A genetic response profile to predict efficacy of adjuvant 5-FU in colon cancer

Ida Kappel Buhl*[1,2], Sarah Gerster*[3], Mauro Delorenzi[3,4], Thomas Jensen[2], Peter Buhl Jensen[2], Nils Brunner[1], Anker Hansen[2], Steen Knudsen[2]

[1] Section for Molecular Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [2]Medical Prognosis Institute, Hoersholm, Denmark; Denmark; [3] Bioinformatics Core Facility, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland; [4]Ludwig Center for Cancer Research and Oncology Department, University of Lausanne, Lausanne, Switzerland.

* Authors contributed equally .

Background

The antimetabolite 5-fluorouracil (5-FU) is the backbone in systemic treatment of primary and metastatic colorectal cancer (CRC), with further activity in a wide range of solid tumors. As not all patients will benefit from 5-FU treatment there is a clinical need for biomarkers of response to adjuvant 5-FU. The current project seeks to validate a predictive 5-FU gene expression profile. A similar model has recently been validated with MD Anderson in three different clinical settings [1] and with researchers from AstraZeneca with fulvestrant [2].

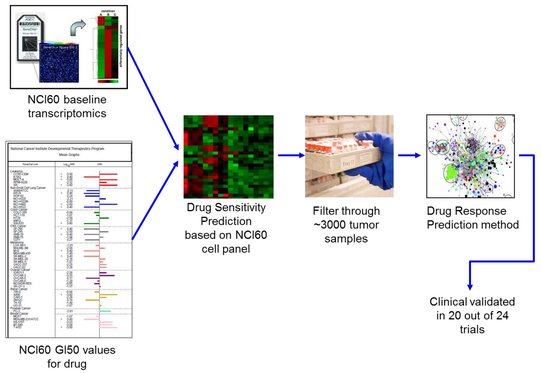

Figure 1: The principle behind the drug response prediction method .

Drug Response Prediction

A validated response prediction method is used in the 5-FU sensitivity prediction. As shown in Fig. 1, the method is based on in vitro sensitivity data and cell line microarray results in a model that also incorporates clinical variables. Biomarker profiles have previously been developed for a number of drugs and validated against 24 published clinical trials.

Table 1A

Gene	Description
APRT	adenine phosphoribosyltransferase
GSR	glutathione reductase
TUFM	Tu translation elongation factor, mitochondrial
MRPS2	mitochondrial ribosomal protein S2
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase
WDR59	WD repeat domain 59
ANP32B	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B
PMM2	phosphomannomutase 2
STOML2	stomatin (EPB72)-like 2
NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex

Table 1B

Gene	Description
NT5E	5'-nucleotidase, ecto (CD73)
CNN3	calponin 3, acidic
ACTN1	actinin, alpha 1
FLNA	filamin A, alpha
ATP2B4	ATPase, Ca++ transporting, plasma membrane 4
CYR61	cysteine-rich, angiogenic inducer, 61
ACTN1	actinin, alpha 1
LGALS1	lectin, galactoside-binding, soluble, 1
RHOC	ras homolog family member C
RAB32	RAB32, member RAS oncogene family

Table 1: The 10 most important genes for predicting 5FU sensitivity (1A) and resistance (1B), respectively

Methods

The 5-FU signature consisted of in total 205 positively and negatively correlated genes mapped to 669 probe sets.

The signature was applied to gene expression profiles of a subset of the PETACC-3 patient cohort obtained previously from pretreatment formalin fixed paraffin embedded (FFPE) tissue from 636 stage III colon cancer (CC) patients treated adjuvantly with 5-FU with or without irinotecan [3].Gene expression data was obtained through a customized Colon DSA gene expression array from ALMAC applied on the PETACC-3 CC population.

It was also tested on ALMAC Colon DSA data obtained from FFPE tissue from 359 stage II CC patients who did not receive adjuvant treatment [4]. The analyses were performed using Cox proportional hazards (CPH) regression models and Kaplan-Meier curves. The log-rank test was used to compare the survival curves.

Results

We found a statistically significant difference between the Kaplan-Meier curves for the PETACC-3 patients stratified by their 5-FU profile score. In this analysis, not including any further covariates, patients with a high 5-FU score showed a statistically significantly better relapse free survival (RFS) (hazard ratio (HR) = 0.54 (95% CI 0.41, 0.71), p < 1e-05, N = 636 stage III patients) and overall survival (OS) (HR = 0.47 (0.34, 0.63), P = 7.4e−07; binary scores). In the untreated cohort no differences were observed in RFS (HR = 0.92 (0.64,1.33), P=0.671) nor in OS (HR = 0.96 (0.67, 1.4), P = 0.849).

