour regression.

It is clear that recent advances have been made in the treatment of HCC, with the introduction of sorafenib (discussed in detail by Jordi Bruix in the last chapter), however given the moderate associated survival benefits, further progress must be made through the development of novel therapeutic agents. It is likely that comparative biomarker analysis of RNA, DNA and the proteome will yield further druggable targets, many of which are likely to be kinases, but in this chapter we will focus on a range of novel and experimental agents including gene therapy, intrahepatic radiotherapy and cytotoxic prodrug treatment.

## 2. Genetic therapy

Gene therapy is a relatively new technique in the therapeutic battle against cancer. In 2007 it was estimated by the Journal for Gene Medicine that 1309 gene therapy clinical trials have been approved (results from 1989 to July 2007 found at [www.wiley.co.uk/genetherapy/clinical/](http://www.wiley.co.uk/genetherapy/clinical/)) [5], the majority of which were for cancer (65%). There are three broad approaches to gene therapy for cancer:

- Correction of underlying genetic abnormalities e.g., substitution of wild type for mutant P53.
- Manipulation of the immune system to cause the cancer cells to become the target of “immune rejection”.
- Delivery of tumour selective cytotoxic insults.

## 3. p53 Gene therapy approaches

As previously discussed by Levero in his chapter, p53 is a tumour suppressor that has many anti-cancer mechanisms:

- Activation of DNA repair proteins.
- Blockade of the cell cycle to aid the repair of a cell.
- Initiation of apoptosis or cellular senescence.

It is theorized that the loss of p53 as an early molecular event plays an important role in initiation of HCC. Therefore finding a way to reactivate, replace or repair the p53 gene could have a beneficial anti-cancer effect through induction of apoptosis or cellular senescence, either singly or in combination with chemotherapy.

Any gene therapy must depend on two essential components, the transgene to be delivered and the vector used to carry the gene, both or either of which should provide some degree of selectivity for the liver cancer cells. The most common viral vector is the adenovirus which carries

its genetic material in the form of double-stranded DNA and is ideally suited to be a vector for delivering therapeutic genes to cancer cells given its size, availability of manufacture and relative ease of genetic manipulation in the laboratory. When the viruses infect a host cell, their genetic material is not incorporated into the host cell's DNA, but exists as an episome, in the host nucleus, from which it is transcribed just like any other gene. Vollmer et al. [6] studied the antitumor activity of single agent E1B-deleted adenovirus (dl1520) and its combination with the cytotoxic drug cisplatin in HCC xenografts. The results showed that dl1520 significantly retarded the growth of these xenografts, although complete tumour regression was rare, that this effect was dependent on mutant p53 as these cells supported the virus' infective life cycle and was augmented by cisplatin, suggesting a rational combination in clinical trial.

Habib and co workers [7] completed a clinical trial of dl1520 gene therapy for hepatocellular carcinoma. This virus can replicate selectively in cancer cells which are devoid of wild type p53 (the majority of HCC specimens), destroying the host tumour cells and allowing released virus to infect and destroy further HCC cells.

They studied 10 patients with post hepatitis cirrhosis, with histologically proven primary liver cancer. The patients were randomly split into two groups, the control receiving a percutaneous ethanol injection, the “active” group receiving gene therapy with E1B-deleted adenovirus, (1 ml,  $3 \times 10^{11}$ ) given by intravenous injection on the first day, followed by a direct intratumoral injection on days 2, 15, 16, 29 and 30. There were no significant side effects (pain/fever for ethanol injection, mild fever or rise in liver enzymes for the gene therapy group). The results of the trial revealed that two patients had stable disease and three showed disease progression in the control group, whereas the gene therapy group showed one patient with a partial response and the remaining four with progressive disease. This trial shows that a replication competent adenovirus can be tolerated by patients, with reduced hepatic function, but is much too small to suggest any real evidence of efficacy.

Peng [8] has designed a recombinant serotype five adenovirus in which the E1 region is replaced by a human wild-type p53 expression cassette (Gendicine). The gene is driven by a Rous sarcoma virus (RSV), promoter with a bovine growth hormone (BGH) poly tail. More than 20 different tumour types have been treated with this recombinant virus, including head and neck squamous carcinoma, recurrent glioma, lung cancer, breast cancer and HCC. Gendicine is well tolerated but can be associated with mild fever, and has been used safely in the treatment of advanced liver cancer, combined with hepatic transcatheter arterial chemoembolization (TACE).

