EXPRESSION OF CYP3A4 AS A PREDICTOR OF RESPONSE TO CHEMOTHERAPY IN PERIPHERAL T-CELL LYMPHOMAS

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ABSTRACT

Peripheral T-cell lymphomas (PTCL) are aggressive tumors in which the current therapy based on multiagent chemotherapy is not successful. Since cytochrome P450 3A subfamily (CYP3A) enzymes are involved in the inactivation of chemotherapy drugs, we hypothesized that CYP3A and P-glycoprotein (MDR1) expression in these lymphomas, could result in a poor clinical response. We measured tumoral CYP3A and MDR1 mRNA content in 44 T-cell lymphomas finding a large variation in CYP3A expression. Multiplex PCR-Analysis and FISH analysis showed genomic gains affecting CYP3A and MDR1 genes in T-cell lines and primary tumors, suggesting that this could be the mechanism underlying the tumoral expression variation. To test whether the tumoral expression of CYP3A and/ or MDR1 could influence PTCL treatment outcome, their expression levels were compared with the clinical response and survival of the patients, finding that a high tumoral expression of CYP3A4 was significantly associated with a lower complete remission rate. This was further investigated with cell lines stably expressing CYP3A4 which exhibited an increased resistance to doxorubicin and etoposide. In conclusion, a high CYP3A4 tumoral expression could be useful to predict poor response to the standard PTCL chemotherapy, in these cases alternative chemotherapy combinations or doses should be explored.
INTRODUCTION

Peripheral T-cell lymphomas (PTCL) constitute a complex group of tumors which are still a challenge for medicine. More than half PTCLs remain classified as unspecified (PTCLu), a group that includes histological and clinically heterogeneous tumors\(^1,2\). Other frequent PTCL subtypes in the current classification are the angioimmunoblastic T-cell lymphomas (AITL) and anaplastic large cell lymphomas (ALCL)\(^3,4\). Regardless of the histological subtype, multiagent chemotherapy is the treatment of choice for most PTCL patients. However, PTCLs are clinically aggressive tumors with poorer response to treatment and shorter survival times than diffuse large B cell lymphomas, typically showing less than 30% 5-years overall survival\(^5-7\). To date, the most effective therapy is a combination chemotherapy regimen, in many cases CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), although a variety of other regimens (CHOEP, VACPE) which add other drugs with important anti-lymphoma activity such as etoposide, have demonstrated similar activity. However, the poor clinical outcome of most patients is disappointing and clearly reveals the need to improve the therapy by identifying factors affecting the response.

Cytochromes P450 (CYPs) of families 1-3 are drug metabolising enzymes involved in the activation and detoxification of a large number of chemotherapeutic drugs\(^8\). Among them, the CYP3A subfamily, constituted in humans by 4 enzymes of similar substrate specificity: CYP3A4, CYP3A5, CYP3A7 and CYP3A43, is involved in the metabolism of more than 50% of all therapeutic drugs in clinical use\(^9-11\). Both genetic polymorphisms and environmental factors have been shown to alter CYP activities resulting in inter-individual differences in drug effects\(^12,13\). In addition to the variations in the constitutive expression, the CYP3A enzymes, which are mainly expressed in the liver, have been found in different tumors\(^14-18\). Because CYP3As inactivate many anti-cancer drugs, an overexpression of CYP3As in tumors could result in an increased intratumoral drug inactivation and consequently decreased drug efficacy\(^17,19\). Similarly, the ABC efflux drug transporter p-glycoprotein (Pgp) can confer tumoral drug resistance and because of the
overlapping substrate specificity of CYP3A and Pgp, functional interactions have been suggested. Interestingly, microarray expression profiling analysis of PTCLs in our group\textsuperscript{20,21} showed large differences in the tumoral CYP3A expression. Furthermore, \textit{CYP3A} and \textit{MDRI} (encoding Pgp) genes are closely located in 7q21, a region recurrently gained in T cell lymphomas\textsuperscript{22}. Thus, in this study, we measured the expression of CYP3As and MDR1 in a panel of 44 PTCL tumors and compared it with clinical parameters such as therapy response and survival.

