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Induction of CDC2 Phosphorylation in Skin Biopsies from Patients with Solid Tumors Undergoing DNA-Damaging Chemotherapy

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[Clinicaltrials.gov; NCT00800865]

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I. Introduction

There remains a significant unmet need for more effective cancer ther apies that can be developed quickly and safely. The identification of biomarkers that can be used in early clinical trials of potential anti-cancer agents remains a critical component of cancer drug development, so that doses used in therapeutic trials are maximally engaging the therapeutic target and provide a robust evaluation of the mechanism. A series of checkpoints exist within the cell cycle to prevent damaged cells from undergoing mitosis. Promoting checkpoint escape when used in combination with cytotoxic standard-of-care regimens is one potential mechanism under investigation in evaluating potential anti-cancer agents. Pathways within G2 arrest are logical therapeutic targets, as cytotoxicity may be improved by permitting DNA-damaged cells to undergo mitosis (Shapiro & Harper, 1999). Wee1 kinase, a regulator of the G2/M checkpoint (Kawabe, 2004; O'Connell, Walworth, & Carr, 2000), phosphorylates CDC2 in response to genotoxic injury, leading to cell arrest and damage repair (Mizuarai et al., 2009). Agents that inhibit Wee1 allow progression of damaged cells to mitosis, progressing to cell death via apoptosis (Mizuarai et al., 2009). Wee1 inhibitors would augment current chemotherapeutic regimens, and Wee1 inhibitors, such as PD0166283 (Hashimoto et al., 2006; Li, Wang, Sun, & Lawrence, 2006; Wang et al. 2001) and MK-1775, a direct substrate of Wee1 kinase in cells (Hirai et al., 2010; Leijen, Beijnen, & Schellens, 2010) have been shown to sensitize cancer cells to cytotoxic agents.

Therapies, such as chemotherapeutic agents that cause damage to DNA, engage the G2/M checkpoint in the cell cycle leading to increased phosphorylation of CDC2 (pCDC2). MK-1775 inhibits phosphorylation of CDC2 at Tyr15 (CDC2Y15). *In vivo*, MK-1775 potentiates tumor growth inhibition by DNA-damaging chemotherapeutic agents such as gemcitabine, carboplatin, and cisplatin. The enhancement of anti-tumor effect by MK-1775 was well correlated with inhibition of CDC2Y15 phosphorylation in tumor tissues and skin hair follicles (Hirai *et al., 2009)*. pCDC2 is normally measured by Western blot (Hirai *et al.*, 2009), gene expression (Mizuarai *et al.*, 2009; Yun *et al.*, 1999), or via qualitative immunohistochemistry methods. Since pCDC2 is a substrate of Wee1 kinase, it is a logical target engagement biomarker to use in the development of Wee1 kinase inhibitors (Mizuarai *et al.*, 2009), particularly if the investigational agents will be used

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concomitantly with DNA-damaging chemotherapy. In normal skin, the measurement of Wee1 inhibitor-mediated decreases in pCDC2 is therefore most accurately assessed in the context of the expected pCDC2 induction by DNA damage from chemotherapy. The pCDC2 biomarker has been studied in preclinical animal models, but because of the species differences, biomarker qualification studies such as this must be conducted in patient populations undergoing various chemotherapies.

This study was designed to quantify pCDC2 in skin punch biopsies from patients receiving DNA-damaging chemotherapy. If a large increase was observed with chemotherapy, then abrogation of the expected increase from Wee1 inhibition could be used to assess target engagement. Immunohistochemistry staining is routinely used in the clinical diagnosis of cancer and is typically a qualitative method. This study employed a quantitative immunohistochemistry assay that was developed to measure skin pCDC2 in patients with solid tumor(s) after they received a single dose of standard-of-care mono- or combination cytotoxic agents.

II. Methods

a) Patients

Patients over 18 years of age were eligible to participate in the study if they had a solid tumor and were being treated with one of the following agents: gemcitabine, cisplatin or carboplatin monotherapy or gemcitabine/cisplatin, gemcitabine/erlotinib, gemcitabine/carboplatin, cisplatin/vinorelbine, cisplatin/pemetrexed, carboplatin/vinorelbine, or carboplatin/pemetrexed combination therapies. The chemotherapy regimen was determined by the investigator. Patients were to have a performance status of ≤2 on the Eastern Clinical Oncology Group Performance Scale (Oken *et al*., 1982) at the first visit to enroll in the study. Patients were excluded if they had undergone chemotherapy or radiotherapy within 2 weeks or biological therapy within 4 weeks prior to study entry, had not recovered from adverse events due to agents administered more than 4 weeks earlier, or were currently participating or had participated in a study with an investigational compound or device within 30 days or 5 half-lives of signing informed consent, whichever was longer. Any patient with a history, or current evidence of any condition, prior or current therapy, psychiatric disorder, or lab abnormality that could confound the results of the study, interfere with participation, or was not in the best interest of the patient was also excluded.

