Experience in Phase I Trials and an Upcoming Phase II Study with uPA Inhibitors in Metastatic Breast Cancer

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Introduction

The majority of advanced solid tumours are only modestly affected by conventional chemotherapy. The most widely utilised chemotherapeutic agents exert their effects by interacting directly with DNA, with cellular enzymes involved in DNA replication such as topoisomerases, or with subcellular structures such as microtubules. The efficacy of these agents is limited in part by the poor selectivity of the drugs for malignant versus normal tissue. This lack of specificity also leads to toxicity. Naturally occurring or acquired drug resistance further limits their efficacy. In addition, most cancer deaths are not caused by the primary tumour but rather result from metastases. Although the underlying mechanisms for metastases are not yet fully understood, a large body of research has shown that the urokinase-type plasminogen activator (uPA) system plays a significant role in this process.

Tumour invasion and metastases depend on the capacity of tumour cells to coordinate cancer cell migration, invasion of cancer cells into surrounding tissues, access to blood and lymphatic vessels and adhesion to and invasion through endothelium, allowing colonisation at distant sites in the organism. This complex scenario requires the concerted and regulated expression of pericellular proteolytic systems, integrins and adhesion proteins.

Degradation of proteins in basement membranes and extracellular matrix is the prerequisite for the invasion of cells and the formation of metastases. It is mediated by various pericellular proteolytic enzymes including serine proteases, metalloproteinases and cystein proteases. There is abundant experimental evidence that the plasminogen activator system plays an essential role in these processes [1–8]. It consists of two serine proteases, uPA and tissue-type plasminogen activator (tPA), the cell surface uPA receptor (uPAR) and the plasminogen activator inhibitors PAI-1 and PAI-2. uPA is the enzyme with major influence on cancer-related processes [9]. Besides its proteolytic activity, uPA, in concert with uPAR, also mediates mitogenic, adhesive and migratory processes [10].

Clinical studies have demonstrated the relevance of uPA, uPAR and PAI-1 in malignant tumours such as ovarian, gastric, pancreatic, head and neck, breast, colon and other cancers. Elevated levels of these factors correlate with increased malignant potential and poor patient outcome [1, 11–14]. These clinical data underline the essential role of the uPA system in tumour biology and suggests that inhibition of its components such as uPA or uPAR may reduce the metastatic potential of cancer cells.

Studies of the invasion markers, uPA and its inhibitor PAI-1, in breast cancer have provided strong evidence of their prognostic value [15–17]. A randomised trial of uPA/PAI-1 in lymph node-negative breast cancer showed that patients with positive expression benefited from adjuvant cyclophosphamide, methotrexate and 5-fluorouracil (CMF) [18]. Larger confirmatory trials support the independent prognostic power of these proteolytic markers [19, 20]. Furthermore, recent data have shown that the combination of both factors, uPA and PAI-1, is superior to either alone with regard to risk stratification [21]. Recently, uPA/PAI-1 expression has also been demonstrated to have prognostic significance independent of HER2/neu expression in lymph node-negative breast cancer [22].

A high level of uPA in the tumour tissue of patients with various malignancies such as breast, ovarian, pancreatic and gastric cancer is associated with an unfavourable course of disease, whereas low levels of uPA tend to correlate with a more favourable prognosis. These data have supported uPA as a significant prognostic factor according to the European Organisation for Research and Treatment of Cancer (EORTC). In addition, the American Society of Clinical Oncology (ASCO) has determined that these data meet the criteria of uPA as both a prognostic and predictive factor in early-stage breast cancer [23]. It also identifies as a potential therapeutic target.

Mechanism-based anticancer agents such as WX-UK1 that target the malignant process more directly may prove to be useful agents in their own right, as well as offering the potential to enhance the efficacy of established cytotoxics. Two examples of the success of this approach in advanced breast cancer have already translated into a significant clinical benefit. First, improvements in survival and response rates have been observed when the HER2/neu-targeted therapy, trastuzumab, was combined with paclitaxel [24]. Furthermore response rates for paclitaxel plus bevacizumab exceeded those of either agent alone [25].