**MATERIALS AND METHODS**

**Tumor samples**

Frozen and paraffin-embedded tumor biopsy samples from a group of 44 peripheral T-cell and NK lymphomas including 23 PTCLu, 14 AILTs and 7 NK lymphomas (NK) were collected through the CNIO tumor bank network from pathology departments of different hospitals in Spain. These lymphomas have been previously analyzed for their expression profiles using cDNA microarrays\textsuperscript{20,21}. Most of the cases were treated with similar therapy protocols based on combination chemotherapy, typically CHOP or CHOP-like protocols. Five reactive lymphadenopathy samples were used as controls. Total RNA and DNA were extracted from frozen samples with standard methods. This study has the approval of the Bioethics Committee of the Instituto de Salud Carlos III and fulfills all ethical requirements.

Eleven T-cell lymphoma cell lines were used were used to analyze genomic gains in chromosome 7q: Karpas-45, HSB-2, Jurkat, KE-37, Molt-4, Molt-13, Molt-16, HPB-ALL, Peer, Hut-78 and Karpas 299.

**Quantitative RT-PCR analysis**

One μg of total RNA was reverse transcribed using MMLV Reverse Transcriptase (Invitrogen) and a random primer. The cDNAs were subjected to quantitative real-time PCR assay with the use
of gene specific double fluorescent labeled probes and the TaqMan Universal PCR Mix in an ABI prism 7900 system (Applied Biosystems) under manufacturer's recommendations. The PCR amplification was carried out with 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C, using the oligonucleotides shown in Supplementary Table 1. Assays-on-Demand Taqman MGB probes (Applied Biosystems) were used for β-actin quantification which was then used as an internal standard and allowed normalization of the samples. To perform relative quantification of the expression of the genes standard curves were constructed with serial 10-fold dilutions of a human liver cDNA. All samples were analyzed in triplicates.

**Specific multiplex-PCR analysis**

A multiplex-PCR analysis was designed to determine the number of copies of *CYP3A4* and *MDR1* genes, both located at 7q21. Ten pairs of primers were designed and labeled (at the 5’end with 6-FAM) to obtain using a multiplex-PCR kit and following standard recommendations (QIAGEN) uniquely sized amplification fragments: 4 for the *CYP3A4* gene (501, 364, 385 and 419 bps) and 1 for *MDR1* (220 bps) (Supplementary Table 1). Five additional fragments were used as controls and were located at 11q23, 5q33, 15q25, 17q21 and 7p14. Briefly, multiplex amplifications were performed using the Qiagen Multiplex PCR kit (QIAGEN) with 25 µl of a mixture containing 1X multiplex-PCR master mix, 0.2 µM of each primer, and 50-100 ng of genomic DNA which was amplified by 22 cycles of 30 s at 94°C, 90 s at 60°C, and 90 s at 72°C. The PCR amplification products obtained were analyzed on an ABI PRISM™ 310 capillary sequencer (Applied Biosystems) and using GeneScan v3.1 software (Applied Biosystems). For each sample the peak area of all fragments was determined and normalized with the values of the control peaks from a control sample (i.e. genomic DNA from a healthy volunteer) 23.

**Fluorescence in situ hybridization (FISH)**

To assess the presence of \textit{CYP3A4} gene gains we performed FISH analysis on several tissue samples. Ensembl Cytoview (www.ensembl.org) was used to select the 4 BAC clones covering the \textit{CYP3A} locus: RP11-757A13, RP11-150A16, RP11-977H6 and RP11-268P20, and 3 BAC clones covering the \textit{MDR1} locus: RP11-212B1, RP11-1149O20 and RP11-647N21. The BACs were obtained from BACPAC Resource Center (BPRC) at the Children's Hospital Oakland Research (Institute in Oakland, CA, USA). Additionally, a commercial Texas-Red labeled probe for the centromeric region of chromosome 7 (Vysis, Downers Grove, IL, USA) was used for ploidy control. FISH assays were carried out as described elsewhere and according to the manufacturer's instructions. In brief, all BACs were labeled directly by nick translation according to the manufacturer's specifications with SpectrumGreen (Vysis). The probes were blocked with Cot-1 DNA (Vysis) to suppress repetitive sequences. Paraffinated tissue slides were deparaffinized and boiled in a pressure cooker with 1 mM EDTA (pH 8.0) for 5-10 min and incubated with pepsin at 37°C for 30 min and dehydrated. The probe was denatured at 75°C for 2 min and hybridized overnight at 37°C in a humid chamber. After post-hybridization washes, the tissue samples were counterstained with 4,6-diamidino-2-phenylindole (DAPI II, Vysis, Downers Glove, IL, USA) for chromatin counterstaining before microscopy. Cell images were captured using a CCD camera (Photometrics SenSys) connected to a computer running the Chromofluor image analysis system (Cytovision). FISH scoring of CYP3A fluorescence signals was carried out in each sample by counting the number of single copy gene and control probe signals in an average of 100 nuclei. Gain or polyploidy status was considered as positive for a sample when the ratio of green/red signals was over 1.5 in > 30% of tumor cells.

