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ORIGINAL ARTICLE

In vitro evaluation of clinical activity and toxicity of anticancer drugs using tumor cells from patients and cells representing normal tissues

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Abstract
Purpose The aim of this study was to evaluate a phenotypic cell panel with tumor cells from various patients and normal cells for preclinical profiles of antitumor efficacy and toxicity of anticancer drugs.
Methods The antitumor activity of fourteen anticancer drugs was tested in over one hundred tumor samples from patients with solid or hematological malignancies. Drug activity against four normal cell types was used for the assessment of normal tissue toxicity. In vitro activity of the drugs was compared with indications approved by the Food and Drug Administration and established adverse event profiles.
Results In general, in vitro drug activity in tumor cells from patients reflected known clinical activity of the drugs investigated. For example, the clinical activity of imatinib in chronic myeloid leukemia was clearly detected in the tumor panel. Further, and in accordance with clinical use, cisplatin and bortezomib showed high activity in ovarian cancer and myeloma samples, respectively. The normal cell models roughly reflected known clinical toxicity profiles and were able to detect differences in therapeutic index, e.g., between targeted drugs and classical cytotoxic agents. For example, the high tolerability of imatinib and the well-known renal toxicity of cisplatin were demonstrated.
Conclusions In preclinical drug development, primary tumor cells from patients can be used for the prediction of cancer diagnosis–specific activity and may aid in the selection of diagnoses for clinical trials. By using tumor and toxicity panels together, information about therapeutic index may be derived, which may be useful when choosing among drug candidates with similar tumor effects.

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Keywords Anticancer drug development · In vitro assays · Toxicity testing · Primary patient tumor cells

Introduction

While there is an urgent need for new and effective anticancer drugs, the development process remains time-consuming and costly. Important advances in drug discovery technologies have been made, with one strategy being high-throughput screening of large or focused compound libraries with structure-based design strategies. The use of new technologies has resulted in a higher yield of potential new agents as well as a reduced time from the selection of a new molecular target to the identification of a drug candidate [1]. The advancement from preclinical models to clinical trials is based on both in vitro and in vivo investigations. However, prediction of clinical outcome from these models is difficult, and the diagnosis–specific activity is still most frequently determined by empirical findings in clinical trials. With this approach, there is a risk of giving a new agent to a large number of patients in which the drug is ineffective, which is problematic from both ethical and financial perspectives. Thus, despite advanced techniques to identify anticancer drug candidates, a large gap remains between preclinical models and the optimal strategy for clinical trials [1–3]. Consequently, preclinical methods able to predict both clinical activity and tolerance of anticancer drugs are highly warranted.

Johnson et al. [4] found only a weak correlation between drug activity in human xenografts with the same human cancer histology and clinical effects. However, for at least one-third of the compounds with in vivo activity, clinical activity was detected in at least some clinical phase II trials [4]. Voskoglou-Nomikos et al. [2] investigated the predictive value of three standard preclinical models; tumor cell lines, human xenografts and murine allografts. The authors concluded that human xenografts were predictive for clinical activity in some diagnoses and that activity in cell lines could be of some use for the prediction of overall clinical activity. With the exception of non-small-cell lung cancer, cell line models could not predict diagnosis–specific activity. Murine allografts appeared to have limited utility [2]. However, Sharma et al. [5] recently showed that the use of large cell line panels (approximately 1,200 cell lines) could reflect the genomic heterogeneity of human cancer and therefore give diagnosis–specific information. Thus, current preclinical methods are far from optimal, and new efficient models for the prediction of clinical utility, including both the activity of and tolerance to anticancer drugs, are needed.

Anticancer drugs not only target neoplastic cells but are also toxic to normal cells and organs [6]. Therefore, there is a need for preclinical assessment of normal tissue toxicity. Efforts have previously been made to develop in vitro toxicity assays for predicting therapeutic index. The "integrated discrete multiple organ cell culture system", for example, used primary cells from liver, kidney, lung, central nervous system, blood vessels and a breast cancer cell line [7]. Bosanquet et al. [8] estimated therapeutic index by using fresh tumor cells and normal cells from the site of the tumor. Furthermore, Li et al. [6] used HepG2 (human hepatocyte carcinoma) and primary renal cells as well as bone marrow progenitor cells and mammary epithelial cells [9] for toxicity assessment of chemotherapeutic drugs.

Although these studies all showed results in concordance with known toxicities from the clinic and can be used for preliminary toxicity profiling in early drug development, in vitro testing is not yet sufficiently predictive to replace in vivo animal studies. Normal cells are often less proliferative and fragile, making in vitro culture something of a challenge. Ideally, primary cells should be used, which is possible with blood cells, while for most other tissues, alternative solutions must be found. In this study, a panel of various types of normal cells was used with the aim to reflect normal tissue toxicity and thus to provide information on therapeutic index.

A correlation between the in vitro activity of anticancer drugs in primary cultures of tumor cells from patients, measured using the non-clonogenic fluorometric cytotoxicity assay (FMCA), and phase II activity was presented in 1999 [10]. Since then, the FMCA has been upgraded from a 96- to a 384-well format, allowing testing of full concentration–effect curves in a highly automated setting instead of single concentrations [11]. The aim of this study was to investigate whether primary cultures of tumor cells from patients together with four normal cell types, assayed using the upgraded FMCA, could reflect the known clinical activity and toxicity of fourteen anticancer drugs.

Materials and methods

Drugs

Fourteen anticancer drugs were tested in the tumor and normal cell panel, including classical cytotoxic agents and targeted drugs. Amsacrine, cisplatin, cytarabine, etoposide, doxorubicin, 5-fluorouracil, melphalan and vincristine were purchased from the local pharmacy (Apoteket AB, Sweden). Bortezomib was purchased from Millennium (Cambridge, MA), and arsenic trioxide and rapamycin were purchased from Sigma-Aldrich (Stockholm, Sweden). Gefitinib was a kind gift from AstraZeneca (Stockholm, Sweden). Imatinib and PKC412 (midostaurin) were gifts from Novartis (Stockholm, Sweden). For most drugs,

commercially available clinical formulations, either powder or solutions for injection/infusion, were used. For the remaining drugs, phosphate-buffered saline (PBS), sterile water or dimethyl sulfoxide (DMSO) was used as solvent for stock preparations. All further dilutions were performed in PBS with a maximum of 1% DMSO in the cell culture; 384-well microplates (Nunclon™ ΔSurface, NUNC Brand Products, Roskilde, Denmark) were prepared with 5 µl of serially diluted drug solutions in duplicates, at five concentrations and at ten times the desired final drug concentrations using the Biomek® 2000 Laboratory Automation Workstation (Beckman Coulter Inc, Fullerton, CA). The plates were stored at −70°C until further use.