One hundred and fifty advanced HCC patients were enrolled at the West China Hospital, of whom 68 patients were treated with the Gendicine/TACE (5-FU, doxorubicin, camptothecin and arterial embolisation with iodised oil) combination administered by intratumoral injection. Gendicine (dose of  $1-4 \times 10^{12}$  VP) was injected once a week for four weeks, 2–5 days after TACE. Eighty two patients treated with TACE alone were considered as a histor-

ical nonrandomized control group. Six months later 76.4% (52 of 68) of the Gendicine/TACE group were alive compared with 23.2% (19 of 82) of the TACE group. The combined treatment seems to have improved the duration and patients' quality of life, although it is important to realise that this was not a randomized trial and therefore likely to be subject to selection bias.

A subsequent trial was performed in Shenzhen, China, in which 38 patients with inoperable advanced HCC received Gendicine treatment alone ( $n = 30$ ) or combined with hyperthermia ( $n = 8$ ). All patients received  $1-2 \times 10^{12}$  virus particles (VP)/week, for 4 weeks by intralesional administration or hepatic arterial infusion. Of the 30 patients treated with Gendicine alone, 26 were judged to have clinically useful responses (two showing partial regression and 24 showing stable disease), the remainder having progressive disease.

These trials suggest that Gendicine may have a role to play in the treatment of liver cancer, but formal, well powered randomised trials need to be carried out, before this treatment enters the therapeutic mainstream.

#### 4. Genetic immunotherapy

Given insights into the molecular basis of antigen presentation and recognition by the immune system, there is growing interest in the concept of cancer vaccination. There are many hurdles to be overcome, particularly over selection of tumour antigens, the expressed protein array that differentiates the cancer from host cells. One way to avoid this problem maybe to make an autologous vaccine from a protein extract of the patient's own cancer, however this is technically complex, cumbersome, and difficult to quality control [9].

Selective intra-tumoural delivery of a cytokine such as granulocyte-macrophage colony stimulating factor (GM-CSF) has the potential to create an autologous vaccine in situ by acting as a chemoattractant for immune effector cells. Simultaneous tumour lysis (induced by TACE for example) will release a host of tumour antigens which can be processed and presented by GM-CSF stimulated dendritic cells and generate lymphocyte directed cancer cytotoxicity.

Wang et al. [10] explored the use of adenovirus mediated interleukin 24 (a broad spectrum tumour suppressor) gene therapy on a human hepatocellular carcinoma cell line. The results of this experiment showed that in vitro addition of the adenovirus induced HCC cell cytotoxicity and apoptosis and perturbed the cell cycle (G2/M arrest). Intratumoral injection of the virus suppressed xenograft growth in athymic nude mice with a mechanism that involved induction of caspase-3 and down regulation of angiogenic factors like VEGF (see Fig. 1).

Kirn and colleagues have constructed a replication competent vaccinia virus which grows selectively in tumour cells, by virtue of deletion of DNA synthetic genes, and synthesises GM-CSF which it releases into the tumour micro-environment prior to cell lysis caused by the viral burst [11]. Although the virus is initially inoculated into the tumour, it can produce a systemic effect by releasing quantifiable and biologically active concentrations of GM-CSF into the venous system, while virus released from the infected cell into the bloodstream is free to infect distant cancer sites and tumour specific cytotoxic t-lymphocytes primed by local production of the cytokine can similarly seek out and destroy distant metastases. This virus has entered clinical trial for HCC, and shown promising initial results [12].

