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The Cancer Research UK experience of pre-clinical toxicology studies to support early clinical trials with novel cancer therapies

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Abstract

Pre-clinical toxicology studies in rodents and Phase I clinical trial data are summarised for 14 novel anticancer therapies. With only one exception, an antifolate antimetabolite, rodent toxicology predicted a safe Phase I trial starting dose and the majority of the dose limiting toxicities, in particular haematological toxicity. For targeted agents with well-defined pharmacodynamic markers, illustrated in the current study by 3 anti-endocrine drugs and one resistance modifier, the definition of a maximum tolerated dose can be avoided. Together with earlier data, the current study confirms that pre-clinical toxicology studies in a non-rodent species are not routinely needed for the safe conduct of early clinical trials with new cancer chemotherapies.

1. Introduction

Pre-clinical toxicology studies play two important and related roles in cancer drug development. Firstly, during the lead optimisation phase of drug development they may be used to prioritise drug candidates for clinical evaluation, and in some cases data from toxicity studies are a primary criterion for final compound selection as, for example, in the development of non-nephrotoxic platinum drugs or non-cardiotoxic anthracylines. Secondly, pre-clinical toxicology studies are required by regulatory authorities as part of procedures that must be followed prior to the initiation of clinical trials. Only this second aspect of toxicology studies will be addressed in the current paper.

Clinical trial approval procedures are not fully harmonised world-wide, although there are moves to do so, and the data reviewed in the current paper relate largely to the experience of the Cancer Research UK Phase I/II Clinical Trials and New Agents Committees, whose activities are supported and co-ordinated by the Drug Development Office of Cancer Research UK. The Phase I/II Committee has been in operation since 1980 and all aspects of cancer drug development; pre-clinical and clinical, have changed dramatically over the intervening 23 years. In particular, guidelines and regulations for pre-clinical studies have changed, and at the current time the details and implications of the new European Clinical Trials Directive [1] are still unclear, although initial perspectives will be discussed at the end of this article. What has not changed in the last 23 years, however, is the need for more effective cancer treatments and the challenge of introducing these into clinical trials. In the latter context, the primary role of pre-clinical toxicology is to identify a safe Phase I trial starting dose, potential toxicities and their reversibility.

Numerous reviews of previous experience of pre-clinical toxicology testing have been published, that of Cancer Research UK by Newell and colleagues in 1999 [2] and that of the Toxicology and Pharmacology Branch of the National Cancer Institute (USA) in the current volume [3]. There is currently a clear consensus that, as with all areas of *in vivo* studies, steps must be continually taken to reduce, refine and replace animal experimentation, and that pre-clinical toxicology packages should be compound specific and designed to mirror the proposed clinical trial as closely as possible, i.e. in terms of drug formulation, route of administration, schedule and toxicity monitoring. Furthermore, wherever possible, pharmacokinetic (PK) and pharmacodynamic (PD) studies should be included to define pharmacological endpoints related to toxicity, as well as efficacy, for use in the design of the Phase I trial.

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Following from the above widely agreed general aims and overall principles for the design of pre-clinical toxicology studies there are many more specific issues which continue to exercise the drug development community, both academic and commercial, as well as the regulatory authorities. For example: How many species should be used in pre-clinical toxicology studies? How closely should the species be "related" to humans; are rodents alone sufficient? How many courses of therapy should be given? At what stage, if at all, should genotoxicity, carcinogenicity and reproductive toxicity be studied? The current guidelines of the Food and Drug Administration (USA) are summarised by Tomaszewski [3] and those of the European Medicines Evaluation Agency have been published [4], as have those used by Cancer Research UK over the past 23 years [5,6]. A recent review of the experience of Cancer Research UK concluded that for 25 agents a single course of dosing in the mouse was able to predict a safe Phase I trial starting dose, and haematological and neurological dose limiting toxicities (DLTs) with a high degree of consistency, i.e. in 7/7 and 3/3 cases, respectively [2]. The preponderance of cytotoxic drugs in the group of compounds studied argued for caution in applying the conclusions to non-cytotoxic or "targeted" agents. Also, limitations in using the mouse to detect effects such as nausea and vomiting, myalgia, pain, flushing, hypotension and asthenia, were noted.