Cells and cell culture

Patient tumor cells to reflect antitumor activity

Tumor samples were obtained by routine surgery, diagnostic biopsy or bone marrow/peripheral blood sampling after approval by the ethical committee at Uppsala University (Regionala etikprövningsnämnden i Uppsala, Sweden, approval number Dnr 21/93—930125). Informed consent was obtained from all patients to store diagnostic samples in a biobank to be used in scientific research. Informed consent was verbal until 2006, which was in agreement with the ethics committee's requirements and was written thereafter. The samples stored in the biobank were coded but labeled with diagnosis.

The samples were obtained from patients with acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelocytic leukemia (CML), lymphoma, multiple myeloma, breast cancer, colon cancer, non-small-cell lung cancer (NSCLC), ovarian cancer and renal cancer. The number of samples assayed for each drug is indicated in Table 1.

Leukemic cells were isolated from bone marrow or peripheral blood using density gradient centrifugation in Ficoll-Paque (1.007 g/ml) (GE Healthcare Life Sciences, Uppsala, Sweden). Tissue from solid tumor samples was processed by mincing with scissors, and tumor cells were then isolated by collagenase dispersion followed by purification using density gradient centrifugation on Percoll (GE Healthcare). Cell viability was determined using a trypan blue exclusion test, and the proportion of tumor cells was assessed after inspection of cytospin preparations stained with May–Grünwald–Giemsa. With the exception of multiple myeloma, for which freshly prepared cells were used, cells were cryopreserved in medium containing 10% DMSO and 90% fetal calf serum (FCS) by initial freezing for 24 h at −70°C, followed by storage at −150°C. The cells were thawed at 37°C in a water bath and suspended in cell culture medium. The medium used throughout was

Table 1 Investigated drugs and diagnoses showing median IC_{50} (µM) and number of samples analyzed

	AML	ALL	CML	CLL	Lymphoma	Myeloma	Breast	Colon	NSCLC	Ovarian	Renal
Amsacrine	0.82 [13]	0.32 [9]	0.26 [2]	2.4 [14]	3.4 [14]	2.0 [3]*	9.6 [4]	>100 [6]	12 [4]	13 [14]	>100 [5]
Arsenic trioxide	1.6 [15]	1.4 [14]	0.76 [10]	1.8 [17]	11 [2]	ND	2.8 [5]	100 [11]	4.9 [4]	7.9 [14]	>100 [7]
Bortezomib	0.058 [14]	0.040 [15]	0.018 [11]	0.041 [15]	0.29 [15]	0.017 [6]*	0.082 [5]	5.4 [12]	0.079 [5]	0.93 [14]	>10 [7]
Cisplatin	24 [15]	17 [11]	2.5 [3]	44 [15]	14 [15]	32 [3]*	21 [5]	23 [8]	13 [4]	9.5 [14]	>100 [7]
Cytarabine	8.1 [13]	11 [10]	1.0 [2]	21 [14]	5.1 [15]	71 [3]*	>100 [4]	>100 [6]	>100 [4]	>100 [11]	>100 [7]
Doxorubicin	0.34 [12]	0.23 [12]	0.090 [2]	0.29 [15]	0.15 [15]	0.57 [3]*	0.92 [5]	0.76 [7]	0.32 [4]	0.60 [13]	>100 [7]
Etoposide	4.6 [13]	2.2 [9]	9.0 [2]	17 [13]	11 [15]	17 [3]*	98 [5]	97 [6]	>100 [4]	>100 [14]	>100 [6]
5-Fluorouracil	340 [14]	250 [11]	35 [3]	2,200 [14]	990 [15]	2,900 [3]*	730 [5]	660 [6]	190 [4]	290 [12]	3,700 [1]
Gefitinib	15 [14]	82 [3]	26 [5]	68 [12]	37 [10]	ND	85 [3]	100 [3]	41 [6]	49 [10]	140 [1]
Imatinib	7.7 [13]	4.2 [10]	0.057 [10]	11 [17]	9.1 [14]	ND	5.7 [5]	100 [12]	21 [6]	13 [14]	>100 [6]
Melphalan	4.6 [15]	3.8 [12]	4.1 [2]	14 [15]	7.0 [15]	11 [3]*	440 [5]	520 [8]	26 [4]	30 [14]	>1000 [5]
PKC412	1.2 [11]	1.2 [9]	0.97 [8]	0.38 [15]	1.1 [14]	ND	4.4 [3]	>100 [2]	4.1 [3]	16 [7]	13 [1]
Rapamycin	20 [10]	5.0 [13]	13 [7]	8.5 [18]	>10 [11]	ND	69 [4]	>100 [11]	40 [5]	40 [11]	>100 [7]
Vincristine	6.9 [13]	1.5 [9]	5.2 [2]	1.2 [14]	1.8 [15]	8.7 [3]*	>10 [5]	>10 [6]	>10 [4]	>10 [14]	>10 [6]

ND Not determined

* Includes freshly prepared samples

RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin (Sigma-Aldrich). Cell plating density was 5,000 cells/well for solid tumors and 12,000 to 40,000 cells/well for leukemic cells [11].

Lymphocytes and CD34$^+$ stem cells to reflect hematological toxicity

Lymphocytes (PBMC) ($n = 4$) from healthy donors were isolated from peripheral blood using density gradient centrifugation on Ficoll-Paque (GE Healthcare). The cells were handled as described for tumor samples from patients before plating at 37,500 cells/well in 384-well plates prepared with drug samples. Four independent experiments were performed with cells from four different donors.