It is thought that many of the molecularly targeted agents will have their greatest impact in combination with cytotoxics and/or other biological therapies, strategically attempting to target malignant cells by perturbing multiple pathways to optimise tumour control and improve both the quality and duration of life. Preclinical investigations combining WX-UK1 with epirubicin and 5-fluorouracil (5-FU) have demonstrated additivity whereas similar experiments with paclitaxel did not, likely reflecting their different mechanisms of action.

Capecitabine (Xeloda®, Hoffman-La Roche, Nutley, NJ, USA) is an oral flouropyrimidine that was rationally designed to generate 5-FU preferentially in the tumour tissue and to mimic continuous infusion of 5-FU. Tumour selectivity is achieved by taking advantage of the

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Table 1. Summary of phase I clinical trials with WX-UK1	Study	Study title
	Phase I: monotherapy	'A double-blind, randomized, three-way cross-over, placebo-controlled, single intravenous dose phase I study to investigate the pharmacokinetics, pharmacodynamics, safety and tolerability of increasing doses of WX-UK1 in healthy male subjects (WX/50–001)'
	Phase I/IIa: monotherapy	'A phase I/IIa open label safety and tolerability study of repeated administrations of the serine protease inhibitor WX-UK1 in patients with advanced solid tumors (WX/50–003)'
	Phase Ib: monotherapy	'A phase Ib open label safety and tolerability study of repeated administrations of the serine protease inhibitor WX-UK1 in patients with advanced head and neck tumors (WX/50–004)'
	Phase I: drug combination	'Phase I study of the antiproteolytic targeting therapy: urokinase plasminogen activator (uPA) inhibitor WX-UK1 in combination with capecitabine in advanced solid malignancies (WX/50–005)'

Table 2. Dose escalation scheme for weekly WX-UK1 infusions given in combination with a fixed dose (2000 mg/m²/d) of capecitabine	Dose level	Dose (mg/kg)	% Difference from prior dose	Dose rate[a] (mg/kg/min)	Solution concentration[b] (mg/ml)	Total amount of WX-UK (mg)[b]	No. of infusions per course
	1	0.3	NA	0.0025	0.024	24	3
	2	0.6	100	0.0050	0.048	48	3
	3	1.0	67	0.0083	0.080	80	3
	4	1.5	50	0.0125	0.120	120	3
	5	2.1	40	0.0175	0.168	168	3
	6	2.8	33	0.0233	0.224	224	3
	7	3.5	25	0.0292	0.280	280	3

[a]Intravenous infusions were given via a central or peripheral catheter. The total volume of 1000 ml was given over 2 h at a rate of 500 ml/h by means of an infusion pump.
[b]For an 80-kg subject.

relatively higher levels of thymidine phosphorylase in many human tumours compared with healthy tissue. Clinical trials have demonstrated that the single agent capecitabine is an active and tolerable oral treatment in metastatic breast cancer (MBC) that has progressed during or after treatment with anthracyclines and taxanes, with response rates of 20–26% and a median survival in excess of 1 year. More recently, capecitabine plus docetaxel resulted in a superior time to progression and survival compared with docetaxel alone in patients with advanced breast cancer [26].

Capecitabine is a versatile well-tolerated orally administered drug that is widely used in the treatment of advanced breast cancer and is likely to have broad utility in other solid tumours such as colorectal cancer. These features make it an attractive chemotherapy partner in the development of combinations with novel targeted therapies such as WX-UK1. In this review, we will summarise completed clinical trials with uPA inhibitors and preview an upcoming phase II study.

WX-UK1 Clinical Trials (Completed)

The uPA inhibitor programme is comprehensive. Table 1 summarises phase I clinical trials using the intravenous formulation of the uPA inhibitor WX-UK1. This includes a phase I trial of monotherapy in a single-rising-dose study in healthy volunteers, a multiple-rising-dose phase I/II study in patients with advanced tumours, and a phase Ib study of multiple rising doses in patients with advanced head and neck cancers. In the dose range tested (0.3–2.1 mg/kg) no significant adverse events, dose-limiting toxicities (DLTs) or safety issues were observed. Plasma C_{max} and area under curve (AUC) were in line with other WX-UK1 clinical studies and largely dose linear. Doses of 0.3 mg/kg or higher achieved concentrations of WX-UK1 in tumour tissue associated with anti-tumour effects in animal studies.

