NUC-1031 in biliary tract cancer: from bench to bedside and back?

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Abstract

In preclinical models of biliary tract cancer, NUC-1031 showed less potency than gemcitabine, no correlation with potential biomarkers and only moderate additive interaction in combination with cisplatin. These findings should prompt further careful pharmacological and translational studies to better define the purported therapeutic advantage of NUC-1031 over gemcitabine. That would be a more cautious approach than the phase III clinical trial which is planning to enrol 828 patients with biliary tract tumours to compare gemcitabine/cisplatin “conventional” treatment with or without NUC-1031.

Biliary tract cancer is an uncommon disease with a notoriously poor prognosis [1]. The only chance of curation is when a patient is eligible for radical surgical resection. However, most are not eligible, as 70–80% of the patients present with advanced disease, for whom only systemic chemotherapy is considered a viable treatment option [2]. The most commonly used treatment consists of gemcitabine and cisplatin and is based on a seminal phase III trial performed by Valle and collaborators [3]. In this phase III study, 410 patients with unresectable or metastatic biliary tract cancer were randomly assigned either gemcitabine and cisplatin, or gemcitabine monotherapy. The primary endpoint of this study was overall survival, with most patients discontinuing treatment due to disease progression. After a follow-up of 8.2 months, the median survival rate of patients in the gemcitabine and cisplatin group was 11.7 vs. 8.1 months in the gemcitabine monotherapy group (HR 0.64; 95% CI 0.52–0.80; $p < 0.001$). This study, supported by subsequent smaller studies reviewed by Park and collaborators [4],

shows that systemic therapy by means of gemcitabine and cisplatin can be beneficial. However, considering the continuously poor prognosis, further research into appropriate and effective chemotherapeutic agents is warranted.

In this issue of Cancer Chemotherapy and Pharmacology, Arora and collaborators evaluate the potential of NUC-1031 in preclinical models of biliary tract cancer [5]. NUC-1031 is a pro-drug based on an aryloxy phosphoramidate derivative of gemcitabine, which was first developed as part of a new class of anti-cancer drugs, termed ProTides, to bypass potential mechanisms of resistance to gemcitabine [6]. Gemcitabine (2',2'-difluorodeoxycytidine, dFdC) is an antimetabolite ranking among the most extensively prescribed drugs in global clinical oncology [7]. Because of its hydrophilic nature, gemcitabine needs a transporter to pass the cellular membrane, predominantly the human equilibrative nucleoside transporter 1 (hENT1). Inside the cell, gemcitabine undergoes a rate-limiting activation step catalysed by deoxycytidine kinase (dCK) and is then phosphorylated to its active diphosphate and triphosphate metabolites (dFdCDP and dFdCTP). These inhibit ribonucleotide reductase (RR) and DNA synthesis, respectively. Conversely, gemcitabine inactivation to 2',2'-difluoro-deoxyuridine (dFdU) is driven by cytidine deaminase (CDA), which is expressed ubiquitously and at very high levels in the liver [8]. Several studies have suggested that hENT1, dCK, RR and CDA are critical factors for gemcitabine activity because their deficiency or over-expression/activity has been involved in acquired resistance/sensitivity in various preclinical models [7]. The expression of hENT1 has been suggested to be a potential predictive biomarker of gemcitabine efficacy in

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different tumour types, including cholangiocarcinoma [9]. However, the results of these studies in different tumour types are conflicting, suggesting the need for a validated standardized technique to assess hENT1 expression [10, 11]. Because of the phosphoramidate moiety on the gemcitabine monophosphate group, NUC-1031 has the following advantageous properties over its parent molecule:

1. it is more lipophilic and enters in tumour cells by passive diffusion, and it is, therefore, less dependent on hENT1,
2. following cellular uptake, the phosphoramidate group is cleaved, releasing dFdCMP, which is rapidly converted to dFdCDP and dFdCTP, bypassing the phosphorylation step by dCK,
3. this agent does not undergo systemic deactivation by CDA.

These three factors should help to overcome resistance to gemcitabine [6]. As a result, a higher accumulation of dFdCTP (at least 100-fold) was found in human lymphocytes in patients treated with NUC-1031 in a Phase I trial compared to various schedules of gemcitabine [12, 13].