The FMCA-GM14 differs from the other normal cell models, and proliferative capacity in the progenitor cell is high. Effects of antiproliferative drugs might thus have a larger impact in FMCA-GM14. Cryopreserved ($n = 3$) human umbilical cord blood CD34$^+$ stem cells were purchased from 3H Biomedical AB (Cat no. 3H-902-10 and 3H-902-1; Uppsala, Sweden). FMCA-GM14 has been described in detail previously [12]. The cells were cryopreserved after isolation (purity 92–95%) and were stored at −150°C in FCS and DMSO. The cells were rapidly thawed at 37°C in a water bath, suspended in a cell thawing media (3H Biomedical AB), then centrifuged at 1,000 rpm for 10 min before suspending the cells in stem cell culture media and plating in 96-well plates. For granulocytopoietic lineage differentiation, the stem cell culture medium was supplemented with stem cell factor, interleukin-3, Flt ligand, granulocyte colony-stimulating factor and granulocyte–macrophage colony-stimulating factor (Cat. no. 900-30-50, 3H Biomedical AB). After 7 days of culture in 96-well plates, the cells were counted and re-plated (250 cells/well) in 384-well plates prepared with drug samples. Cell survival was analyzed using FMCA after 7 days of drug exposure under standard culture conditions. Three experiments were performed using cells from two different donors.

Cells to reflect epithelial and renal toxicity

Human telomerase reverse transcriptase subunit-retinal pigment epithelial cells (hTERT-RPE1) from Clontech Laboratories (Palo Alto, CA) were cultured in Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham (cat. no D6421) supplemented with 10% heat-inactivated FCS, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin (Sigma-Aldrich). Human renal proximal tubular epithelial cells (HRPTEpiC) were cultured in Epithelial Cell Medium (cat. no 4101) where 500 ml medium was supplemented with 10 ml FCS (cat. no. 0010), 5 ml epithelial cell growth supplement (cat. no. 4152) and 5 ml penicillin/streptomycin solution (cat. no 0503). The culture flasks were coated with poly-L-lysine (cat. no 0403/0413). All products for culturing of HRPTEpiC were purchased from 3H Biomedical AB. HRPTEpiC were cultured according to the manufacturer's instructions, and hTERT-RPE1 was sub-cultured twice a week. hTERT-RPE1 and HRPTEpiC were plated (2,500 cells/well) in 384-well plates prepared with drug samples, and cell survival was analyzed using the FMCA. Three independent experiments were performed for each cell type.

Measurement of cytotoxicity

Cell survival was measured using FMCA, described previously in detail [11, 13]. The FMCA is based on the measurement of fluorescence generated from the hydrolysis of fluorescein diacetate to fluorescein by cells with intact plasma membranes. Cells were seeded in 384-well microplates prepared with drug samples and incubated at 37°C in the presence of 5% CO_2 for 3 days (with the exception of the FMCA-GM14 that was incubated for 7 days). Wells without drugs, i.e., medium only, served as blanks. After incubation, the FMCA was performed using the automated Optimized Robot for Chemical Analysis (Orca, Beckman Coulter) programmed with SAMI software (Beckman Coulter). Medium and drugs were aspirated, the cells were washed twice with PBS, and fluorescein diacetate was added. After 50 to 70 min of incubation, fluorescence was measured at 485/520 nm in a fluorometer (Fluostar Optima, BMG Technologies, Germany). The emitted fluorescence is proportional to the number of living cells. Cell survival is presented as the survival index, defined as fluorescence in experimental wells as a percentage of fluorescence in control wells, with blank values subtracted. The mean fluorescence in the control wells had to be at least five times the mean blank value (signal/noise ratio), and a coefficient of variation had to be less than 30% in the control wells for the assay to be approved.

Data analysis and presentation

Statistical analysis

All tumor cell data were stored in an Accord HTS database (Accelrys Inc, San Diego, CA). For tumor cells and epithelial, lymphocyte and renal toxicity assays, IC_{50} values (drug concentration resulting in 50% cell survival compared with controls) were calculated using non-linear curve fit with constraints set to a minimum value of 0 and a maximum value of 100 in the Hill's equation, using

GraphPadPrism software (GraphPad Software, Inc. San Diego, CA). For the FMCA-GM14, data were processed in Microsoft Excel using a log-linear interpolation to calculate the log IC_{50} values. For both the normal cell models and primary tumor samples where an IC_{50} could not be determined because a too low or too high drug concentration range was tested, the IC_{50} value was reported as the highest or lowest tested concentration, respectively. For tumor cell data, the median IC_{50} was used and the mean value was used for normal cells. In mean graphs, diagnoses were included when at least four samples were available. Delta was defined as the difference between median log IC_{50} of an individual cell type and mean log IC_{50} for all cell types and provides a 'fingerprint' of the activity pattern of a drug.

Solid/hematological (S/H) activity ratio

Solid/hematological (S/H) activity ratio was calculated for each drug to investigate whether the FMCA results could predict solid or hematological tumor specificity. The response rate for the hematological and the solid tumor group was defined as the fraction of samples with IC_{50} below the median IC_{50} of both groups in each tumor type. S/H ratio is defined as the response rate of solid tumors divided by that of the hematological samples. S/H ratios greater than 1 and less than 1 indicate a relatively higher activity and lower activity, respectively, in solid tumors compared with hematological malignancies.

Clinical data

Approved indications from the Food and Drug Administration (FDA) were used as references for clinical activity. Information on approved indications was collected from the FDA database, "Daily Med, Current Medication Information" [14]. Toxicity profiles (bone marrow and kidney) were collected from summaries of the Swedish Drug Compendium and clinical experience [15, 16].

The GM14 index

The GM14 toxicity index was defined as the ratio of the mean IC_{50} in FMCA-GM14 to the median IC_{50} of the most sensitive tumor type in vitro ($\geq$4 samples). Thus, a higher GM14 index corresponds to a better therapeutic index.

