Race and smoking status associated with paclitaxel drug response in patient-derived lymphoblastoid cell lines

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The use of ex-vivo model systems to provide a level of forecasting for in-vivo characteristics remains an important need for cancer therapeutics. The use of lymphoblastoid cell lines (LCLs) is an attractive approach for pharmacogenomics and toxicogenomics, due to their scalability, efficiency, and cost-effectiveness. There is little data on the impact of demographic or clinical covariates on LCL response to chemotherapy. Paclitaxel sensitivity was determined in LCLs from 93 breast cancer patients from the University of North Carolina Lineberger Comprehensive Cancer Center Breast Cancer Database to test for potential associations and/or confounders in paclitaxel dose-response assays. Measures of paclitaxel cell viability were associated with patient data included treatment regimens, cancer status, demographic and environmental variables, and clinical outcomes. We used multivariate analysis of variance to identify the in-vivo variables associated with ex-vivo dose-response. In this unique dataset that includes both in-vivo and ex-vivo data from breast cancer patients, race ($P = 0.0049$) and smoking status ($P = 0.0050$) were found to be significantly associated with ex-vivo dose-response in LCLs. Racial differences in clinical dose-response have been previously described, but the smoking association has not been reported. Our results indicate that in-vivo smoking status can influence ex-vivo dose-response in LCLs, and more precise measures of covariates may allow for more precise forecasting of clinical effect. In addition, understanding the mechanism by which exposure to smoking in-vivo effects ex-vivo dose-response in LCLs may open up new avenues in the quest for better therapeutic prediction. ***Pharmacogenetics and Genomics*** **31: 48–52**

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Introduction

An important challenge in the development of anticancer therapies is the paucity of robust model systems for precise translation from the preclinical to clinical setting. One approach uses human B cell lymphocytes from peripheral blood that are infected *ex vivo* with the Epstein Barr virus (EBV) to create immortalized human lymphoblastoid cell lines (LCLs) [1]. LCLs are a widely used model system in several fields, including immunology, toxicogenomics, and pharmacogenomics [2]. LCLs have been successfully used in genetic and functional studies to find associations with genetic variations and to investigate the response of numerous chemotherapeutics, offering insights into the mechanism of action or resistance [3–5]. Several such discoveries using LCLs have led to successful clinical translations [6,7].

The model is highly scalable, efficient, and cost-effective with fewer known confounding issues compared with other preclinical models [3]. LCLs, such as those in the 1000 Genomes Project [8], are created from individuals from a wide range of demographic populations and exposures. As with any model system, its success will be dependent on properly understanding confounders. Research has demonstrated that growth rate, batch effects and the sex, ethnicity, and race of the samples can influence dose-response in LCLs [9–12]. These known confounders can be controlled for by experimental design and execution, quality control, and appropriate control of covariates in the statistical analyses [3]. However, to the best of our knowledge, the effect of other in-vivo factors, such as in-vivo exposures, on ex-vivo dose-response in LCLs has not been studied.

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Here, we used patient-derived LCLs from breast cancer patients to examine the effect of a number of demographic and clinical in-vivo variables on ex-vivo paclitaxel dose-response.

Materials and methods

The patient cohort consisted of 93 breast cancer patients from a prospective trial of genetic heterogeneity (LCCC 9830), the University of North Carolina (UNC) Lineberger Comprehensive Cancer Center (LCCC) that included LCLs created from pretreatment blood drawn from newly diagnosed breast cancer patients. These 93 were selected from the overall study cohort because of receiving paclitaxel as part of their systemic therapy, as described in detail in Hertz *et al.* [13]. The in-vivo patient data included treatment regimens, cancer status, demographic and environmental variables, and prospectively ascertained clinical outcomes for each patient. Informed consent was obtained from all participants. This study adhered to the 1964 Declaration of Helsinki and its later amendments and the protocol was approved by the UNC Institutional Review Board.

In brief, a 30-mL blood sample was collected from each patient pretreatment, at the time of enrollment. Lymphocytes from blood samples were infected with EBV to create immortalized human LCLs, as previously described [14]. The LCLs were then grown on 384 well plates, and percent viability for each cell line was measured at each of 10 concentrations (nM) of paclitaxel (100, 250, 350, 450, 500, 550, 600, 700, 900, and 1200). Details of these methods are found in the Supplemental Material, Supplement digital content 1, *http://links.lww.com/FPC/B381*.