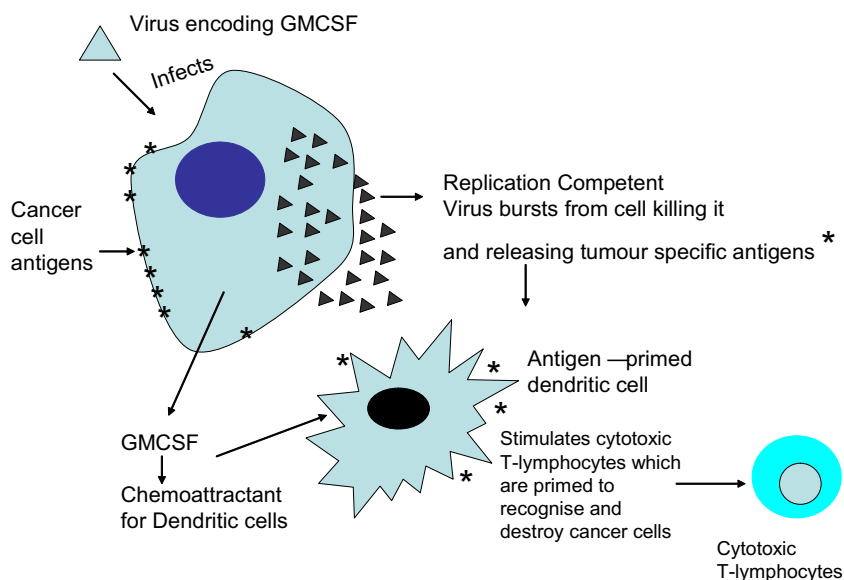


Fig. 1. Genetic immunotherapy.

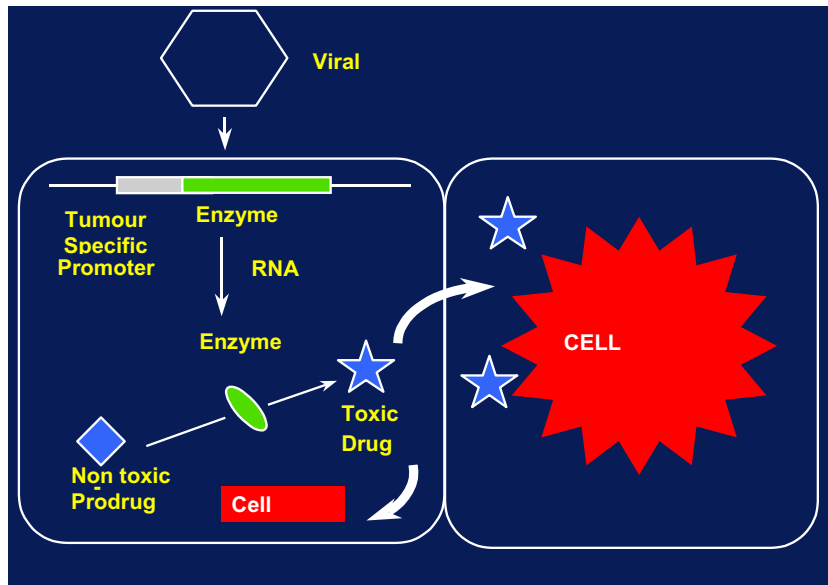


Fig. 2. Viral delivery of enzymes for prodrug therapy.

### 5. Virus directed enzyme prodrug therapy (VDEPT)

The premise underlying VDEPT is relatively simple in that a virus is used to deliver a gene encoding an enzyme capable of catalysing conversion of a nontoxic prodrug to a short lived cytotoxic species [13]. If gene delivery can be located selectively to cancer cells, then this should increase the ratio of cancer to normal host cell death. The weak monofunctional alkylating agent, CB1954, is a substrate for the *Escherichia Coli* enzyme, nitroimidazole reductase (ntr). This has been genetically engineered into replication deficient type 5 adenovirus, under the control of the CMV promoter (Ad-ntr) (Fig. 2).

A series of experiments have shown that a range of human cancer cell lines from various tissue lineage including HCC can be sensitised by 100,000-fold following transfection by Ad-ntr. There is a correlation between the extent of cellular ntr expression and the concentration of drug which kills 50% of cells ( $IC_{50}$ ) and it proved possible to achieve virtually 100% cell kill with only 5% of the total population of cancer cells transfected. This illustrates the so-called bystander effect, an important element of stoichiometry, as it is extremely unlikely that any viral vector could infect 100% of the tumour cell population. The bystander effect is probably mediated by transport of the activated cytotoxic species via intercellular junctions or by diffusion into the tumour interstitium in concentrations sufficient to induce apoptotic cell death [14].