Results

Tumor cell activity

Eleven diagnoses and 14 drugs were included in the study (see Table 1). The activity of the tested drugs in solid compared with hematological tumors in vitro is shown as S/H ratios in Fig. 1a. 5-Fluorouracil, cisplatin and gefitinib had the highest relative effects in solid tumors, in agreement with the clinical use of these drugs. The lowest ratios among registered drugs were seen for etoposide, amsacrine and cytarabine, which for the last two is consistent with approved indications (i.e., hematological malignancies).

The activity 'fingerprint' of the 14 drugs on the tumor types tested is shown as mean graphs in Fig. 1b, and the three most sensitive diagnoses are compared with FDA-approved indications in Table 2. In general, the in vitro activity was in accordance with the approved clinical use. Myeloma samples were sensitive to bortezomib and relatively sensitive to melphalan (not shown in Fig. 1b due to too few samples, see Table 1), which are important drugs in the treatment for myeloma.

Ovarian cancer samples were clearly more sensitive to cisplatin compared with cells from other diagnoses, which is consistent with the drug's main clinical use. Ovarian cancer samples were relatively sensitive to melphalan compared with other solid tumor diagnoses, also in line with the approved indication. Doxorubicin is approved for use in both solid and hematological malignancies, which corresponds with its in vitro results. Solid tumor samples were relatively resistant to etoposide, which does not agree with the indicated diagnoses but is in accordance with current use of this drug [16].

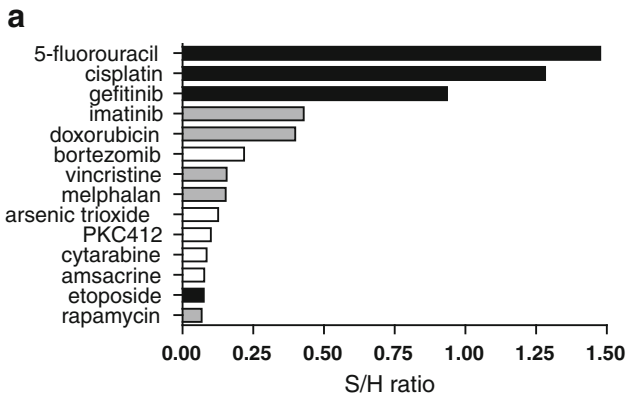


Fig. 1 **a** Solid/hematological drug activity ratios in vitro for fourteen different anticancer drugs. The S/H ratio is defined as the ratio between the in vitro response rate of solid and hematological samples. Drugs approved for use in solid tumors only are indicated by *black bars*, drugs approved for use in both hematological and solid tumors are indicated by *gray bars*, and drugs approved for use in hematological tumors only are indicated by *white bars*. **b** Drug activity in the patient tumor cell panel. Drug activity was displayed as mean graphs with delta values defined as differences between median log IC_{50} and log IC_{50} for the individual tumor type. *Bars* to the *right* and *left* indicate diagnoses more and less resistant, respectively, compared with the mean of all samples. Diagnoses were included when four or more samples were available ($n = 4$ to 15, see Table 1)