In the current paper the further experience of Cancer Research UK is reviewed based on 14 additional agents.

2. Materials and methods

The 14 drugs included in the current analysis are listed in Table 1. Table 1 also summarises the properties of the compounds, the general class to which each compound belongs and references to the reports describing the Phase I clinical trials. The clinical studies were all approved by, and performed under the auspices of, Cancer Research UK and each trial was approved by the relevant local ethics committee (institutional review board). Pre-clinical toxicology studies were in the main performed according to the protocols previously published [5,6], and the full reports are held on file at the Drug Development Office, Cancer Research UK, London, UK. Experiments were conducted according to local animal welfare regulations and, where appropriate, were covered by a UK Home Office project license.

3. Results

3.1. Pre-clinical toxicology studies

The quantitative pre-clinical rodent toxicology data on the 14 compounds studied are given in Table 2. In 5/14 cases, the rodent species used was the mouse, in 2/14 cases the rat and in the remaining cases both rats and mice were studied. The route of administration was that used in initial clinical studies with only 1 exception, AMD-473, where the pre-clinical route was i.p., and not i.v., due to the poor solubility of the drug and hence the need to administer large volumes. Table 2 also lists the rodent maximum administered (i.e. the highest dose given) or tolerated (i.e. the highest dose tolerated without lethality) doses (MAD or MTD), the Phase I trial starting doses and the ratios of the Phase I trial starting dose to the mouse MAD or MTD.

In four cases, rogletimide, CB7630, idoxifene and Patrin 2, a rodent MTD was not defined because the primary Phase I trial endpoint was pharmacodynamic (PD), and the PD endpoint was readily achieved in rodents at well tolerated doses. In all other cases, an MTD was defined, with potencies ranging from 21 mg/m^2/d $\times 5$ (SPAG- rat) to 5000 mg/m^2/d (limonene–mouse). For the 10 drugs that did not have a Phase I trial PD endpoint, the median (range) ratio of the Phase I trial starting dose to the mouse MTD was 0.1 (0.0005–0.25, 9 drugs),

Table 1
Details of compounds investigated in Phase I trials

Compound	Alternative names	Compound properties	Compound class	References
Rogletimide	Pyridoglutethimide	Aromatase inhibitor	Anti-endocrine agent	[7]
CB7630	Abiraterone	17αHydroxylase/$C_{17,20}$-lyase inhibitor	Anti-endocrine agent	[8]
Idoxifene		Estrogen receptor antagonist	Anti-endocrine agent	[9]
AG2034		Antipurine antifolate	Antimetabolite	[10]
CA4P	Combretastatin A4 prodrug	Tubulin binding agent prodrug	Anti-vascular agent	[11]
DMXAA		Cytokine modulator	Anti-vascular agent	[12]
PaTrin 2	O′-Bromothenylguanine	MGMT inactivator	Drug resistance modifier	[13]
AMD-473	ZD 0473	Platinum complex	Platinum complex	[14]
PK2		Polymer-doxorubcin conjugate	Polymer targeted cytotoxic	[15]
CT2103	Xyotax™	Polymer-paclitaxel conjugate	Polymer targeted cytotoxic	[16]
MAG-CPT		Polymer-camptothecin conjugate	Polymer targeted cytotoxic	[17]
Limonene		Protein isoprenylation inhibitor	Signal transduction inhibitor	[18]
SPAG	Substance P antagonist G	Mitogenic neuropeptide antagonist	Signal transduction inhibitor	[19]
Biotransdox	SP1049C	Doxorubicin pluronic polymer mixture	Topoisomerase inhibitor	[20]