The combination of CB1954 and Ad-ntr was sufficiently efficacious (HCC, ovarian and pancreatic xenografts) and safe (detailed toxicology assessment) to proceed to clinical trial. This was performed in three stages, CB1954 alone, Ad-ntr alone and then the two agents in combination. The phase I and pharmacokinetic trial of CB1954 administered by brief intravenous infusion every 3 weeks, revealed that

dose limiting toxicities were diarrhoea and asymptomatic hepatic transaminitis, there was no myelosuppression and plasma drug concentrations of 2–3  $\mu\text{M}$  were achieved, well within the range which was active in vitro [15]. In the second trial, Ad-ntr was administered by direct intra-tumoural (IT) injection under ultrasound guided control to patients with isolated hepatic metastases; 2 days before resection. This was a dose escalating study, and found that the maximum was delivered dose of  $2.5 \times 10^8$  viral particles was well tolerated and most importantly, that there was a dose related increase in expression of ntr in tumour cells in the resected specimen. The average diameter of the resected hepatic metastases was 3 cm and at the top dose of Ad-ntr up to 30% of cells expressed ntr, well above the 5% transfection rate that was shown to elicit significant cancer cell kill in vitro. The next phase of the clinical study combined the virus delivered by IT injection and CB1954 by intravenous infusion, in patients with inoperable HCC or colorectal cancer hepatic metastases [16]. This meant that those tumour nodules which were not injected could serve as “internal controls”. Sequential imaging of the liver showed that there was stabilisation or reduction of tumour size in three patients. Clearly, although presenting proof of principal, in that the virus did infect a significant proportion of tumour cells and cause them to express ntr, Ad-ntr is unlikely to be further developed as a cancer therapeutic given the highly localised method of administration, the inability of the virus to undergo more than one round of replication and the antibody mediated host immune response that neutralises the virus and militates against retreatment with the same vector. More modern generations of viral vectors will be selectively replication competent, constrained by molecular switches to grow only within cancer cells, carry surface modifications to reduce immunoreactivity and target the vectors to tumour associated receptors on the cell surface.

## 6. Intrahepatic radiotherapy

### 6.1. Yttrium-90

In the past external radio therapy has not been considered an effective liver cancer treatment, because the dose of radiation given to the tumour is reduced by the tolerance of the noncancerous part of the liver. Most tumours need a dose of radiation of approximately 120 Gy to be killed whereas normal liver can only handle 30 Gy before radiation hepatitis develops.

Radioembolisation (RE) is a technique that has been developed to target multiple sites of disease within the liver. SIR-Spheres microspheres contain the pure  $\beta$ -emitter, yttrium-90, which has a physical half-life of 64.1 h.

Since RE delivers high doses of ionising radiation selectively to the tumour compartment, whilst maintaining radiation exposure of the normal liver to a tolerable level, it can be regarded as a form of brachytherapy [17,18]. Many theoretical and clinical aspects of the use of SIR-Spheres for the treatment of primary and metastatic liver cancer have been published, including ideal microsphere characteristics [19], the relationship between the amount of yttrium-90 administered and radiation dose received by the tumour/normal liver compartments [20–23], the tolerance of the liver to yttrium-90 radiation [24], dosimetry [25], and clinical response rate to treatment in first- and second-line therapy [26–31].

Lau and Co workers [32], evaluated the use of yttrium-90 microspheres in nonresectable hepatocellular carcinoma. Seventy one patients were treated with hepatic arterial infusion of the microspheres, using a Seldinger approach by hepatic angiography or via an implanted arterial portacath. The patients in the trial were initially treated with an activity of 0.8–5 GBq, with a maximum of five treatments depending on the results of monitoring the serum tumour markers, alpha fetoprotein or ferritin. The median survival duration for patients was 9.4 months (range 1.8–46.4 months). The treatment was well tolerated and radiation pneumonitis was observed in the minority of patients who had significant pulmonary shunting. Confirmatory randomised trials in HCC are required before the treatment could be considered standard.