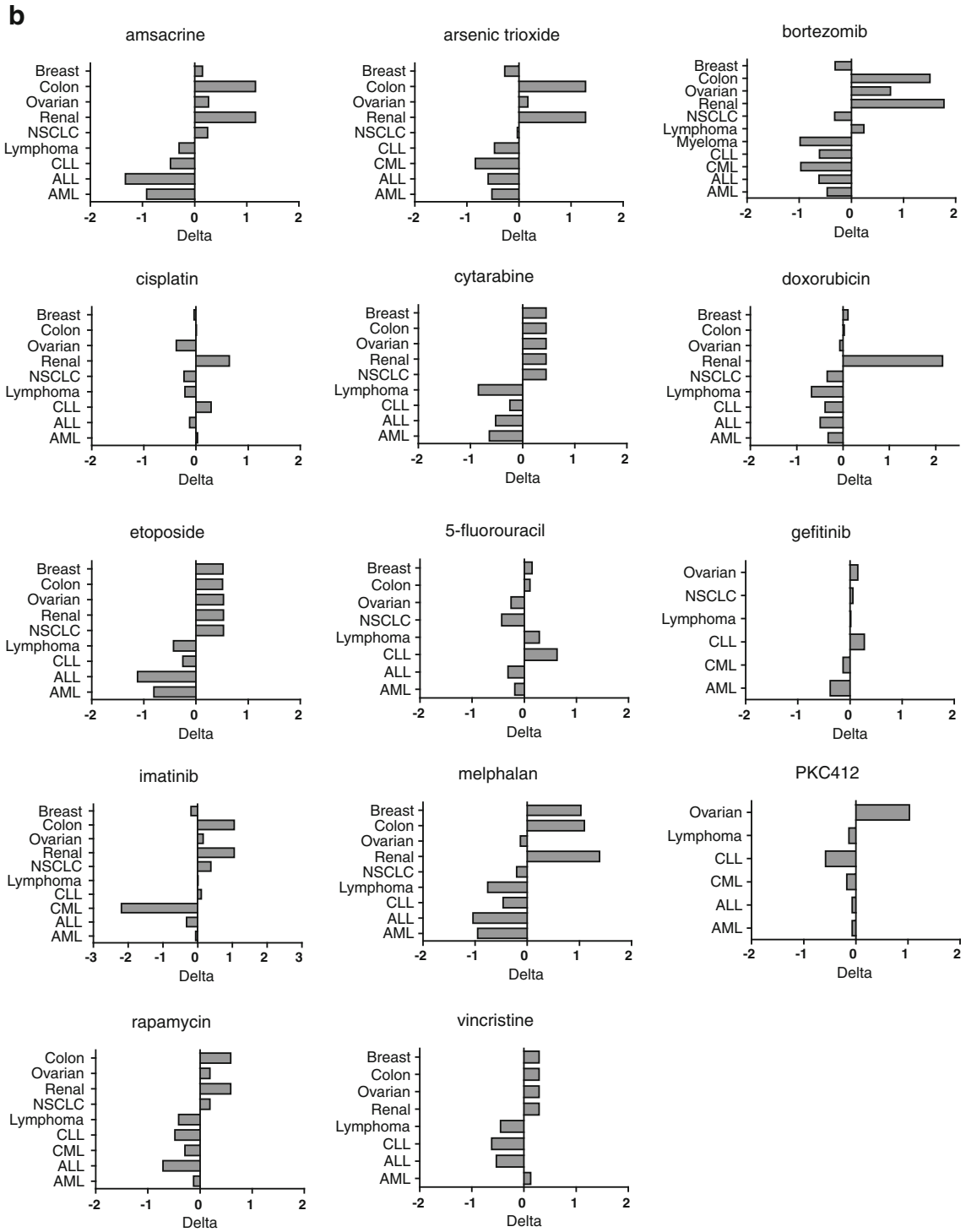


Fig. 1 continued

5-Fluorouracil showed a solid tumor activity profile in accordance with its clinical use. For gefitinib, which is an epidermal growth factor receptor (EGFR) inhibitor, AML was clearly the most sensitive diagnosis, which is not an approved indication. Gefitinib is used in the treatment for NSCLC, which was relatively sensitive in vitro. Imatinib has been successfully used in the treatment for CML, which is reflected in pronounced in vitro activity in this diagnosis. A high response in leukemias was detected for PKC412, currently in phase III studies in AML. The same

Table 2 Approved indications by Food and Drug Administration (FDA) and the most drug-sensitive diagnoses in vitro, followed by the second and third most sensitive diagnoses in vitro (>4 samples), are rated for each drug included in the study

Drug	3 most sensitive diagnoses in vitro			Approved indications by FDA
Amsacrine	ALL	AML	CLL	(ALL)*
Arsenic trioxide	CML	ALL	AML	APL
Bortezomib	Myeloma	CML	ALL	Multiple myeloma, mantle cell lymphoma
Cisplatin	Ovarian	NSCLC	Lymphoma	Bladder, ovarian and testicular cancer
Cytarabine	Lymphoma	AML	ALL	AML, ALL, CML
Doxorubicin	Lymphoma	ALL	CLL	ALL, AML, Wilm's tumor, neuroblastoma, sarcoma, breast, lung, ovarian, bladder, thyroid and gastric cancer, Hodgkin's and non-Hodgkin's lymphoma
Etoposide	ALL	AML	Lymphoma	Lung and testicular cancer
5-Fluorouracil	NSCLC	ALL	Ovarian	Breast, colon, gastric and pancreatic cancer
Gefitinib	AML	CML	Lymphoma	NSCLC
Imatinib	CML	ALL	Breast	Ph^+CML, Ph^+ALL, MDS/MPD, ASM, HES, CEL, Kit^+GIST
Melphalan	ALL	AML	Lymphoma	Multiple myeloma, ovarian cancer
PKC412	CLL	CML	Lymphoma	In clinical trials for AML treatment
Rapamycin	ALL	CLL	CML	Model substance for mTOR inhibitors
Vincristine	CLL	ALL	Lymphoma	Acute leukemia, Hodgkin's and non-Hodgkin's lymphoma, rhabdomyosarcoma, neuroblastoma, Wilm's tumor

APL Acute promyelocytic leukemia, *MDS/MPD* Myelodysplastic/myeloproliferative diseases, *ASM* Aggressive systematic mastocytosis, *HES* Hypereosinophilic syndrome, *CEL* Chronic eosinophilic leukemia, *GIST* Gastrointestinal stromal tumors

* Previously approved for ALL

pattern was seen for rapamycin, an immunosuppressant included as a model substance for mammalian target of rapamycin (mTOR) inhibitors.