b) Study design

This Phase 1b, open-label, 2-part study was conducted at 4 sites in the United States and Europe. Institutional review board or ethics committee approval and informed consent were obtained prior to the initiation of study procedures. Each part of the study was to enroll approximately 15 patients; Part 2 commenced upon full enrollment of Part 1. The study duration was approximately 23 days from the first visit to the last visit for a total of 5 to 6 study visits.

c) Collection of skin biopsy samples

Each patient underwent 3 biopsies. In Part 1, patients underwent skin biopsies at baseline and at 24 and 48 hours post-chemotherapy. Skin biopsies were obtained from patients in Part 2 at 24, 32, and 48 hours post-chemotherapy. Punch skin biopsies were to be at least 3 mm and were obtained per institutional standards from hair rich areas behind the ears. The administration of cytotoxic agents followed standard-of-care dosing, route of administration, and duration. Patients in both parts returned to the clinic or to their own physician for a follow-up safety visit 7 days after the final biopsy to assess the biopsy site and remove any sutures. A follow-up phone call occurred approximately 14 days after the last visit. Safety assessments included procedurerelated adverse event collection, physical exams, electrocardiograms, vital signs, and laboratory evaluations.

d) Immunohistochemistry methods

Representative images of epidermis, hair follicle, and hair bulb samples from a patient (Part 1, number 23) are illustrated in Figure 1. A multiplex immunohistochemistry assay for total CDC2 (sc-54 antibody, Santa Cruz Biotechnology, Inc., Santa Cruz CA) and pCDC2 [Y15] (AF888 antibody, R&D Systems, Inc., Minneapolis MN) was used to evaluate formalin-fixed, paraffinembedded skin samples at Mosaic Laboratories (Lake Forest CA). Samples were stained using proprietary multiplex chromogenic immunohistochemistry methods. Vulcan Red Chromogen (Biocare Medical Inc., Concord CA) was used to stain CDC2, 3, 3' diaminobenzidine tetrahydrochloride (DAB, Dako North America Inc., Carpinteria CA) was used to stain pCDC2, and hematoxylin functioned as a counterstain. Regions of epidermis, follicles, and hair bulbs were imaged at 20 times using the Nuance FX2 Multispectral Imaging System (Cambridge Research & Instrumentation, Inc., Woburn MA) attached to a Leica DMLS2 brightfield microscope. Multispectral imaging was performed from 420 to 720 nm in 20 nm increments, and the image stack was quantitatively unmixed using spectral absorption patterns for each chromogen to produce quantitative grayscale images.

Evaluation of multiplex staining results was performed by two methods, manual review and histogram analysis. Manual review of re-colored images was performed to identify the frequency of CDC2 and pCDC2 in the cell type of interest (epidermis, hair follicles, or hair bulbs). This method also allowed the reviewer to exclude melanin or off-target membrane

staining in some specimens, which was observed with the pCDC2 antibody. Using the Nuance software, CDC2, pCDC2, and hematoxylin images were used to create a pseudo-colored darkfield composite image with CDC2 colored red, pCDC2 colored green, and hematoxylin colored blue (Figure 1B). Cells within the cell type of interest were enumerated as positive for CDC2, pCDC2, both, or neither. The percentage of cells positive for CDC2, pCDC2, and both epitopes was enumerated by a trained technician followed by secondary quality control review. Pixel-based image analysis was performed on 8-bit images using ImageJ version 1.38w (National Institutes of Health, Bethesda MD). Histogram analysis of staining intensity in the region of interest was determined for CDC2 and pCDC2, and co-localization analysis was performed to determine the percentage of the CDC2-positive area that was positive for pCDC2 at three staining intensity thresholds. All low, medium and high optical density (OD) staining thresholds were set for both CDC2 (>20, 20-70, 70-90, >90 OD units) and pCDC2 (>20, 20-40, 40-60, >60 OD units). For co-localization analysis, the percentages of CDC2-positive (>20 OD units) pixels that demonstrated all, low, moderate and high pCDC2 staining were determined.

e) Primary endpoints

A total of 16 immunohistochemistry parameters were identified in the protocol (Table 1) with two being defined for analysis in the primary objective: Parameters 3 (% CDC2 + pCDC2 [manual enumeration]) and 13 (%CDC2 [all] with pCDC2 [all] [histogram analysis]). These two parameters were chosen because they trended well with preclinical Western blot data (correlation coefficients 0.85 and 0.91, respectively). Prior to performing the statistical analysis and upon visual assessment of the raw images for Part 1 patients, Parameter 4 (proportion of total CDC2 positive cells that are positive for pCDC2 from manual enumeration) was chosen as an additional primary parameter as this parameter corrects for variability in the frequency of CDC2 positive cells across samples. The manual review of images allowed the reviewer to exclude melanin or off-target membrane staining in some specimens, which was observed with the pCDC2 antibody.