In the present study, the authors investigate the preclinical activity of NUC-1031 compared to gemcitabine and evaluated the potential mechanisms of action in multiple cellular assays. This was done by employing a panel of 10 biliary tract cancer cell lines. Furthermore, they investigated candidate biomarkers that could predict sensitivity or resistance, including hENT1, RR and CDA expression, and performed combination studies with cisplatin. In addition, *in vivo* efficacy of NUC-1031 and gemcitabine was evaluated, using a cholangiocarcinoma patient-derived xenograft with high CDA transcript levels. These efforts showed that NUC-1031 had less potency than gemcitabine and showed only moderate additive interaction in combination with cisplatin. The level of additive interaction was similar to the additive interaction of gemcitabine and cisplatin. This is possibly due to the low concentrations and the simultaneous exposure of the drugs used in the present study, as a sequential schedule in which gemcitabine is administered before cisplatin has shown a higher synergy in various other study models [7]. However, the efficacy of both drugs was not correlated to the expression levels of the potential biomarkers, undermining the rationale of using NUC-1031 to enhance the effects of gemcitabine and overcome putative mechanisms of resistance. In addition, despite equivalent efficacy, the body weight loss of the mice treated with NUC-1031 was significantly higher and two out of five mice died, whereas zero died in the equimolar gemcitabine cohort. This effect of weight loss and death may be related to the higher accumulation of dFdCTP in normal cells, as was seen for peripheral lymphocytes in the Phase I study [12, 13], and warrants further investigation.

Most importantly, patients are now being recruited for the largest ever-planned phase III clinical trial in biliary tract cancer, which will compare treatment consisting of NUC-1031 combined with cisplatin to gemcitabine combined with cisplatin. The author's findings suggest that further studies are needed to determine the effects of NUC-1031 before implementation in such a clinical trial.

As bile duct carcinomas are rare, it is extremely difficult to perform thorough randomized controlled clinical trials. When randomized trials are performed this should preferably be done on the basis of thorough and evidence-based preliminary research. There are few available publications that assess NUC-1031 both *in vitro* and *in vivo* in cancer in general, and in biliary tract cancer specifically. The first studies on the application of ProTide technology on anti-cancer drugs led to the development of pro-drug NUC-1031, which showed its ability to overcome drug resistance in preclinical models, including multiple cellular assays and *in vivo* in pancreatic cancer xenografts [6]. In 2018, two preliminary studies reported controversial results on the clinical use of NUC-1031. One of these studies compared NUC-1031 with gemcitabine in patients with advanced pancreatic carcinoma and was suspended due to the minor potential of NUC-1031, based on unpublished information provided by Arora and collaborators [5]. The other study, named ABC-08, combined NUC-1031 with cisplatin and compared this to gemcitabine and cisplatin combination therapy in a small group of patients with biliary tract cancer. An objective response rate of 64% with 1 complete response and 6 partial responses in 11 biliary tract cancer patients was found [3, 15, 16]. The biggest randomized clinical trial that is underway for biliary tract cancer which has started recruiting patients in the United States of America, aiming to enrol 828 patients [ClinicalTrials.gov Identifier: NCT04163900], is based upon this open-label phase 1b study. As is common in studies of rare malignancies, the number of potential patients in this study is small and combined with the limited amount of adequate preliminary research, it is debatable whether this is a safe basis for such a substantial clinical trial for patients with biliary tract cancer. A preferable alternative would be to establish the efficacy of NUC-1031 in more appropriate *in vivo* models before patient recruitment.

In addition, the identification of biomarkers that are associated with sensitivity and resistance to NUC-1031 will be of value to further define the clinical deployment of this agent. With these biomarkers, we can potentially improve our understanding of the mechanisms of resistance to this drug and thereby select the most appropriate therapy for each patient. Studies on these biomarkers should be informed by current findings on emerging genetic and epigenetic biomarkers which are being evaluated in biliary tract cancers [16, 17]. An ideal panel of these predictive/prognostic biomarkers should facilitate the choice of the best

systemic treatment and be drawn from minimally invasive samples, such as biliary brush cytology and blood. Liquid biopsies are particularly appealing in biliary tract cancers because of the limited availability of tissue biopsies as well as the fact that they offer the potential to (1) characterize tumour heterogeneity and (2) monitor tumour progression and response to treatment.

In conclusion, in light of the study by Arora and collaborators [5], further research should be conducted on NUC-1031 and other gemcitabine derivatives before entering clinical studies. This is due to the lack of preclinical data surrounding its efficacy. A preferable type of evaluation should focus on solid preclinical data characterized by the identification of biomarkers for prediction of sensitivity and resistance to NUC-1031. This type of data will be of value to further define the clinical deployment of this agent, which in turn will be used to treat patients with biliary tract cancer more effectively.

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Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

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