Normal cell toxicity

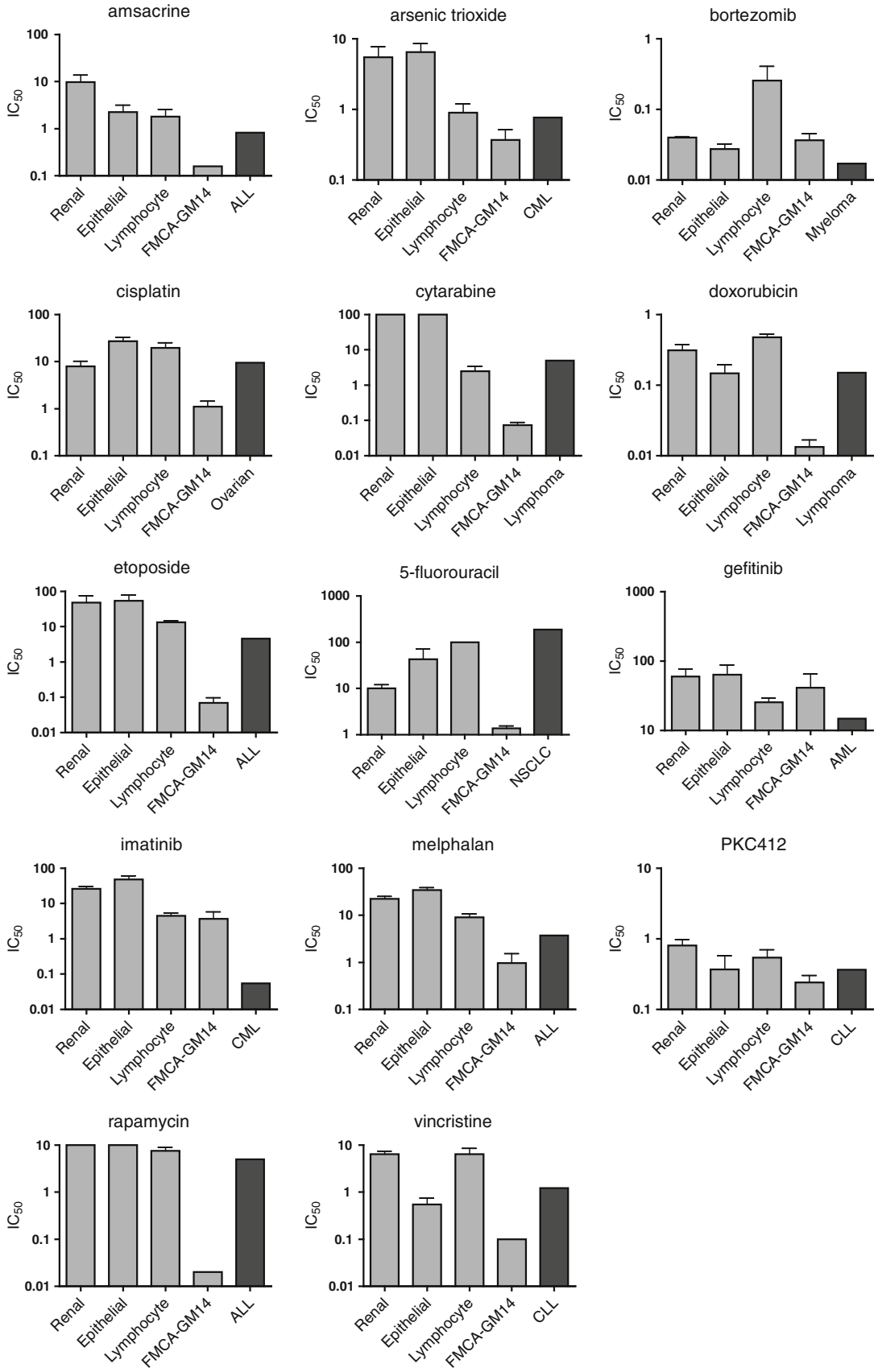
The normal cell panel was able to roughly predict the clinical toxicity profile when comparing in vitro toxicity with established side effects (Fig. 2; Table 3). The response of the normal cells varied considerably between drugs (Fig. 2). In agreement with clinical experience, epithelial and renal cells were relatively resistant to amsacrine, cytarabine and melphalan, while lymphocytes and FMCA-GM14 were more sensitive. Myeloma cells were more sensitive to bortezomib compared with all normal cell types. Platinum agents, such as cisplatin, are well known for their renal toxicity [6, 17], and renal cells were relatively sensitive in vitro. CML cells were clearly more sensitive than any of the normal cells to imatinib, which is in agreement with its low clinical toxicity [18]. Gefitinib and PKC412 are both signal transduction inhibitors with favorable toxicity profiles [19, 20], which is reflected in the panel for gefitinib. However, for PKC412, lymphocytes and FMCA-GM14 cells were more sensitive than CLL samples, the most sensitive tumor type.

The GM14 index, defined as the ratio of IC_{50} for FMCA-GM14 and median IC_{50} for the most sensitive diagnosis for each drug, is displayed in Fig. 3. The highest GM14 index was seen for the targeted drugs imatinib, bortezomib and gefitinib, reflecting a broad therapeutic window with respect to myelotoxicity. In clinical trials, PKC412 has been relatively well tolerated [19], which was also reflected in FMCA-GM14 index. A lower GM14 index was seen for conventional cytotoxic agents and the immunosuppressant, rapamycin. With the exception of 5-fluorouracil and rapamycin, this is in reasonable agreement with clinical tolerance.

Discussion

There is a need for improved methods to guide in the transition from promising preclinical results of anticancer drugs to optimized clinical drug development. Using tumor cells from patients and a panel of normal cells, we investigated whether data on clinical activity profiles as well as toxicity could be obtained from relatively simple in vitro models.

In general, in vitro drug activity in tumor cells from patients reflected the known clinical activity of the drugs investigated, which is in agreement with a previous report from our group including a more limited number of standard drugs [10]. The activity in leukemias for typical hematological drugs such as amsacrine, arsenic trioxide, bortezomib, cytarabine, melphalan and vincristine was well reflected in vitro, which is shown in both the S/H ratio and individual tumor type sensitivities, including the very specific effect of imatinib on CML. In addition, the solid


amsacrine
IC50
100
10
1
0.1
Renal
Epithelial
Lymphocyte
FMCA-GM14
ALL
arsenic trioxide
IC50
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Renal
Epithelial
Lymphocyte
FMCA-GM14
CML
bortezomib
IC50
1
0.1
0.01
Renal
Epithelial
Lymphocyte
FMCA-GM14
Myeloma
cisplatin
IC50
100
10
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Renal
Epithelial
Lymphocyte
FMCA-GM14
Ovarian
cytarabine
IC50
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NSCLC
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rapamycin
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ALL
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1
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Epithelial
Lymphocyte
FMCA-GM14
CLL

◀ **Fig. 2** Drug activity in the normal cell panel. Mean IC_{50} ($n = 3$ to 4 and ±SEM) from the renal, epithelial, lymphocyte and FMCA-GM14 normal cell models are shown together with the median IC_{50} of the most sensitive tumor type for each drug

tumor effects of cisplatin and 5-fluorouracil were well reflected. The in vitro effect on hematological malignancies for etoposide was not in concordance with approved indications by the FDA, but corresponds with clinical use in Sweden [16], where AML and lymphoma, as well as lung and testicular cancer, are approved indications. The response of gefitinib in NSCLC is strongly associated with EGFR mutations [20], in this study, the samples were not characterized regarding EGFR mutation status which may explain why response in NSCLC samples was not apparent. AML is not an approved indication for gefitinib today, but the high in vitro activity in this cell type has been reported previously [21–23] and the tyrosine kinase SYK has been suggested as target [24]. Hematological cells were generally sensitive to PKC412, which is consistent with effects seen in clinical trials where PKC412 has shown cytotoxic acitivty in patients with FLT3-positive AML [25]. The mTOR inhibitor rapamycin is currently used as an immunosuppressant following renal transplantations and not in cancer, but its analogs, everolimus and temsirolimus, were recently approved for the treatment of advanced renal carcinoma [14, 26, 27]. Temsirolimus has also shown activity in mantle cell lymphoma [28], which is an approved indication in Europe [29]. Effects on renal carcinoma were poorly reflected by rapamycin in this tumor panel, but the effect on lymphoma was detected.