From the skin biopsy samples, several subtypes of skin tissues were identified, including, hair follicles and bulbs, cells lining the sebaceous glands, and epidermis. Distinct tissue types for analysis were not prespecified in the protocol. Epidermis was chosen as the primary tissue for analysis due to a consistent presence of sufficient material for analysis in samples before and after treatment.

Part 1 data were analyzed prior to Part 2 patients completing enrollment. We recommended (and documented in the Part 1 results memo) Parameter 4 in epidermis tissue as the preferred endpoint. Consequently, Part 2 data were analyzed based on the preferred endpoint (Parameter 4 in epidermis tissue), consistent with the Part 1 recommendations. For completeness, the results of Parameters 3, 4 and 13 are also reported.

f) Statistical methods

Statistical analyses were performed separately for each tissue type and each part of the study after log-transformation of the data. The longitudinal analysis of variance (ANOVA) model included patient and time. An unstructured covariance matrix was used to model the correlation among repeated measurements over time. As the majority of patients received gemcitabine, sensitivity analyses for patients with or without gemcitabine therapy were also performed. The sensitivity analysis model included patient, time, chemotherapy (with or without gemcitabine), and time-by-chemotherapy. Geometric means for each time point, geometric mean ratios between two time points, 90% confidence intervals, and nominal 1-sided *P*-values were calculated. Safety data were summarized for all patients who received at least one dose of standard chemotherapy during the study. Statistical analyses were performed using SAS v 9.1 (SAS Institute Inc., Cary NC). The sample size of 15 patients per study segment was to allow a precise estimate of the within patient standard deviation.

For Part 1 analysis on all patients, the multiplicity adjustment for the 3 key parameters was based on the Hochberg procedure and those significant results before adjustment ($P<0.05$) remained significant after adjustment (adjusted $P<0.05$). The adjustment was made across the parameters but separately for each of the three comparisons (24 hours versus baseline, 48 hours versus baseline, and 48 hours versus 24 hours). Part 2 analysis was based on Parameter 4 in epidermis tissue and no multiplicity adjustment was required. Only the raw *P*-values are reported here.

III. Results

a) Patient characteristics

A total of 31 patients aged 29 to 88 years were enrolled and completed the study; no patients discontinued prematurely. Nineteen (61.3%) were males and 12 (38.7%) were females. Gemcitabine monotherapy or combination therapy was administered to 12/15 patients (80.0%) in Part 1 and 11/16 patients (68.8%) in Part 2. The treatment regimens for the three patients in Part 1 that did not include gemcitabine were comprised of carboplatin monotherapy and cisplatin/pemetrexed combination therapy. The treatment regimens for the five patients in Part 2 that did not include gemcitabine consisted of carboplatin monotherapy. Patients tolerated the study well and no serious

adverse reactions were reported. Six patients (19.4%) reported a total of ten procedure-related adverse experiences including pain, bleeding, and swelling at the biopsy site. All events were of mild intensity and all but one had resolved at the end of the trial.

b) pCDC2 characterization and changes

Expression of CDC2 and pCDC2 was observed in subtypes of skin tissues including hair follicles and bulbs, cells lining the sebaceous glands, and epidermis. However, many skin specimens contained mostly epidermis but few other skin structures leading to insufficient measurements of tissue types (other than epidermis) from each patient over time. For example, patient number 23 was deficient in hair bulbs at the 24-hour time point (Figure 1A). Although induction in pCDC2 was observed in all tissue types, epidermis was the only tissue structure that had sufficient measurements across baseline and post-chemotherapy time points in the majority of the patients. For this reason, epidermis was selected as the key tissue component and results presented are from epidermis only.

Results are presented for Parameters 3, 4 and 13 in epidermis tissue. Consistent results were obtained among the primary parameters. Sample photomicrographs of CDC2/pCDC2 staining are presented in Figure 1B. Results for all three parameters in the epidermis by time point for Part 1 and Part 2 patients are presented in Table 2 and Table 3, respectively. In Part 1, Parameters 4 and 13 both showed a 1.4 fold induction from baseline to 24 hours postchemotherapy ($P = 0.012$, both parameters), and a 2.05 and 1.47 fold increase from baseline to 48 hours ($P < 0.001$, $P = 0.013$), respectively. Parameter 3 showed a 1.45 fold increase from baseline to 24 hours post-chemotherapy ($P = 0.070$) and a 4.01 fold increase from baseline to 48 hours ($P < 0.001$). Parameter 4 was significantly higher at 48 hours than that at 24 hours in both Part 1 ($P = 0.012$) and Part 2 ($P = 0.046$) of the study, suggesting pCDC2 induction continued to increase between 24 and 48 hours post-chemotherapy. Similar results were observed for the gemcitabine-treated subgroup.