**Statistical analysis of the clinical data**

Since different subgroups of T-cell lymphomas could have different clinical behaviour, clinical analysis was carried out only in the group of PTCLu. After the initial therapy patients were classified as non responders (NR), having partial remission (PR) or complete remission (CR).
Pearson Chi-square and Fisher exact tests were performed to carry out comparisons between cases with high or low expression of \textit{CYP3A4} and the rate of clinical responses. Kaplan-Meier survival curves were used to compare overall survival (OS) between lymphomas with higher or lower expression of \textit{CYP3A} and \textit{MDR1} genes. Overall survival time was measured from the date of diagnosis to the date of death or last follow-up. The Log-Rank test was used to compare survival curves. The SPSS version 12.0 software was used for these analyses.

\textbf{Construction and cytotoxicity analysis of HEK293 cells stably overexpressing CYP3A4}

Based upon sequences from their corresponding cDNAs, we designed primers CYP3A4FW 5’-GAAAGCTAGCATGGCTCTCATCCCAGACTTGGCCA-3’ and CYP3A4RV 5’-CTGGGCGGCTTCAGGCTCCACTTACGGTCG-3’ to amplify CYP3A4 coding sequence from human liver cDNA and introducing NheI and NotI cleavage sites. The product was cloned into pIRESpuro2 vector (Clontech) and sequenced. To generate stable cell lines, HEK293 cells were electroporated and 24 h later grown with puromycin (Sigma) for selection. CYP3A4 resistant clones were analyzed for CYP3A4 expression by Western blot using a primary antibody recognizing CYP3A4 (299223, Daiichi Pure Chemicals LTD). Control cells (HEK293-pIRES) were selected similarly as above and all resistant clones were pooled together. Stable transfectants were maintained in DMEM supplemented with 10% FBS, penicillin, and streptomycin with puromycin at a concentration of 0.5µg/ ml.

Citotoxicity tests for doxorubicin, etoposide and vincristine (Sigma) were performed in HEK293-CYP3A4 and control cells. Briefly, 24 h after trypsinization the cells were incubated with the drugs for 96 h, each concentration was assayed in triplicate, and then incubated with MTT substrate. The resulting absorbance was measured by means of a microplate reader (Bio-Rad), and the cytotoxic effect of each treatment was assessed by IC50 value (concentration of the drug leading to 50% cell survival).

\textbf{RESULTS}
PTCLs show a variable expression of \textit{CYP3A}s and \textit{MDR1}

In a previous study by our group, expression profiling analysis using microarrays allowed us to detect overexpression of different \textit{CYP3A} genes in a subset of PTCLs (data not shown). To confirm that \textit{CYP3A}s and \textit{MDR1} are expressed in PTCL tumors, quantitative RT-PCR was used in 44 PTCL tumors and compared to 5 reactive lymph nodes. A large inter-sample variability in the expression of the \textit{CYP3A}s was found, with about one third of the cases showing \textit{CYP3A4}, \textit{CYP3A5}, \textit{CYP3A7} and \textit{CYP3A43} mRNA levels at least 2-fold higher than the reactive lymph nodes. On the contrary, \textit{MDR1} mRNA levels showed less variation and most tumors (85%) had lower mRNA content than the reactive lymph nodes. Because T cell lymphomas include a range of different histological subtypes, we investigated whether the heterogeneity found in the \textit{CYP3A} expression could be associated to a specific tumor subtype. In general, the proportion of tumors overexpressing \textit{CYP3A} genes was similar for PTCLus and AITLs tumors, in average 35 and 20%, respectively, while NK tumors had a higher \textit{CYP3A} expression, with 57% of the tumors showing increased levels of \textit{CYP3A4} and \textit{CYP3A43} but only 29 and 14% showing increased \textit{CYP3A7} and \textit{CYP3A5} expression, respectively. Figure 1A shows the expression levels of \textit{CYP3A4} in the different tumor subtypes.