Cell lines, with advantages such as unlimited number of cells and very high accessibility, are widely used to investigate the effects of new compounds. Cell lines have also proven useful in mechanistic studies of novel compounds [30] but may be limited in their capacity to reflect tumor diagnosis–specific activity. In tumor cells from patients, the diagnosis–specific phenotype is largely intact with respect to drug sensitivity and these cells may therefore be better predictors of clinical activity. However, working with patient samples requires good cooperation with interested clinicians, logistics and laboratory skills [10].

Using approved indications to measure clinical activity of drugs, as demonstrated here, is simple and robust, but is limited by a high off-label use of the older drugs. Another option would have been to use phase II data, but it was difficult to obtain comparable data for the fourteen drugs tested in the eleven diagnoses. Indeed, not all drugs are tested clinically in all diagnoses and drug development in medical companies is driven not only by preclinical results but also by strategic considerations. It should also be emphasized that although in vitro drug activity in a specific tumor type reflects clinical efficacy fairly well, the correspondence is far from

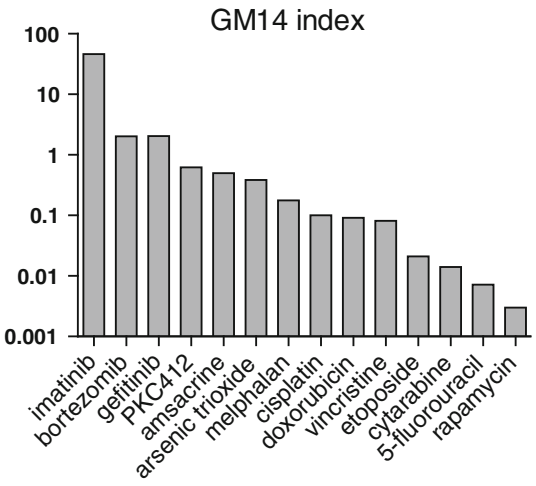

Fig. 3 GM14 index for in vitro assessment of stem cell toxicity potential. The GM14 index is defined as the ratio between the mean IC_{50} from the FMCA-GM14 and the median IC_{50} for the most sensitive tumor type in vitro. The most sensitive tumor type for each drug was as follows: amsacrine—ALL, arsenic trioxide—CML, bortezomib—myeloma, cisplatin—ovarian, cytarabine—lymphoma, doxorubicin—lymphoma, etoposide—ALL, 5-fluorouracil—NSCLC, gefitinib—AML, imatinib—CML, melphalan—ALL, PKC412—CLL, rapamycin—ALL and vincristine—CLL

perfect since clinical efficacy depends on a number of factors not included in the assay, such as tumor cell interactions with stroma, the extracellular milieu of tumors, achievable plasma drug concentrations, mutations, distribution to tumor tissue and dose-limiting toxicity [30].

In the toxicity assays, the great clinical benefit of imatinib in CML was seen as a large difference in sensitivity between CML cells and normal cells and the well-known renal toxicity of cisplatin was detected in the cell types used. Although some of the tissue-specific toxicities were well predicted, the toxicity assay panel will probably be most useful in giving a rough prediction of therapeutic index rather than giving information on each specific organ. Toxicities such as cardiomyopathy after doxorubicin treatment, allergic reactions and neurological toxicity would not be detected in this type of panel, and it will always be difficult to predict toxicities with low frequency. In the epithelial, stem cell and renal normal cell models, cells proliferate at a higher rate than the very low proliferative primary cells, and these models would theoretically be more sensitive to strictly antiproliferative drugs. This should be kept in mind when comparing results from different cell models, but in general both cytotoxic and antiproliferative drugs in our study show in vitro activity corresponding to the clinical activity and toxicity pattern.

The FMCA-GM14 seemed to be a powerful tool to rank drugs according to stem cell toxicity and could distinguish between conventional cytotoxic drugs and less toxic drugs targeted to bone marrow. However, the thrombocytotoxic

Table 3 Toxic reactions typical in bone marrow and kidney, arranged after mechanism of action. Information is based on adverse event reports in the Swedish drug compendium and clinical experience [15, 16]

Mechanism of action	Drugs included in the study	Targets of toxicity	
		Bone marrow	Renal
Alkylating agents	Melphalan	++	+
Platinum agents	Cisplatin	+	++
Topoisomerase II inhibitors	Amsacrine, etoposide, doxorubicin	++	−
Antimetabolites	Cytarabine, 5-fluorouracil	++	+
Tyrosine kinase inhibitors	Imatinib, gefitinib, PKC412	+	−
Vinca alkaloids	Vincristine	+	−
mTOR inhibitors	Rapamycin	+	−
Proteasome inhibitors	Bortezomib	+	+
Other	Arsenic trioxide	+	−

++ Common toxicity

+ Toxicity is less common

− Toxicity is uncommon or does not occur

effect of bortezomib was not reflected in our methods, which may be due to the fact that cells in the GM14 assay belong to the granulocytopoietic lineage [12, 31]. Another exception was rapamycin, which had a surprisingly low GM14 index. Rapamycin is used clinically as an immunosuppressant, which might explain the sensitivity of the progenitor cells, although its analogs have been generally well tolerated [26, 27]. In addition, 5-fluorouracil was ranked as the conventional cytotoxic drug most toxic to stem cells, which is not in accordance with clinical experience. For 5-fluorouracil and rapamycin, analyses based on IC_{50} are generally suboptimal due to a flat dose–response relationship in many cell types, making it difficult to determine IC_{50} based on Hill's equation.

In this study, we aimed to develop normal cell models of human origin, suitable for a high-throughput setting that is reflected in the choice of models. Primary renal cells of high quality are commercially available, may be reliably cultured and reflect the in vivo situation [6]. Genetically altered normal cell lines may also be used as an in vitro model for normal tissue. The epithelial cell line used in this work was immortalized by the introduction of human telomerase reverse transcriptase. In the lymphocyte toxicity assay, cells from healthy donors were used. The golden standard for the prediction of bone marrow toxicity, the colony-forming unit-granulocyte–macrophage (CFU-GM) assay [32], has the disadvantages of being labor intensive and subjective in its endpoint. An alternative high-throughput method, the FMCA-GM14 assay, was thus recently developed and correlated well with the CFU-GM assay [12]. This in vitro toxicity assay has high-throughput potential and may possibly be used to evaluate large numbers of drug candidates earlier in the drug development process. Although costly and time-consuming in vivo toxicity studies cannot presently be replaced, normal cell assays similar to the example described here are important additions to preclinical toxicity testing. One promising application for the normal cell models is as a means to rank compounds prior to testing in vivo (Fig. 3).