A QC pipeline, described in detail in Brown *et al.*, was applied prior to statistical analysis [4,15,16]. To avoid issues with imbalanced classes and increase statistical power for multivariate analysis of variance (MANOVA), observations whose categorical level had a frequency ≤10 were filtered out, and highly correlated responses were removed (Supplemental Methods, Supplemental Figs. S1 and S2, Supplement digital content 1, *http://links.lww.com/FPC/B381* and Supplemental Tables S1 and S2, Supplement digital content 1, *http://links.lww.com/FPC/B381*). Multivariate analyses using the complete dose-response profile have more power and maybe more robust than univariate summary measures, such as the AC_{50} [16]. Hence, to identify in-vivo variables associated with ex-vivo dose-response in LCLs, we conducted a one-way MANOVA using four minimally correlated dose-response variables as the response and each categorical in-vivo variable of interest as predictor variables. When the in-vivo variables were ordinal or continuous, multivariate response regression was used. We used the Benjamini–Hochberg method [17] with a false discovery rate of $q<0.10$ to ascribe significance. Additional supporting methods and analyses of the AC_{50} response, a two-way MANOVA with race and smoking status, and comparisons of ex-vivo response to in-vivo clinical outcomes are described in Supplemental Material, Supplement digital content 1, *http://links.lww.com/FPC/B381*. Analyses were conducted using R [18].

Results

Results from the one-way MANOVA analyses are shown in Table 1. Race and smoking status *in vivo* were found to be independently, significantly associated with ex-vivo dose-response with moderate effect size. Table 2 shows estimated contrasts for smoking status and their significance after a Bonferroni correction. We observe that individuals who never smoked and former smokers were less sensitive to paclitaxel *ex vivo* than current smokers at higher concentrations. However, there is no significant difference between never and former smokers. This is also shown in Fig. 1a, which illustrates that current smokers have lower cell viability than former smokers and never smokers, at 600 nM of paclitaxel. Analyses evaluating racial differences in response followed similarly, and results are shown in Fig. 1b and Table 3. Figure 1b

Table 1 Results from one-way multivariate analysis of variance with the selected four dose-response variables as the response and each in-vivo variable of interest as predictor variables

	Variable	*P* value	Corrected *P* value
1	Race	0.0049	0.0501
2	Smoke	0.0050	0.0501
3	Neuropathy	0.2966	0.9359
4	ER	0.3771	0.9359
5	Regimen	0.4029	0.9359
6	ER or PR	0.4621	0.9359
7	Total weeks	0.5052	0.9359
8	Menopause	0.5108	0.9359
9	Age	0.5187	0.9359
10	Grade	0.5515	0.9359
11	Myalgia	0.6231	0.9359
12	StagePre	0.6687	0.9359
13	Neutropenia	0.7683	0.9359
14	Follow-up status	0.7696	0.9359
15	NumCycles	0.7958	0.9359
16	Dose interval	0.8109	0.9359
17	StageFinal	0.8366	0.9359
18	Response nontaxane	0.8423	0.9359
19	Response taxane	0.9708	0.9715
20	HER2	0.9715	0.9715

The Benjamini–Hochberg method was used to control for multiple testing correction with a false discovery rate of $q<0.10$. Race and smoking status were found to be significantly associated with ex-vivo dose-response in LCLs after multiple testing correction.

Age, age at diagnosis (in years); dose interval, paclitaxel dose interval; ER, estrogen receptor status (positive or negative); ER or PR, estrogen or progesterone receptor-positive (yes or no); follow-up status, follow-up status; grade, grade of tumor at diagnosis; HER2, human epidermal growth factor receptor 2 status (positive or negative); menopause, menopause status at diagnosis (pre or post); myalgia, myalgia status (yes or no); neuropathy, grade 3+ neuropathy status (yes or no); neutropenia, neutropenia status (yes or no); NumCycles, number of paclitaxel chemotherapy cycles; race, self-reported race; Regimen, paclitaxel regimen (first or second); response nontaxane, response to nonpaclitaxel therapy; response taxane, response to paclitaxel therapy; smoke, smoking status (current, former or never smoker); StageFinal, final cancer stage; StagePre, cancer stage before start of treatment; total weeks, total weeks of treatment; LCLs, lymphoblastoid cell lines; MANOVA, multivariate analysis of variance

Table 2 Estimated pairwise contrasts and their *P* values for the multivariate dose-response profile and smoking status

Contrast	1200 nM	600 nM	500 nM	100 nM	*P* value	Bonferroni corrected *P* value
Former smoker–never smoked	0.17	4.15	3.53	1.90	0.0720	0.2160
Former smoker–current smoker	3.50	13.78	0.23	0.41	0.0141	0.0424*
Never smoked–current smoker	3.33	9.62	−3.30	−1.48	0.0135	0.0404*

The multivariate dose-response analysis used four minimally correlated responses at 100, 500, 600, and 1200 nM of paclitaxel out of the ten measured responses (see Supplemental Methods, Supplement digital content 1, *http://links.lww.com/FPC/B381* for details).
Number of individuals in each category: current smoker = 12, former smoker = 27, never smoker = 54.

shows that at 600 nM of paclitaxel, lower cell viability is observed in individuals who are black compared with individuals who are white. The entire dose-response profile for all individuals is shown in Supplemental Fig. S5, Supplement digital content 1, *http://links.lww.com/FPC/B381* segregated by (a) smoking status and (b) race.