### 6.2. Rhenium sulphide

The radio isotope Rhenium ( $\text{Re}^{188}$ ) is easily formed using a  $^{188}\text{W}/^{188}\text{Re}$  generator. It is a  $\beta$  particle and gamma emitter with a half-life of 16.9 h, implying that if injected intratumoral injection it has the potential to selectively kill the cancer but minimise toxicity to the rest of the body. It has been shown by Junfeng et al. [33] that Rhenium is more effectively given as a radio therapy drug when it is prepared as a sulphide suspension. This isotope is made from the reaction of sodium thiosulphate and potassium perrhenate in acid solution. Thirty nude mice with transplanted human liver cancer xenografts were split into six groups, two groups were used as a control, one receiving nonradioactive rhenium sulphide the other a salt solution, by intratumourally. The other 4 groups were treated with 0.1 ml of rhe-

nium sulphide, each receiving a different dose (3.7, 7.4, 18.5, 29.6 MBq). The results showed tumour inhibition ratios as high as 89%, at a total delivered dose of 507.6 Gy. This pre-clinical study provides an evidentiary basis for taking Rhenium Sulphide forward to the clinic [34].

## 7. Cytotoxic drugs

Many conventional cytotoxic agents lack any intrinsic anti-tumour selectivity. They mostly act on cells that are in cycle, or in some cases, in a specific phase of the cell cycle. Thus, the limiting toxicity of the majority of anti-cancer agents is the result of anti-proliferative effects on the normal host tissues that are rapidly dividing, such as bone marrow, gut mucosa and hair follicle cells. Because of dose limiting host toxicity, treatment may have to be continued at levels that are below the dose that would be required to kill all viable tumour stem cells. This relative non-specificity of anti-cancer agents has been long recognised and attempts to improve tumour targetting and allow greater doses to be administered have been numerous [35].

Prodrugs are chemicals that are toxicologically and pharmacodynamically inert but may be converted in vivo to active products. NAD(P)H,quinone oxidoreductase 2 (NQO2) [36] is one such enzyme which has been shown to activate the prodrug CB 1954 [37] to its cytotoxic species a bifunctional alkylating agent, in the presence of a vitamin B derived co-factor (EPO152R) [33] (see Fig. 3). As previously described, CB1954 is also a substrate for the bacterial enzyme, ntr, used in our gene therapy approach.

The finding that NQO2 can catalyse activation of CB1954 only in the presence of this co-factor, opens up a completely novel therapeutic avenue. A range of pre-clinical studies have shown that activation of NQO2 can sensitise a wide range of cancer cell lines in vitro and in vivo, when grown as xenografts by more than 10,000-fold. Because the activated derivative of CB1954 has a very short intracellular half-life (seconds) very little of the toxic species is sufficiently stable to escape into the bloodstream and cause side effects [38,39].

The activity of the target enzyme, NQO2 has been shown to be greatly elevated in some human cancers, especially HCC, establishing a convincing basis for tumour selectivity (Table 1). Given the provenance of CB 1954, the favourable distribution of NQO2 and the lack of acute toxicity of the co-factor EP-0152R, this prodrug approach is low risk and suitable for clinical development.

Traditionally, phase I clinical trials, the portal of entry of any novel cytotoxics into the clinic, and depend heavily on observation of empirical toxicity. However, given the mechanistic basis on which this prodrug treatment is predicted, there is an opportunity to include pharmacodynamic endpoints. The following trial outline illustrates how experimental anti-cancer agents might be taken into proof-of-principle phase I trials and provides a general model for developing novel anti-cancer agents for HCC:

- Establish the maximum tolerated dose (MTD) of CB1954 and EP-0152R, administered every 3 weeks by intravenous infusion.