In the 8 patients not receiving gemcitabine, no significant increases in pCDC2 were observed in Parameter 4: 1.06 fold increase from baseline to 24 hours ($P = 0.412$) in Part 1; 1.68 and 1.00 fold increases from 24 to 48 hours in Part 1 ($P = 0.068$) and Part 2 ($P = 0.499$). Parameter 4 results in scalp punch biopsy epidermis from all patients and the gemcitabine subgroup in both parts of the study are displayed in Figure 2, adjusted to the common 24-hour time point.

IV. Discussion

This is the first clinical study to evaluate pCDC2 with quantitative multiplex immunohistochemistry methods at multiple time points in patients with solid tumors receiving DNA-damaging chemotherapy. Immunohistochemistry is a subjective, semi-quantitative assay scored on a discrete scale. We had no previous knowledge regarding the lower limit of detection and quantitation. No reports regarding the expected magnitude or time course of pCDC2 induction in patients with or without chemotherapy existed. By developing a parameter analysis strategy, we were able to gain experience in quantitatively evaluating the pCDC2 signal in skin samples in this study.

Greater expression of both CDC2 and pCDC2 was typically observed in the hair follicles, cells lining the sebaceous glands, and hair bulbs. Ideally, there would be sufficient measurements of the same tissue types from the same patients across different time points for the assessment of changes induced by treatment. However, due to the scarce presentation of hair bulbs, very few hair bulbs in each specimen from each patient were identified. Epidermis was the only tissue type that, by itself, had sufficient measurements across baseline and postchemotherapy time points in the majority of the patients. The epidermis therefore became the tissue of choice. This determination was made based solely on evaluable tissue without any knowledge of pCDC2 results.

Whether one should combine different tissue types for the assessment of changes may depend on 1) whether or not there are sufficient measurements of the same tissue types and 2) how the measurements are combined (equal or unequal weights). In this analysis, similar conclusions from both epidermis and 'all tissue type combined' were obtained (data not shown). Restricting analysis to epidermis does not require assumptions to inform weighing of the various tissues, another reason that epidermis was the tissue type selected for analysis of pCDC2 levels. In the clinical setting where hair follicles could be reliably sampled, the sensitivity of this measure could be increased.

In preclinical studies, pCDC2 levels peaked at about 32 hours after chemotherapy administration (Hirai *et al.*, 2010). In this study, cytotoxic chemotherapy significantly induced the epidermal pCDC2 level up to 48 hours; statistically significant increases from baseline were noted at 24 hours with levels continuing to increase between 24 to 48 hours. In a preclinical study, the inhibition of CDC2 phosphorylation by MK-1775 correlated with antitumor efficacy. Although MK-1775 was not used in this study, given the magnitude of pCDC2 induction after chemotherapy administration, we believe relative decreases of pCDC2 in the presence of MK- 1775 could be measured as a tool for assessing the degree of target engagement.

In the subgroup analysis, the gemcitabine-treated group showed significant induction of pCDC2 24 and 48 hours after cytotoxic chemotherapy. In the subgroup of eight patients receiving therapeutic

regimens that did not contain gemcitabine, no significant increase in pCDC2 was observed. An increase from baseline was observed in Part 1 but no change was observed in Part 2. The reason for this observation is unknown; it may be related to the time course, sample size, or sensitivity to cisplatin/carboplatin.

In a preclinical study, MK-1775 inhibited phosphorylation of CDC2 with an EC50 value of 85 nmol/L in cells pretreated with gemcitabine, whereas EC50 values of pCDC2 inhibition for carboplatin- and cisplatin-treated cells were 180 and 159 nmol/L, respectively (Hirai *et al.*, 2009). This suggests that a higher concentration of non-gemcitabine treatment is required to achieve comparable pCDC2 inhibition. Alternatively, gemcitabine is a deoxycytidine analogue with a mechanism of action distinct from other cytotoxics, and the differential observed here may be due to its unique action on cellular regulatory processes (Plunkett *et al*., 1995).