Since the different \textit{CYP3A} genes were simultaneously overexpressed in specific tumors, we performed correlation analysis and found that \textit{CYP3A4} expression correlated with \textit{CYP3A5} and \textit{CYP3A7} (Figure 1B). When \textit{MDR1} expression was analyzed a significant correlation was found with \textit{CYP3A7}, \textit{CYP3A43} and \textit{CYP3A4}. Therefore, the four \textit{CYP3A} enzymes and \textit{MDR1}, although showing differences in their mRNA levels, have a coordinated expression in PTCL tumors, which suggests a common regulatory mechanism.

\textbf{T-cell lines and PTCLs show recurrent gains of the 7q21 region containing \textit{CYP3A} and \textit{MDR1} genes}
To identify the molecular mechanisms involved in the altered CYP3A4 tumoral expression, we set up a MPA technique able to detect changes in *CYP3A4* and *MDR1* copy numbers using genomic DNA (Figure 2A). Initially, we used T-cell lymphoma cell lines because they are homogeneous tumoral cell populations with no contaminating non-tumoral genomic DNA, which facilitates the technique. We included 11 cell lines in the study and found that 9 showed simultaneous increases in *CYP3A4* and *MDR1* values, indicating that a region containing both genes (located at 7q21 and separated by 12 Mbp) was gained. Most cell lines also showed 7p gains, although to a lower degree. In general, the cell lines followed two different gain patterns: i) with both *CYP3A4* and *MDR1* genes in 7q21 gained but a minimal changes in the 7p *AQP1* gene, such as MOLT4 and MOLT16; and ii) with all 7q21 and 7p genes gained, such as Karpas 299, HPB-ALL, Hut-78 and Peer (see Figure 2B). When we used this technique to study PTCL tumors with high content of tumoral cells, we could also detect in some cases clear gains affecting *CYP3A4* and *MDR1* genes by MPA. For example, PTCL_21 had increased number of the 7q21 genes while no changes in 7p could be detected (Figure 2A and Figure 2B, last column).

We selected 4 T lymphoblastic cell lines (Jurkat, KE-37, MOLT 16 and Peer) and one peripheral T-cell line (Hut-78) to confirm the gains in *CYP3A4* copies using FISH analysis. Between 3 and 6 *CYP3A* copies were found by FISH in these cell lines, in contrast Karpas 45 cells which by MPA showed a normal number of *CYP3A* copies were also normal by FISH (data not shown). As shown in Figure 3A-D, MOLT 16 FISH analysis showed 3 *CYP3A* copies due to an isochromosome 7, while Peer cells had 6 *CYP3A* copies, likely by an increased number of chromosomes 7, since also 6 centromeres were detected. We could also confirm by FISH the *CYP3A4* gains in primary T-cell lymphomas with a high percentage of tumor cells, demonstrating that the 7q21 gains are not specific for the cell lines, but rather a common event in T-cell tumorogenesis. As shown in Figure 3E, about 8 *CYP3A* copies could be observed in the tumor cells of PTCL_21 while the number of chromosome 7 centromeres was lower. The reason for the relatively small changes seen by MPA for PTCL_21 are probably caused by contamination of the total genomic DNA by non-tumoral
cells that decrease the effect observed by MPA. An additional, PTCL case FISH is shown in Figure 3F. We obtained FISH data from 19 PTCL cases for which we had measured CYP3A content (11 PTCLus, 8 AITLs and 5 NK cell lymphomas). We observed that 7/8 (87%) of the cases with increased CYP3A copies showed high CYP3A4 mRNA expression, while only 1/11 (9%) of the cases with no alterations in CYP3A copy number had a high CYP3A4 mRNA expression (Figure 3G).