In conclusion, the results support that tumor cells from patient samples can be used for the prediction of cancer diagnosis–specific activity in the preclinical stage of drug development. The primary tumor cell panel may aid in the selection of diagnoses for clinical trials and a normal cell panel may reflect expected toxicity. When both tumor and toxicity panels are used together, important information about therapeutic index may be derived, which may be useful when choosing among drug candidates and diagnoses for clinical drug development.

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References

1. Damia G, D'Incalci M (2009) Contemporary pre-clinical development of anticancer agents–what are the optimal preclinical models? Eur J Cancer 45:2768–2781
2. Voskoglou-Nomikos T, Pater JL, Seymour L (2003) Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clin Cancer Res 9:4227–4239
3. Peterson JK, Houghton PJ (2004) Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development. Eur J Cancer 40:837–844
4. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM et al (2001) Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer 84:1424–1431
5. Sharma SV, Haber DA, Settleman J (2010) Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nature reviews 10:241–253
6. Li W, Lam M, Choy D, Birkeland A, Sullivan ME et al (2006) Human primary renal cells as a model for toxicity assessment of chemo-therapeutic drugs. Toxicol In Vitro 20:669–676
7. Li AP, Bode C, Sakai Y (2004) A novel in vitro system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast cancer cells. Chem Biol Interact 150:129–136

8. Bosanquet AG, Bell PB (2004) Ex vivo therapeutic index by drug sensitivity assay using fresh human normal and tumor cells. J Exp Ther Oncol 4:145–154
9. Li W, Lam MS, Birkeland A, Riffel A, Montana L et al (2006) Cell-based assays for profiling activity and safety properties of cancer drugs. J Pharmacol Toxicol Methods 54:313–319
10. Fridborg H, Jonsson E, Nygren P, Larsson R (1999) Relationship between diagnosis-specific activity of cytotoxic drugs in fresh human tumour cells ex vivo and in the clinic. Eur J Cancer 35:424–432
11. Lindhagen E, Nygren P, Larsson R (2008) The fluorometric microculture cytotoxicity assay. Nat Protoc 3:1364–1369
12. Haglund C, Aleskog A, Hakansson LD, Hoglund M, Jacobsson S et al (2010) The FMCA-GM assays, high throughput non-clonogenic alternatives to CFU-GM in preclinical hematotoxicity testing. Toxicol Lett 194:102–107
13. Larsson R, Kristensen J, Sandberg C, Nygren P (1992) Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia, using a fluorometric microculture cytotoxicity assay (FMCA). Int J Cancer 50:177–185
14. FDA (2010) http://dailymed.nlm.nih.gov/dailymed/about.cfm. Food and Drug Administration. Available via Food and Drug Administration http://dailymed.nlm.nih.gov/dailymed/about.cfm. Accessed October 2010
15. Ramström H (ed) (2009) Läkemedelsboken. 17 edn. Apoteket AB, Stockholm
16. FASS (ed) (2009) FASS. The Swedish Drug Compendium, Läkemedelsindustriförenningen AB
17. Kintzel PE (2001) Anticancer drug-induced kidney disorders. Drug Saf 24:19–38
18. Wolf D, Rumpold H (2009) A benefit-risk assessment of imatinib in chronic myeloid leukaemia and gastrointestinal stromal tumours. Drug Saf 32:1001–1015
19. Sanz M, Burnett A, Lo-Coco F, Lowenberg B (2009) FLT3 inhibition as a targeted therapy for acute myeloid leukemia. Curr Opin Oncol 21:594–600
20. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R et al (2008) Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 372:1809–1818
21. Lindhagen E, Eriksson A, Wickstrom M, Danielsson K, Grundmark B et al (2008) Significant cytotoxic activity in vitro of the EGFR tyrosine kinase inhibitor gefitinib in acute myeloblastic leukaemia. Eur J Haematol 81:344–353
22. Boehrer S, Ades L, Galluzzi L, Tajeddine N, Tailler M et al (2008) Erlotinib and gefitinib for the treatment of myelodysplastic syndrome and acute myeloid leukemia: a preclinical comparison. Biochem Pharmacol 76:1417–1425
23. Stegmaier K, Corsello SM, Ross KN, Wong JS, Deangelo DJ et al (2005) Gefitinib induces myeloid differentiation of acute myeloid leukemia. Blood 106:2841–2848
24. Hahn CK, Berchuck JE, Ross KN, Kakoza RM, Clauser K et al (2009) Proteomic and genetic approaches identify Syk as an AML target. Cancer Cell 16:281–294
25. Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E et al (2005) Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. Blood 105:54–60
26. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C et al (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449–456
27. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R et al (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356:2271–2281
28. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M et al (2009) Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol 27:3822–3829
29. EMEA EMA Document Number EMA/783677/2009. Accessed 2010/09/30 2010
30. Holbeck SL, Collins JM, Doroshow JH (2010) Analysis of Food and Drug Administration-approved anticancer agents in the NCI60 panel of human tumor cell lines. Mol Cancer Ther 9:1451–1460
31. Lonial S, Waller EK, Richardson PG, Jagannath S, Orlowski RZ et al (2005) Risk factors and kinetics of thrombocytopenia associated with bortezomib for relapsed, refractory multiple myeloma. Blood 106:3777–3784
32. Pessina A, Albella B, Bayo M, Bueren J, Brantom P et al (2003) Application of the CFU-GM assay to predict acute drug-induced neutropenia: an international blind trial to validate a prediction model for the maximum tolerated dose (MTD) of myelosuppressive xenobiotics. Toxicol Sci 75:355–367