Discussion

It is well established that LCL growth rate, batch effects, and the sex and race of the individuals affect the dose-response phenotype in LCLs [9–12]. However, to the best of our knowledge, the effect of other in-vivo factors on ex-vivo dose-response in LCLs has not been studied. Our results show an expected and confirmatory effect of race [13] and an unexpected and surprising effect of an in-vivo environmental factor, smoking status, on ex-vivo dose-response in LCLs. Interestingly, the effect size of smoking status (partial $\eta^2 = 0.096$) is on the same order of magnitude as that of the race (partial $\eta^2 = 0.13$) (Supplemental Table S3, Supplement digital content 1, *http://links.lww.com/FPC/B381*), indicating that the influence of smoking status on dose-response is comparable to that of racial ancestry.

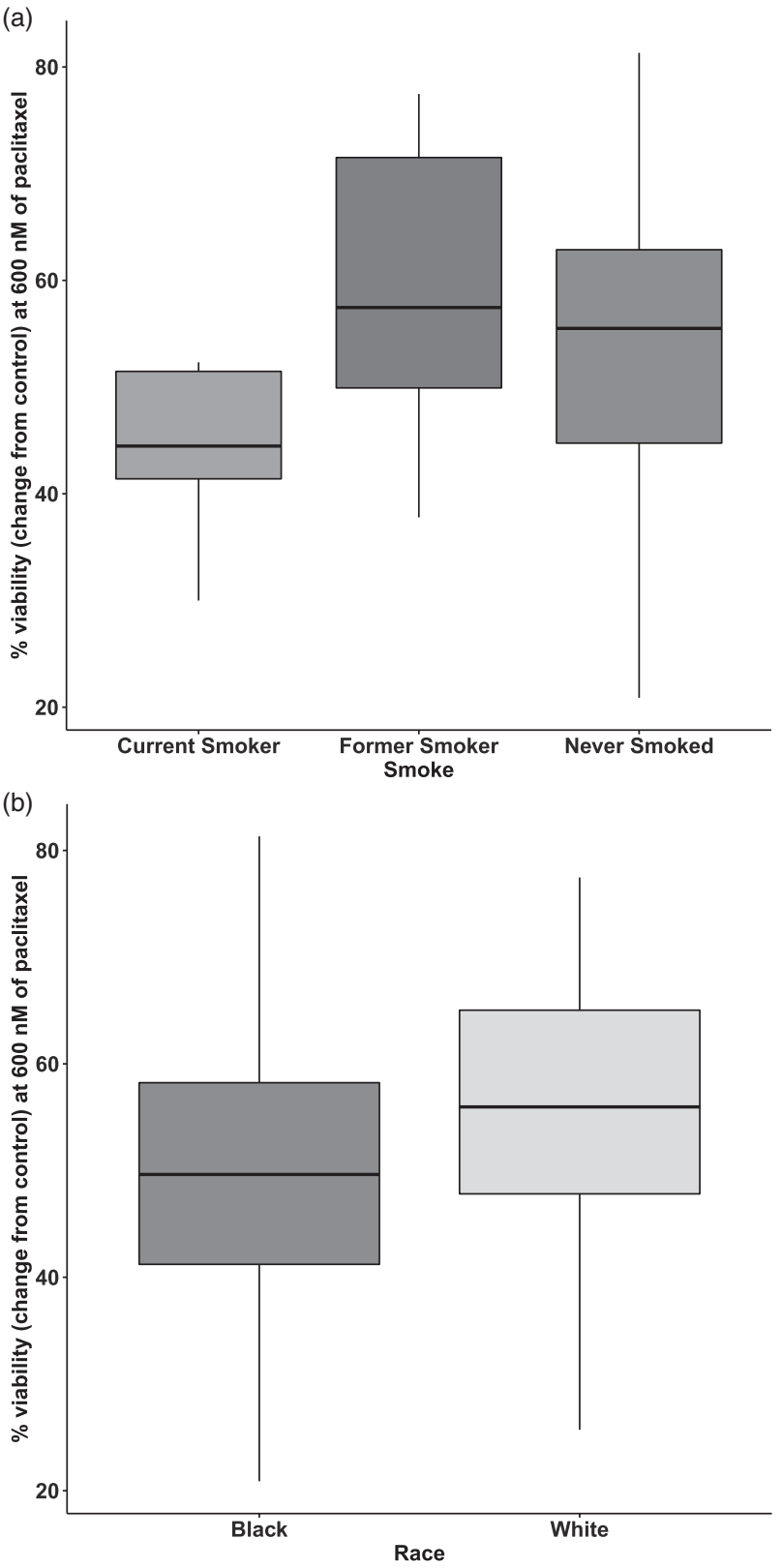
While smoking status was not significantly associated with the clinical outcome in our study, it is known that smoking can impact patient outcomes. Studies have shown that smoking can worsen patients' prognoses and survival outcomes after anticancer therapy [19,20]. Studies have also shown resistance to chemotherapeutics in cancer cells exposed to nicotine [21,22]. However, our results suggest that LCLs from active smokers were more sensitive *ex vivo* to paclitaxel, at higher concentrations. This potential discordance between the clinical observation and laboratory sensitivity patterns needs to be better understood as the use of ex-vivo model systems expands.