**CYP3A4 expression influences chemotherapy response and survival.**

Because CYP3As and Pgp can influence therapy outcome, we studied whether the expression levels of CYP3A and MDR1 genes could predict the treatment response and/or survival of T-cell lymphoma patients. Since the different histological T-cell lymphoma subtypes have differences in treatment response and survival rates, to avoid affecting the results by the intrinsic features of the tumors, we performed survival analysis only in the PTCLu subgroup. Clinical data and CYP3A4, CYP3A5, CYP3A7, CYP3A43 and MDR1 expression was compared, finding that CYP3A4 expression had a significant association with treatment response, while no significant differences were found for the other genes. In the group of patients with tumors over-expressing CYP3A4 (over 2-fold the expression in reactive tissue), only 1/6 cases (16%) had complete remission, in contrast with 7/9 (77%) complete remissions and 1/9 (11%) partial remission in the group with low CYP3A4 expression (p=0.011). As shown in Figure 4A, in the high CYP3A4 group all patients except one did not respond to the treatment while in the low CYP3A4 group only one did not respond. No significant differences were found in the distribution of common clinical variables such as age, sex, stage of disease, or LDH levels in the group of PTCLu cases showing low or high expression of CYP3A4 (Table 1), indicating that difference in response was not likely effected by clinical parameters typically influencing PTCL survival. Most of the cases with high CYP3A4 had advanced disease stages and did not respond to therapy, however, in the low CYP3A4 expression group, 3 out 5 cases in stages III and IV, reached complete remission.
Furthermore, Kaplan-Meier curves of cases in the high CYP3A4 expression group showed a tendency to lower survival times than patients with tumors with low CYP3A4 expression (P=0.075) (Figure 4B). Although these analyses were not carried out for other histological subtypes, it is remarkable that the NK tumors, which have the highest CYP3A4 expression, usually do not respond to the standard treatments and have very short survival times.

CYP3A4 expression increase cells resistance to doxorubicin and etoposide

To test whether an increased CYP3A4 expression could result in altered chemotherapy sensitivity we constructed HEK293 cells stably expressing CYP3A4 and incubated these and control cells with drugs commonly used in T-lymphoma treatment: vincristine, doxorubicin and etoposide. The HEK293 cells showed a constitutive resistance to vincristine (data not shown) and, thus, the effect of CYP3A4 could not be assessed in this system. However, previous data had already shown that vincristine is a CYP3A4 substrate and that cells overexpressing CYP3A were more resistant to vincristine 25. As shown in Figure 5 insert, the different stable transfected clones used in the study had a large difference in CYP3A4 protein content, and were classified as medium and high CYP3A4 expression. As shown in Figure 5, we found that the concentration of doxorubicin and etoposide that caused 50% cell death (IC50) was higher in the cells overexpressing CYP3A4 than in the control cells. In the case of doxorubicin the expression levels of CYP3A4 modulated the response with 2 and 7.5-fold increase in cell death resistance for medium and high CYP3A4 expressing cells, respectively. For etoposide full effect was achieved already at intermediate CYP3A4 expression levels and in average CYP3A4 caused a 39-fold drug resistance increase. This data, therefore, supports the idea that an increased CYP3A4 expression can cause chemoresistance.

DISCUSSION
To date, there is very limited data available regarding the biological factors that affect prognosis and PTCL survival. Thus, it is clear that, in addition to the classical factors included in the International Prognostic Index (IPI), more molecular markers are needed to accurately predict the course of PTCL disease and treatment response after chemotherapy. The low incidence of PTCL, the difficulty to collect simultaneously frozen and paraffined tissues and clinical data of the patient contributes to the scarcity of these type of studies. In this study, we gain insight into the mechanisms underlying the poor response rates to the common chemotherapy regimens used in these lymphomas.