	HR	CI 95 %
5FU predict 1 unit = 1 iqr	0.69	0.58, 0.82
T stage T4 vs T3	2.03	1.4, 2.95
N stage N2 vs N1	2.01	1.45, 2.79
grade G34 vs G12	1.81	1.07, 3.07
KRAS mut vs wt	1.89	1.34, 2.67
MSI, MSI vs MSS	0.42	0.21, 0.81

Table 2: Results from a multivariable Cox Proportional Hazards model. We used overall survival (OS) as endpoint. We report the hazard ratio (HR) estimates and their confidence interval for all variables with a p-value < 0,05. Further variables included in the model are: BRAF mutation, treatment group, age, sex, tumor site and T-stage 1 and 2.

The effect of the 5-FU profile remained significant in the PETACC-3 subpopulation when performing an analysis with a multivariable Cox Proportional Hazards model. Several relevant clinicopathological parameters, including microsatellite instability (MSI) vs stability (MSS) and KRAS mutation status, were included as covariates (see Table 2).

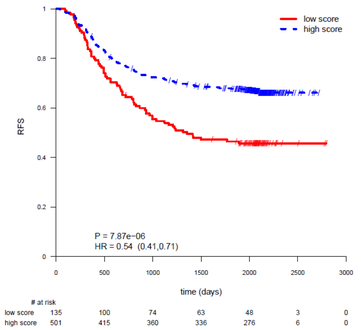

Figure 2: Kaplan-Meier curves – subpopulation from PETACC-3 study stratified by the 5-FU profile score. The endpoint is relapse free survival (RFS).

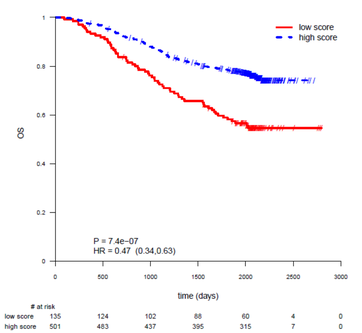

Figure 3: Kaplan-Meier curves – subpopulation from the PETACC-3 study stratified by the 5-FU profile score. The endpoint is overall survival (OS).

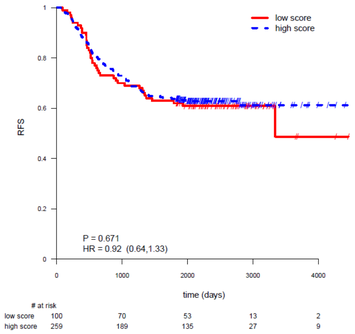

Figure 4: Kaplan-Meier curves – study of non-treated patients stratified by the 5-FU profile score. The endpoint is relapse free survival (RFS).

Conclusions

The finding of this study was that the 5-FU response profile significantly separated the PETACC-3 patients into groups of good and poor survival independently from selected clinicopathological parameters such as stage, age and MSI/MSS-status. Due to the design of the PETACC-3 study it is not possible to precisely validate a predictive value of the 5-FU response profile on this cohort, since patients in both treatment groups received 5-FU. We therefore also applied the 5-FU response profile to an untreated CC patient population in order to validate the prognostic association of the profile in untreated patients.

In contrast to the data derived from the PETACC-3 study, we did not observe any statistically significant prognostic effect of the 5-FU profile in the untreated CC patient cohort. These findings support a potential predictive value of the 5-FU profile. However, the presented results needs further validation.

Competing Interests

The authors have read ESMO's policy and have the following conflicts. Authors IKB TJ PBJ NB AH SK declare advisory role or past or present primary employment or ownership in a company (Medical Prognosis Institute) that has a potential to benefit from these results. Medical Prognosis Institute holds a patent on the subject matter. This does not alter the authors' adherence to all policies on sharing data and materials.

References

[1] Wang W et al., JNCI 2013;105:1284-1291
[2] Knudsen S et al., PLoS One 2014;9:e87415
[3] Van Cutsem E et al., JCO 2009;27:3117-3125
[4] Kennedy RD et al., JCO 2011;29:4620-4626

Correspondence to ikb@medical-prognosis.com