When examining pharmaceutical R&D productivity, attrition in phase 2/3 of compound development cycles is a key cause in productivity decreases. Finding ways to reduce this attrition is a cornerstone of effective R&D planning (Paul *et al.,* 2010). The careful use of biomarkers in proof-of-concept trials augments target selection and increases the probability of success (Tan *et al.,* 2009). The methodology reported here allows for the quantitative measurement of pCDC2 and has provided recommended analysis parameters and tissue types for future studies. The study procedures were minimally invasive and were well tolerated by study participants. Our data indicate that pCDC2 is an appropriate target engagement biomarker for assessing pCDC2 inhibition in early clinical evaluations of Wee1 kinase inhibitors such as MK- 1775.

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Figure 1

Figure Legends

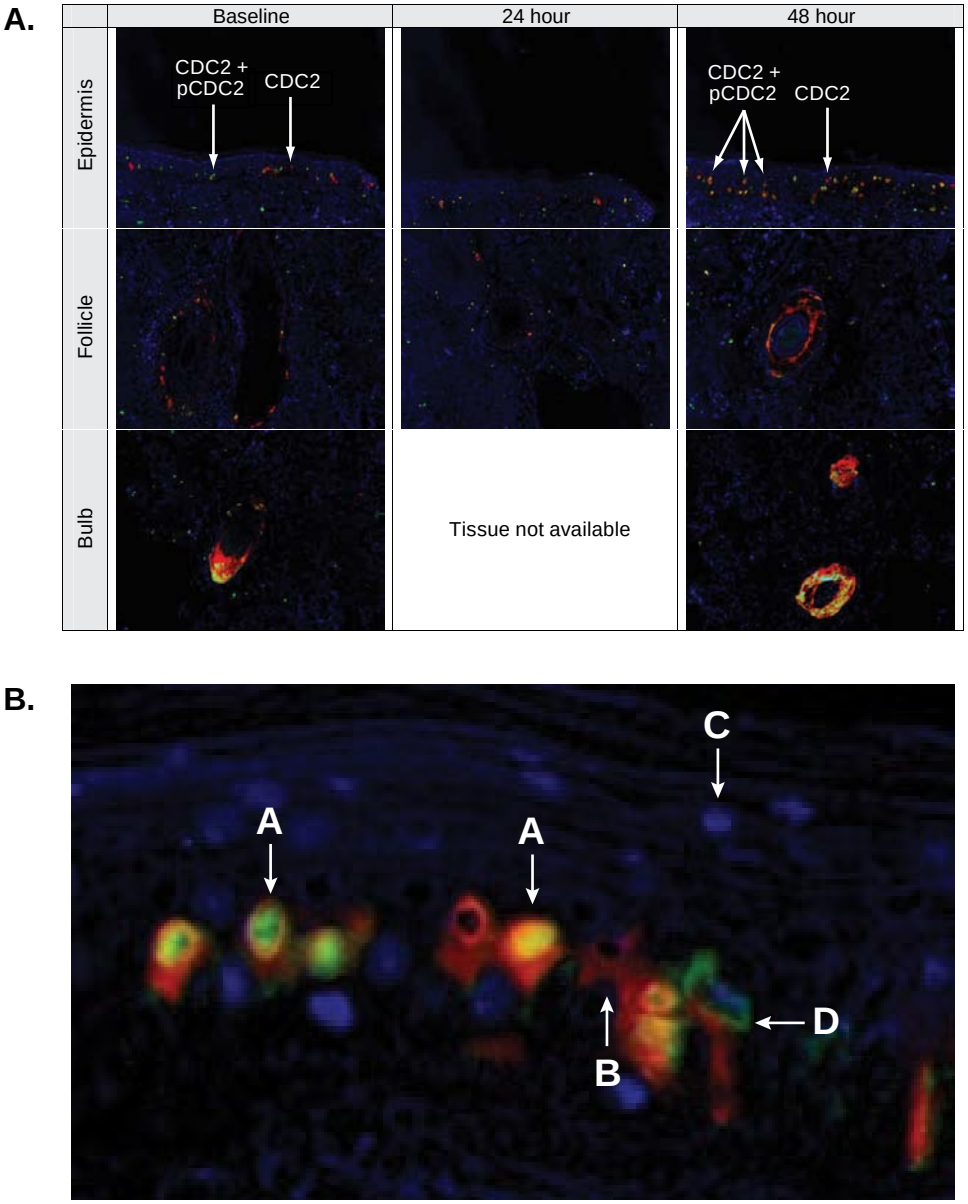

Fig. 1 : Representative images of epidermis, hair follicle, and hair bulb samples from a patien (Part 1, number 23). Panel A: Samples collected at Baseline, 24 hours and 48 hours after chemotherapy administration. Panel B: Recolored image for manual enumeration of an epidermis sample at 48 hours. A: CDC2 + pCDC2 (yellow); B: CDC2 only (red); C: negative cells (blue); D: melanin (green). For this sample, 74% of the CDC2 positive cells expressed pCDC2 (parameter 4 shown).