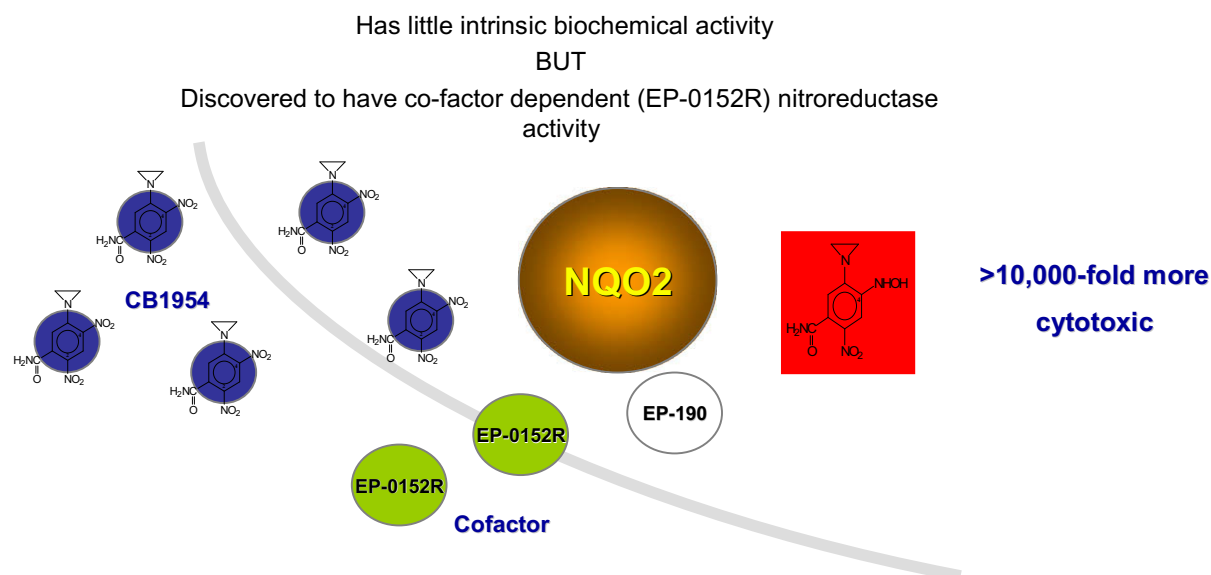


Fig. 3. NAD(P)H quinone oxidoreductase 2 (NQO2).

**Table 1**  
NQO2 concentration in the liver.

Tissue	No. samples	NQO2 activity (U/g protein $\pm$ SEM)	Range	Comment
Liver of cancer patient	6	2.11 $\pm$ 0.19	1.28–2.26	Chemoresistant tissue
Liver of healthy donor	6	2.47 $\pm$ 0.22	1.88–3.11	Chemoresistant tissue
Hepatoma	6	9.13 $\pm$ 0.95	6.17–12.63	NQO2 activity up to 10 $\times$ higher than matched normal tissue

- Determine and establish the toxicity profile of CB1954 and EP-0152R and to identify the dose limiting toxicity (DLT).
- Propose a safe dose for phase II evaluation.
- Investigate the pharmacokinetic (PK) and pharmacodynamic behaviour of CB1954 and EP-0152R in man using plasma and peripheral blood lymphocytes.
- Document possible anti-tumour activity in patients using pharmacodynamic assays in tumour biopsies.

Approximately 18–40 patients with solid tumours will be entered into this study with efforts made to enrich this population with HCC patients. The starting dose will be 12 mg/m<sup>2</sup> (CB1954) administered as a 15 min IV infusion at the midpoint of a 4 h IV infusion of EP-0152R (starting dose of 200 mg/m<sup>2</sup>). Initially one patient will be enrolled per dose level and the dose of EP-0152R will be doubled until drug related Grade 2 toxicities are observed. Then dose levels of EP-0152R will be escalated by 33% increments in order to achieve and maintain plasma concentrations of >100  $\mu$ M, similar to the concentration capable of maximally enhancing NQO2 in vitro.

If there is no associated toxicity, despite dose escalating EP-0152R to achieve plasma concentrations of >100  $\mu$ M then the trial will be continued by dose escalating CB1954 by 33% increments in cohorts of three patients, until the maximum tolerated dose is reached.

Descriptive PK studies will be performed for both agents, singly and in combination, to determine if there is any correlation with toxicity, biochemistry etc. Plasma concentrations will be compared with in vitro and in vivo concentrations found to be effective in pre-clinical studies. The COMET assay will be used to estimate CB1954 induced DNA damage in tumours biopsied 24 h post CB1954 and 24 h post administration of the combination treatment to offer mechanistic proof of principle.

Following definition of the dose-schedule for CB1954 and EP-0152R, this targeted prodrug combination will go forward to phase II trials recruiting only HCC patients in the UK and the Gambia.

## 8. Conclusions

This article has highlighted the areas of research that look promising for the future treatment of primary liver cancer. These are all at an early stage of clinical development but show sufficient promise to warrant further investment and progression to large, well powered randomised clinical trials. It is equally obvious that the global clinical community must unite to form the necessary collaborative study groups to take these agents forward, singly and in combination, using sophisticated factorial trial designs which are likely to require several hundred patients.

## Conflicts of interest

None declared.

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