Whether any such association is considered a confounder or a covariate is unclear, and could be contextual on the basis of study design, but our results demonstrate for the first time that smoking status of LCL donors is linked to ex-vivo response. Unfortunately, for many of the publicly available LCL results, this data is not available. Our results suggest that smoking status of donors from whom LCLs are cultured should be recorded and considered in analysis if possible. Although factors, such as batch effect, that are clearly only associated with ex-vivo response need to clearly be adjusted for, other factors that may be true indicators of clinical in-vivo response should be modeled in a hypothesis-specific fashion.

This begs the question of how in-vivo smoking affects ex-vivo dose-response because the LCLs are no longer exposed to the environmental factor. We hypothesize that the effect of smoking *in vivo* could persist in the LCLs through epigenetic mechanisms, such as DNA or histone methylation. One such pharmacoepigenetic study by Zhang and Zhang identified epigenetic variations associated with clofarabine-induced cytotoxicity in LCLs [23]. Recent studies of the acquired environmental genetic change clonal hematopoiesis of indeterminant potential suggest that these acquired genetic changes can influence neutropenia risk after chemotherapy for solid tumors [24]. Functional experiments using chromatin immunoprecipitation assays and RNA-sequencing followed by differential gene expression analyses would be required to investigate this hypothesis and understand the mechanism by which exposure to smoking in-vivo effects ex-vivo dose-response.

Although our study has identified an impactful result of the influence of an in-vivo environmental exposure on ex-vivo drug response in LCLs, these results need to be interpreted in context of several limitations. We were unable to detect strong associations between in-vivo clinical outcomes and ex-vivo drug response due to limited sample size and power, in addition to other general limitations to the model, which have been previously discussed in detail [3,7]. Notably, the LCLs used in our study were created from lymphocytes of breast cancer patients, who all received multifaceted treatment strategies, including combination chemotherapy and anti-HER2 treatment, when indicated [3,7]. The taxane response outcome on the basis of tumor size was collected prior to and after each line of treatment, such that the taxane response would be independent of other chemotherapy (anthracycline/cyclophosphamide) but would include concurrent HER2 targeted treatment (~12 weeks). The LCL model cannot capture the complex interplay of multiple treatment strategies with clinical outcomes. Additionally, smoking status was patient-reported and reflects a categorical simplification of what is likely a continuum of biological risk.

Fig. 1



Boxplots showing paclitaxel response stratified by (a) smoking status and (b) race. Percent viability (change from control) at 600 nM, as an example concentration of paclitaxel, in the patient-derived cell lines stratified by (a) smoking status and (b) race. (a) Current smokers have significantly lower cell viability compared with the other smoking categories. The difference in cell viability between former smokers and never smokers was not found to be significant. Number of individuals in each category: current smoker=12, former smoker=27, never smoker=54. (b) Significantly lower cell viability is observed in individuals who are black compared with those who are white. Number of individuals in each category: black=24, white=64.

Table 3 Estimated pairwise contrasts and their *P* values for the multivariate dose-response profile and race

Contrast	1200 nM	600 nM	500 nM	100 nM	*P* value
White–black	−1.35	5.64	−0.58	0.91	0.0049**

The multivariate dose-response analysis used four minimally correlated responses at 100, 500, 600, and 1200 nM of paclitaxel out of the ten measured responses (see Supplemental Methods, Supplement digital content 1, *http://links.lww.com/FPC/B381* for details).
Number of individuals in each category: black=24, white=64.

Conclusion

This study shows that in-vivo smoking status is significantly associated with ex-vivo dose-response in the LCLs, with a moderate effect size. These results identify an in-vivo environmental exposure, smoking, that is an important factor for LCL dose-response assays and, hence, should be recorded and considered in dose-response analyses when possible. The findings from this study identify an influential, but as of yet unstudied covariate that significantly affects individual variation in dose-response in LCLs.

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Conflicts of interest

There are no conflicts of interest.

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