Figure 2

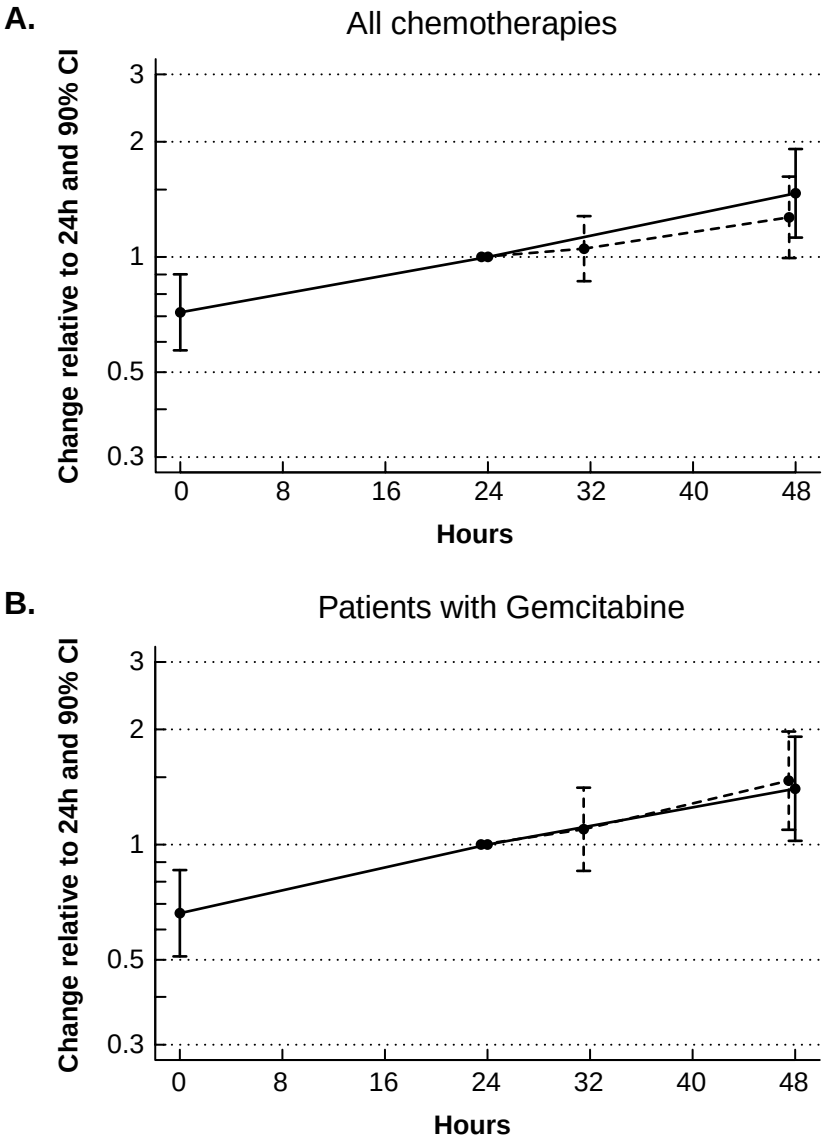

Fig. 2 : Expression of pCDC2 measured by the percentage of CDC2 positive cells that are pCDC2 positive in epidermis scalp punch biopsies for Parts 1 and 2 of the study, adjusted to the common 24-hour time point. Panel A: All patients (n=31); Panel B: Subgroup of patients taking gemcitabine (n=23). The overlap of the two lines indicates strong agreement between Part 1 and 2 results.

Table 1 : Immunohistochemistry parameters

	Description	Methodology
1	**Percentage of all cells CDC2 positive**	**Manual Enumeration**
2	**Percentage of cells pCDC2 positive**	
3[a]	**Percentage of cells CDC2 positive co-localized with pCDC2**	
4[a]	**Percentage of CDC2-positive cells that express pCDC2**	
5	**Percentage of all positive pixels for CDC2 > 20 OD**	
6	**Percentage of positive pixels for CDC2 > 20 OD and < 70 OD (weak)**	
7	**Percentage of positive pixels for CDC2 > 70 OD and < 90 OD (moderate)**	