Table 2
Quantitative rodent toxicology of the compounds investigated

Compound	Species	Route	Sex	Schedule	MTD/MAD (mg/kg/d)	MTD/MAD (mg/m^2/d)[a]	Phase I start dose	Start dose[b]: MTD/MAD ratio
Rogletimide[c]	Mouse	oral	M	Single	>500	>1500	500 mg ×2	<0.4
CB7630[c]	Rat	oral	M	Single	>400	>2400	10 mg	<0.002
Idoxifene[c]	Mouse	oral	F	Single	>100	>300	10 mg/d	<0.02
AG2034[d]	Mouse	iv	M/F	Single	630	1890	1 mg/m^2	0.0005
CA4P[e]	Rat	iv	M/F	Weekly×4	50	300	5 mg/m^2	0.02
DMXAA	Mouse/Rat	iv	M	Weekly×5	20/>200	60/>1200	6 mg/m^2	0.1/<0.005
PaTrin 2[c]	Mouse/Rat	iv	M	Daily×5	>20/>10	>60/>60	10 mg/m^2	<0.2/<0.2
AMD-473	Mouse/Rat	ip	M/F	Single	40/>20	120/>120	12 mg/m^2	0.1/<0.1
PK2[f]	Mouse	iv	M	Single	45	135	20 mg/m^2	0.1
CT2103[f]	Mouse/Rat	iv	M/F	Single	>81/41	>243/246	11 mg/m^2	<0.05/0.05
MAG-CPT[f]	Mouse/Rat	iv	M/F	Single	40/80	120/480	30 mg/m^2	0.25/0.06
Limonene	Mouse	oral	F	Single	1666	5000	500 mg/m^2	0.1
SPAG	Mouse/Rat	iv	M	Daily×5	20/3.5	60/21	2 mg/m^2	0.03/0.1
Biotransdox[f]	Mouse/Rat	iv	M/F	Single	15/7.5	45/45	5 mg/m^2	0.1/0.1

[a] mg/kg doses in mice and rats were converted to mg/m^2 using conversion factors of 3 and 6, respectively.
[b] Where human doses were not based on surface area a value of 1.7m^2/subject has been used.
[c] Phase I trial starting dose chosen on the basis of pharmacodynamic data in rodents.
[d] Phase I trial starting dose was 1/60th of the NOEL in dogs.
[e] Phase I trial starting dose based on 33% of the daily ×5 toxic dose low in dogs, i.e. 15 mg/m^2/d.
[f] Doxorubicin (PK2, Biotransdox), paclitaxel (CT2103) or camptothecin (MAG-CPT) equivalent doses.

and for the rat 0.06 (<0.005–0.1, 7 drugs). In one case, the antipurine antifolate AG2034, the Phase I trial starting dose (1 mg/m^2) was approximately 2000-fold lower than the mouse single dose LD_{10}. The clinical trial starting dose was 1/60th of the no-observed-effect level (NOEL) in dogs (i.e. 60 mg/m^2) (10). When given daily for 5 days, the NOEL for AG2034 in mice and dogs was 9 and 4 mg/m^2/d ×5, respectively (10), and on the same schedule the MTD in mice was 120 mg/m^2/d ×5. However, when mice were placed on a low-folate diet to reduce plasma folate to levels more representative of those found in humans, the MTD of AG2034 decreased markedly to 0.6 mg/m^2/d ×5 (10). In the 7 cases where both mice and rats were studied, the quantitative toxicity observed was similar in both species with only one exception, DMXAA, where the mouse was markedly more sensitive.

The qualitative murine toxicology data for the 25 drugs studied are summarised in Table 3. Three general categories of toxicology were recorded; clinical, macroscopic tissue pathology/histopathology, and haematology/chemical pathology. In addition to listing the toxicities observed with each drug, Table 3 also indicates the dose level at which the toxicity was observed. Although in the majority of cases the effects were observed at or below the iv/po/ip MTD, toxicities were in some cases only observed at higher dose levels or following repeated administration.