Overexpression of Pgp and CYP3A enzymes in tumoral tissue has been associated to resistance to drug treatment: p-glycoprotein by pumping cytotoxic drugs out of the tumor cells and CYP3A enzymes by inactivation of anticancer drugs \(^{17,19,26}\). Interestingly, previous work from our group showed by microarray expression profiling analysis of this PTCL series that CYP3A expression was variable among the samples. In this work we used quantitative RT-PCR for the four different CYP3A enzymes to validate the microarray data. We found that all CYP3A enzymes showed a large tumoral expression variation in all histological PTCL subtypes and that some PTCL tumors had an over-expression of the \textit{CYP3A} genes with respect to the non-tumoral tissue (Figure 1). In addition, there was a correlation in the tumoral expression of CYP3As and MDR1, suggesting that there is a common mechanism responsible for the overexpression and that a synergistic drug-resistance mechanism could be expected in the tumors with high expression, in fact, both MDR1 and CYP3A detoxifying proteins have a large substrate specificity overlap. Since \textit{CYP3As} and \textit{MDR1} genes are located in 7q21 and gains involving this region have been previously detected in 30% of T-cell tumors examined\(^ {22}\), we hypothesized that this could be the mechanism underlying the overexpression. Gains of 7q were found in an important proportion of T cell lines. Although these cells mainly corresponded to lymphoblastic T-cell lines, which are not representative of PTCL, two peripheral T cell lines (Hut-78 and Karpas 299) were also analyzed and the 7q21 gains occurred in both lymphoblastic and peripheral T cells. Despite the fact that
MPA and FISH techniques are hampered by the contamination of the tumoral samples with normal cells, we were able to detect 7q21 gains not only in the T-cell lines, but also in PTCL primary tumors. Furthermore, FISH analysis showed that 87% of the cases with CYP3A4 mRNA expression 2-fold higher than the normal tissue had more than two CYP3A signals per cell, while only 9% of the cases with low CYP3A4 mRNA expression showed an increased number of CYP3A signals by FISH (see Figure 3G). This data supports that somatic chromosomal aberrations in 7q21 are frequent and could be involved in the tumoral overexpression of CYP3A genes in PTCLs.

We hypothesized that tumoral CYP3A and P-glycoprotein expression could result in resistance to treatment. In fact, disease progression of PTCLs has been frequently observed during anthracycline-based chemotherapy, suggesting an inherent resistance of these tumors to conventional systemic therapy. When we compared the treatment response and survival of PTCLu patients with the tumoral MDR1 and CYP3A expression, we found that patients with tumors of high CYP3A4 expression had a significantly lower response to treatment and a tendency to shorter survival times, when compared to tumors with low CYP3A4 expression. This data suggests that CYP3A4 tumoral expression could be a marker predicting treatment response and that it could likely influence PTCL survival. Interestingly, in the NK/T cell lymphomas, which are one of the most aggressive lymphomas, the expression of CYP3A4 was the highest (see Figure 1). To further investigate the effect of CYP3A4 on drug treatment, we constructed cell lines overexpressing CYP3A4 and showed that they exhibited an increased resistance to doxorubicin and etoposide, in agreement with previous data performed with other cytotoxic drugs such as vincristine. Because these drugs are commonly used to treat lymphomas, we propose that tumoral CYP3A4 expression has a direct effect on PTCLs response to chemotherapy.

In conclusion, CYP3A4 is a key drug metabolizing enzyme differently expressed in T-cell tumors. A poor response to standard chemotherapy was found in patients with tumors with high CYP3A4 expression, suggesting that tumoral CYP3A4 expression could be a marker for treatment
response. Thus, patients with an increased CYP3A4 tumoral expression might benefit from a different chemotherapy regimen, such as increased doses of cytotoxic drugs or drugs that follow an inactivation pathway independent from CYP3A4/ Pgp.
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The authors declare no competing financial interests.

REFERENCES


FIGURE LEGENDS

Figure 1. CYP3As and MDR1 mRNA quantification in PTCL tumors. A) CYP3A mRNA content in PTCL tumors. CYP3A4 mRNA expression was quantified by realtime RT-PCR in 45 PTCL tumors: 23 PTCLu, 15 AITL and 7 NK. Results are expressed relatively to CYP3A4 expression in control tissue (reactive lymph nodes). B) Correlation between CYP3As and MDR1 expression in PTCL tumors. The mRNA expression of CYP3A4, CYP3A5, CYP3A7, CYP3A43
and MDR1 in 45 PTCL was compared by correlation analysis. The results obtained for CYP3A4 are shown with the correlation coefficients and P values.