We have also completed a phase Ib study of multiple rising doses of WX-UK1 in combination with capecitabine in patients with advanced malignancies at the Fox Chase Cancer Center. The study was designed to assign escalating doses of WX-UK1 in combination with a fixed dose of capecitabine in advanced malignancies of diverse histologies. Seven dose levels were planned. Each treatment course was a 3-week cycle with 14 days of oral capecitabine and a once-weekly WX-UK1 infusion for 3 weeks. Cycles were repeated until disease progression or unacceptable toxicity. The study endpoints were safety of the drug combination, pharmacokinetics (PK) of WX-UK1 and capecitabine, to determine the recommended phase II dose for further study, and to evaluate for preliminary evidence of efficacy. Doses of WX-UK1 ranged from 0.3 to 2.8 mg/kg (table 2). The goal was 3 patients per cohort with 6 patients for the last cohort.

Treatment duration ranged from 1.5 to 7.5 months. Escalation stopped at 2.8 mg/kg because PK analysis of the patients' plasma demonstrated that adequate drug exposure had been achieved based on comparison with animal pharmacological data.

Phase I WX-UK1/Capecitabine Study

For this study, 38 patients were screened and 30 patients were allocated to the treatment. Of these, 23 patients completed at least cycle 1. The average number of cycles per patient was 4.8. The maximum individual study duration was 15 completed courses. A total of 110 treatment courses were given.

Table 3. Summary of phase I clinical trials with WX-671

Study	Study title
Phase I: monotherapy	'An open label phase I study to investigate the bioavailability and pharmacokinetics of oral WX-671 given at three dose levels pre- and post-prandially to healthy male subjects (WX/60–001)'
Phase I: monotherapy	'A randomized, double blind, placebo controlled phase I study designed to evaluate the tolerability and pharmacokinetic (PK) profile of three multi-dose regimens of oral WX-671 in healthy male subjects (WX/60–002)'
Phase Ib: monotherapy	'Phase I study to investigate the pharmacokinetics, safety and tolerability of multiple oral doses of the serine protease inhibitor WX-671 in patients with advanced head and neck carcinoma (WX/60–003)'
Phase I: monotherapy	'14C-WX-671 – a phase I, open label study of absolute bioavailability, metabolism, and excretion following a single oral dose to healthy male subjects (WX/60–005)'

Safety and Tolerance

The combination of WX-UK1 and capecitabine was safe and well tolerated. 7 patients discontinued treatment prior to completion of cycle 1, 5 due to serious adverse events (SAEs) all of which were unrelated to the study drugs, and 2 due to non-serious adverse events (AEs). Another 4 patients discontinued therapy prior to the first WX-UK1 infusion.

The incidence of AEs was unrelated to the WX-UK1 dose level. No SAE related to the study drugs was observed. There were no acute systemic, allergic, or local intolerance reactions (exception: 1 case of refractory diarrhoea due to capecitabine). AEs were mostly gastrointestinal, respiratory, skin and subcutaneous, or lab events mostly typical of capecitabine treatment (e.g. hand and foot syndrome, diarrhoea) or underlying disease. These were mostly of mild or moderate intensity (common toxicity criteria (CTC) 1 or 2) and had no explicit relationship to WX-UK1.

Efficacy

A higher than expected number of cycles/patient suggests clinical benefit response as observed by prolonged stable disease. The histologies of tumours included breast (10), pancreas (3), biliary tract (3), unknown primary cancer (3), rectum (2), cervix, gastric (1), adenocystic (1), thyroid (1), duodenum (1), oesophageal (1), and lung tumours (1). There were 20 evaluable patients, among which 4 patients had partial responses (PR), 7 patients had stable disease (SD) with an average of 4.7 months on study average (3–9 months), at a dose range of 0.3–2.1 mg/kg, and 9 patients experienced progressive disease (PD) of their tumours.