8	**Percentage of positive pixels for CDC2 > 90 OD (strong)**	**Histogram Analysis**
9	**Percentage of all positive pixels for pCDC2 > 20 OD**	
10	**Percentage of positive pixels for pCDC2 > 20 OD and < 40 OD (weak)**	
11	**Percentage of positive pixels for pCDC2 > 40 OD and < 60 OD (moderate)**	
12	**Percentage of positive pixels for pCDC2 > 60 OD (strong)**	
13[a]	**Percentage of all cells CDC2 positive co-localized with all cells pCDC2 positive**	**Histogram and Co-Localization Analyses**
14	**Percentage of all cells CDC2 positive co-localized with weak pCDC2**	
15	**Percentage of all cells CDC2 positive co-localized with moderate pCDC2**	
16	**Percentage of all cells CDC2 positive co-localized with strong pCDC2**	

[a]Primary endpoint parameter

OD=optical density; pCDC2=phosphorylated CDC2

Table 2 : pCDC2 changes post-chemotherapy, Part 1, epidermis tissue

Parameters	n	Geometric mean or ratio[a]	90% CI	*P* value[b]	SD	Median
Parameter 3: % CDC2 + pCDC2 (manual enumeration)						
All patients in Part 1						
Baseline	15	2.2	(1.3, 3.8)		3.2	3.4
24h post-chemotherapy	14	3.3	(1.9, 5.5)		2.8	5.2
48h post-chemotherapy	15	9.0	(6.3, 12.9)		2.2	9.0
24h post-chemotherapy/baseline	14	1.45	(0.95, 2.22)	0.070	2.45	1.50
48h post-chemotherapy/baseline	15	4.01	(2.40, 6.70)	<0.001	3.09	4.89
48h/24h post-chemotherapy	14	2.76	(1.79, 4.24)	<0.001	2.49	3.26
Gemcitabine subgroup						
Baseline	12	1.9	(1.0, 3.4)		3.5	2.5
24h post-chemotherapy	11	2.7	(1.5, 4.9)		3.1	4.6
48h post-chemotherapy	12	8.5	(5.6, 12.9)		2.1	7.7
24h post-chemotherapy/baseline	11	1.46	(0.89, 2.40)	0.100	2.78	1.48
48h post-chemotherapy/baseline	12	4.52	(2.52, 8.11)	<0.001	2.93	4.72
48h/24h post-chemotherapy	11	3.10	(1.91, 5.04)	<0.001	2.25	3.21
Parameter 4: % of CDC2 that are pCDC2+ (manual enumeration)						
All patients in Part 1						
Baseline	13	24.6	(19.0, 31.8)		1.7	26.3
24h post-chemotherapy	13	34.3	(25.6, 46.0)		1.9	31.1

48h post-chemotherapy	15	50.3	(39.9, 63.5)		1.7	59.4
24h post-chemotherapy/baseline	13	1.40	(1.11, 1.75)	0.012	1.59	1.37
48h post-chemotherapy/baseline	13	2.05	(1.56, 2.69)	<0.001	1.78	2.47
48h/24h post-chemotherapy	13	1.47	(1.12, 1.91)	0.012	1.73	1.38
Gemcitabine subgroup						
Baseline	10	25.4	(18.7, 34.5)		1.8	27.4
24h post-chemotherapy	10	38.4	(27.7, 53.2)		1.9	47.1
48h post-chemotherapy	12	53.8	(41.4, 69.8)		1.5	62.8
24h post-chemotherapy/baseline	10	1.51	(1.17, 1.96)	0.008	1.64	1.56
48h post-chemotherapy/baseline	10	2.11	(1.53, 2.93)	<0.001	1.48	2.34
48h/24h post-chemotherapy	10	1.40	(1.02, 1.91)	0.040	1.59	1.36
Parameter 13: %CDC2 (all) with pCDC2 (all) (histogram analysis)						
All patients in Part 1						
Baseline	15	37.6	(26.3, 53.7)		2.2	47.5
24h post-chemotherapy	14	52.6	(42.2, 65.5)		1.6	60.5
48h post-chemotherapy	15	55.2	(42.0, 72.6)		1.8	76.7
24h post-chemotherapy/baseline	14	1.40	(1.11, 1.77)	0.012	1.68	1.24
48h post-chemotherapy/baseline	15	1.47	(1.12, 1.93)	0.013	1.83	1.60
48h/24h post-chemotherapy	14	1.05	(0.79, 1.39)	0.382	1.86	1.21
Gemcitabine subgroup						
Baseline	12	39.8	(26.4, 60.1)		2.4	51.4
24h post-chemotherapy	11	58.8	(46.8, 73.8)		1.6	63.0
48h post-chemotherapy	12	55.5	(40.3, 76.4)		1.9	78.5
24h post-chemotherapy/baseline	11	1.48	(1.13, 1.92)	0.011	1.75	1.26
48h post-chemotherapy/baseline	12	1.39	(1.02, 1.91)	0.042	1.79	1.43
48h/24h post-chemotherapy	11	0.94	(0.70, 1.28)	0.628	1.80	1.10