**Figure 2. CYP3A4 and MDR1 copy number analysis by multiplex-PCR analysis (MPA).** Ten DNA fragments with sizes ranging from 150 to 501 bps were amplified by PCR using genomic DNA and specific primers: 4 fragments corresponded to CYP3A4 (7q21), 1 to MDR1 (7q21) and 5 to additional fragments used as controls (C-) and located in different chromosomes. A) The chromatogram of a DNA sample corresponding to PTCL_21 (red) was normalized and compared with a control DNA sample (blue). Fragments showing gains are marked with an asterisk. B) MPA data was obtained for 11 T-cell lines, the chromatograms were normalized with a control DNA sample for the control DNA peaks (as above) and the mean peak fluorescence area was calculated. The values represent the ratio between the T-cell line peak area divided by the control peak area after subtracting 1 ± S.D. (i.e. no gains result in a ratio of 1 and subtraction of 1 will result in a value of 0).

**Figure 3. FISH analysis of CYP3A.** Hybridization of the CYP3A probe (labeled in green) and a chromosome 7 centromeric probe (labeled in red) in tumoral T-cells. A) and B) Representative FISH images of MOLT16 cells. C) and D) FISH images of PEER cells. E) and F) FISH images from 2 paraffin embedded PTCLs, showing cells with multiple CYP3A and centromeric 7 signals. The case PTCL_21 corresponds to panel E. G) CYP3A4 mRNA content of 19 PTCL cases classified according to the number of CYP3A copies per cell, as assessed by FISH (CYP3A=2 corresponds to 2 copies per cell; CYP3A>2 corresponds to cases with more than 2 copies per cell). The mean CYP3A4 mRNA content for PTCL cases with two CYP3A copies and for those with more than two CYP3A copies is shown with horizontal dashed lines (0.9 and 11.6 -fold change relative to lymph nodes, respectively, P<0.001).
**Figure 4. CYP3A4 tumoral expression predicts therapy response and survival of PTCLu patients.** Twenty three patients with PTCLu were divided in two groups: with CYP3A4 mRNA tumoral expression <2-fold the control samples content (low CYP3A4) and with CYP3A4 mRNA >2-fold control samples content (high CYP3A4). A) After the initial therapy patients were classified as non responders (NR), having partial remission (PR) or complete remission (CR). The therapy response of the patient groups was compared by Pearson Chi-square (P=0.02) and Fisher exact (p=0.041) tests. B) Kaplan-Meier curves were constructed to analyze the survival of the two groups (p=0.075).

**Figure 5. Effect of CYP3A4 expression on doxorubicin and etoposide toxicity.** The survival of HEK293 cells stably expression medium or high levels of CYP3A4 and control cells was measured for doxorubicin and etoposide at various concentrations and measured by MTT assay. The concentration of the drug causing 50% of cell death (IC50) is plotted and a table showing the mean ± SD IC50 values is included. A western blot (WB) showing the different CYP3A4 expression levels in the cells used is shown.
Table 1. Clinical features of PTCLu cases with low or high expression of CYP3A4.

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<td>63</td>
<td>2/4</td>
<td>50</td>
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Figure 1

A

B

Figure 2

A

B
Figure 5

<table>
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<tr>
<th>Drug</th>
<th>IC50 (µM)</th>
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<tbody>
<tr>
<td>Control</td>
<td>6.4 ± 3.2</td>
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<tr>
<td>CYP3A4-Medium</td>
<td>11.2 ± 1.8</td>
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<tr>
<td>CYP3A4-High</td>
<td>40.7 ± 25.9</td>
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<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (µM)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.03 ± 0.01</td>
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<tr>
<td>CYP3A4-Medium</td>
<td>1.40 ± 0.42</td>
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<tr>
<td>CYP3A4-High</td>
<td>0.66 ± 0.31</td>
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</table>
Expression of CYP3A4 as a predictor of response to chemotherapy in peripheral T-cell lymphomas

Cristina Rodriguez-Antona, Susanna Leskela, Magdalena Zajac, Marta Cuadros, Javier Alves, Maria Victoria Moneo, Carmen Martin, Juan Cruz Cigudosa, Amancio Carnero, Mercedes Robledo, Javier Benitez and Beatriz Martinez-Delgado