Evidence of efficacy was observed in 4 patients with responses, particularly in some whose liver metastases disappeared. In addition, in MBC patients, more cycles were delivered than expected. Of the 4 PRs, 3 were in MBC and 1 was observed in a patient with a carcinoma of unknown primary. All three MBC patients had tumours that were ER positive, PR positive and HER2 negative. All also had prior hormone therapy and chemotherapy for metastatic disease. One patient with MBC had a PR of liver metastases at 0.3 mg/kg WX-UK1 and received a total of 9 cycles of therapy. Another patient with MBC had disease in the liver and bone, had a near complete response (CR) in the liver at a dose level of 1.5 mg/kg and received 16 cycles of treatment. The third MBC patient with a PR had disease involving bone, mediastinum, lymph nodes and liver at the 2.8-mg/kg dose level and received 14 cycles of combined therapy. Finally, the patient with carcinoma of unknown origin was a male with metastases involving lymph nodes of the neck, mediastinum, and para aorta treated at the 2.8-mg/kg dose for 14 cycles.

Pharmacokinetics

WX-UK1 plasma AUCs appeared to be dose-linear. There were no reciprocal drug-drug interactions observed at the doses assessed and there was no negative influence of WX-UK1 on the standard therapy. In summary, we can conclude from the WX-UK1 phase I programme that WX-UK1 is safe and well tolerated in combination with capecitabine. Achievement of drug levels in plasma and tumour was adequate to achieve antitumour effects. In addition, there were encouraging signs of activity in MBC patients, 3 PR in MBC and 1 PR in a tumour of unknown primary. Moreover, more than the anticipated number of cycles were delivered per patient, particularly in MBC patients (average = 7). Responses were observed irrespective of dose, and one patient at 1.5 mg kg had a PR.

Future Directions

Since capecitabine is an orally delivered drug, combining it with an oral formulation of a biological agent would have numerous advantages including patient acceptance, convenience, and a potential improvement in quality of life. WX-671 is an oral pro-drug of WX-UK1 and is converted to active WX-UK1. Table 3 summarises the completed trials with WX-671.

Extensive preclinical safety studies of acute toxicology, local tolerability, 4-week subchronic toxicity, 3-month subchronic toxicity in rats/dogs, safety pharmacology, genotoxicity, in vitro metabolism, and animal metabolism have all demonstrated good safety and tolerability. Phase I WX-671 monotherapy clinical trials have been completed in single and multiple rising doses in healthy volunteers and in a neoadjuvant study of head and neck cancer.

PK studies show successful conversion of WX-671 to WX-UK1 and delivery of sustained WX-UK1 levels over 24 h, thus supporting that the pro-drug concept is applicable in man. In addition, there are large orders of magnitude between the curves for WX-671 and WX-UK1 and the bio-distribution showed more WX-UK1 in tissue than in plasma, which is consistent with animal data showing 1–2 orders of magnitude more WX-UK1 in tissue. Escalating oral doses of WX-671 have been shown to lead to increases in available plasma levels of WX-UK1, and daily oral dosing of WX-671 delivers WX-UK1 AUCs in the same range as in the WX-UK1 studies.

The pro-drug WX-671 was also found to be efficient at delivering equivalent AUCs without the high C_{max} seen with WX-UK1. In summary, daily oral WX-671 administration delivers equivalent levels of the active metabolite WX-UK1, repeated dosing is safe and well tolerated, and a stable capsule formulation has been developed.

Current Clinical Trials with WX-671

Ongoing clinical trials investigating WX-671 include a phase II study in pancreatic cancer and a phase II double-blind, multicentre, randomised study of the combination of oral WX-671 plus capecitabine compared to capecitabine alone in first-line Her2-negative MBC (WX/60–006). This phase II study in MBC is chaired by Lori J. Goldstein and Nadia Harbeck. The plan is to randomise 100 evaluable patients. The primary endpoint is the comparison of progression-free survival of WX-671 plus capecitabine vs. capecitabine monotherapy. The secondary endpoints include rates of clinical benefit (CR + PR + SD) at 12 and 24 weeks, overall survival, safety, and limited PK to include full PK for 6 patients per arm to exclude drug-drug interactions and trough levels of WX-671/WX-UK1 for all patients. The phase II MBC Study WX/60–006 has received regulatory approval in several countries and recently began accrual.

Conclusion

Advances in biologically targeted therapy in oncology will lead to more rationally designed treatment and tumour selection. Targeting the uPA pathway in the treatment of malignancies is supported by robust preclinical models. Clinical trials of inhibitors of the uPA pathway have thus far demonstrated that the current agents are safe and tolerable. Current trials investigating the potential efficacy of such agents, which in this case has also been formulated into a convenient oral dose, hold much promise for the future.

Acknowledgements

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