[a] Back-transformed least squares mean from log scale: Geometric mean for individual time points and mean ratio between two time points

[b] 1-sided *P* value

CI = confidence interval; h=hours; pCDC2=phosphorylated CDC2; SD=geometric (between-patient) standard deviation

Table 3 : pCDC2 changes post-chemotherapy, Part 2, epidermis tissue

Parameters	n	Geometric mean or ratio[a]	90% CI	*P* value[b]	SD	Median
Parameter 3: % CDC2 + pCDC2 (manual enumeration)						
All patients in Part 2						
24h post-chemotherapy	16	5.1	(3.5, 7.3)		2.3	5.6
32h post-chemotherapy	16	4.6	(3.6, 5.9)		1.7	4.2
48h post-chemotherapy	16	6.6	(4.6, 9.5)		2.3	7.4
32h/24h post-chemotherapy	16	0.92	(0.62, 1.35)	0.651	2.42	1.02
48h/24h post-chemotherapy	16	1.31	(0.91, 1.90)	0.109	2.33	1.10
48h/32h post-chemotherapy	16	1.43	(1.01, 2.03)	0.045	2.21	1.25
Gemcitabine subgroup						
24h post-chemotherapy	11	5.6	(3.6, 8.9)		2.7	5.8
32h post-chemotherapy	11	5.1	(3.8, 6.8)		1.9	4.2
48h post-chemotherapy	11	9.2	(6.4, 13.2)		2.1	9.9
32h/24h post-chemotherapy	11	0.91	(0.56, 1.47)	0.635	2.74	0.75
48h/24h post-chemotherapy	11	1.64	(1.07, 2.50)	0.031	2.48	1.69
48h/32h post-chemotherapy	11	1.80	(1.22, 2.66)	0.009	2.29	2.12
Parameter 4: % of CDC2 that are pCDC2+ (manual enumeration)						
All patients in Part 2						
24h post-chemotherapy	16	23.0	(17.4, 30.3)		1.9	28.8
32h post-chemotherapy	16	23.7	(17.5, 32.1)		2.0	22.4
48h post-chemotherapy	16	29.9	(23.2, 38.6)		1.8	30.7
32h/24h post-chemotherapy	16	1.03	(0.84, 1.27)	0.399	1.60	0.97
48h/24h post-chemotherapy	16	1.30	(1.01, 1.69)	0.046	1.80	1.31
48h/32h post-chemotherapy	16	1.26	(0.99, 1.62)	0.058	1.75	1.15
Gemcitabine subgroup						
24h post-chemotherapy	11	26.1	(18.8, 36.3)		2.0	34.0
32h post-chemotherapy	11	28.6	(20.3, 40.4)		2.1	37.4
48h post-chemotherapy	11	38.4	(30.2, 48.8)		1.6	45.5

32h/24h post-chemotherapy	11	1.10	(0.85, 1.41)	0.266	1.62	0.98
48h/24h post-chemotherapy	11	1.47	(1.08, 2.00)	0.022	1.75	1.36
48h/32h post-chemotherapy	11	1.34	(0.99, 1.82)	0.056	1.81	1.21
Parameter 13: %CDC2 (all) with pCDC2 (all) (histogram analysis)						
All patients in Part 2						
24h post-chemotherapy	16	18.7	(9.8, 35.6)		4.4	29.2
32h post-chemotherapy	16	20.3	(13.4, 30.8)		2.6	19.2
48h post-chemotherapy	16	21.4	(12.2, 37.3)		3.6	30.0
32h/24h post-chemotherapy	16	1.09	(0.69, 1.70)	0.376	2.78	0.79
48h/24h post-chemotherapy	16	1.14	(0.54, 2.40)	0.379	5.43	1.16
48h/32h post-chemotherapy	16	1.05	(0.61, 1.82)	0.437	3.52	1.03
Gemcitabine subgroup						
24h post-chemotherapy	11	31.6	(16.1, 62.3)		2.4	36.6
32h post-chemotherapy	11	29.3	(19.2, 44.7)		2.1	26.0
48h post-chemotherapy	11	30.8	(16.5, 57.6)		2.1	32.9
32h/24h post-chemotherapy	11	0.93	(0.54, 1.60)	0.595	2.22	0.67
48h/24h post-chemotherapy	11	0.97	(0.39, 2.44)	0.520	2.65	1.06
48h/32h post-chemotherapy	11	1.05	(0.53, 2.10)	0.451	1.96	1.00

[a] Back-transformed least squares mean from log scale: Geometric mean for individual time points and mean ratio between two time points

[b] 1-sided *P* value

CI=confidence interval; h=hours; pCDC2=phosphorylated CDC2; SD=geometric (between-patient) standard deviation