3.2. Phase I trial results

The quantitative details of the Phase I trials initially performed with the 14 drugs are presented in Table 4. The median number of patients in the trials was 27, ranging from 10 (rogletimide) to 46 (DMXAA), and the median number of dose levels was 7, ranging from 2 (rogletimide) to 15 (DMXAA). It is notable that trials of agents with Phase I PD endpoints (rogletimide, CB7630, idoxifene and Patrin 2) involved fewer dose levels. Table 4 also compares the human MAD and the rodent MAD/MTD, and gives the ratios of these two values. For the mouse the median and range of human MAD/mouse MTD ratios was 1.2 (0.006–82), and for the rat 1.1 (0.1–19). For the mouse the two extreme values were for AG2034 (0.006) and DMXAA (82), and as discussed above the value for AG2034 can be explained by the difference in folate levels between rodents and humans. For DMXAA, the mouse was markedly more sensitive than the rat (>20-fold), and the rat proved to be more predictive of the quantitative human toxicity of the compound.

The qualitative human toxicology data for the 14 compounds studied are presented in Table 5. Toxicities are distinguished as being either dose limiting or not on the basis of the Phase I trial reports (7–20). By comparing the data in Table 5 with those in Table 3, the ability of the pre-clinical rodent studies to predict human toxicities was determined. In comparing the human and rodent data, it was recognised that a number of the human toxicities are difficult or impossible to evaluate in rodents, e.g. nausea and vomiting, malaise/asthenia/lethargy, flushing, headache, hypersensitivity, pain, visual disturbances, depression, abnormal taste and myalgia. For the 9 drugs where DLT was observed, 17 toxic events were described as being dose limiting. The most common dose limiting

Table 3
Qualitative rodent toxicology of the compounds investigated

Compound	Clinical toxicity	Macroscopic pathology-histopathology	Haematology-chemical pathology
Rogletimide	B[a]. Neurotoxicity	A. GIT[b] irritation. B. Thymus, testes, liver C. Liver	
CB7630	A. None	C. Liver, testes, prostate and spleen	C. Platelets, serum albumin, liver enzymes
Idoxifene	C. Weight loss	A. Ovary and uterus	
AG2034	B. Weight loss	B/C. GIT, thymus	B/C. WBC
CA4P	A. Weight loss, diarrhoea	A. Testes, LN, bladder, thymus, GIT	A. WBC B. RBC, blood urea nitrogen
DMXAA	B. Acute fatality	A. Spleen, thymus, LN, ileum, colon, caecum, liver, BM	A. WBC, AST, ALT
PaTrin 2	A. None	A. Stomach, spleen, thymus	A. Platelet, WBC, creatinine
AMD-473	A. Weight loss	A. Spleen, thymus, LN, BM, GIT, testes	A. WBC, RBC, platelets
PK2	A. Weight loss	A. Thymus, testes, spleen, BM C. Liver, LN	A. WBC, RBC, platelets, AST, ALT
CT2103	A. Injection site, weight loss C. Neurotoxicity	A. Testes, thymus, BM, liver, GIT, spleen, ovary C. Nerves	A. Bilirubin, AST, ALT, cholesterol B. WBC, platelets
MAG-CPT	A. Acute reduction in urine output, diarrhoea	A. GIT, prostate, seminal vesicles	A. WBC, platelets B. AST, ALT C. Creatinine
Limonene	B. Weight loss		
SPAG	A. Neurotoxicity	A. Spermatocytes	A. WBC
Biotransdox	A. Weight loss	A. Testes, BM, GIT, skin, kidney	A. Glucose, cholesterol, billirubin, ALT, urea, RBC, WBC, platelets

[a] Dose route/level/schedule at which the toxicity was reported: A. At or below the iv/po/ip MAD/MTD. B.Only at greater than the iv/po/iv MTD. C. Only after repeated dosing.

[b] Toxicities-BM-bone marrow, LN-lymph node, GIT-gastrointestinal tract, RBC-red blood cells, WBC-white blood cells, AST aspartate transaminase, ALT-alanine transaminase.

Table 4
Phase I trial details, maximum doses administered and ratios of human maximum administered (MAD) to rodent maximum administered/tolerated doses (MAD/MTD)

Compound	Schedule	Starting dose	Patients	Dose levels	Human MAD	Rodent MAD/MTD[d] ($mg/m^2/d$)	Human MAD[e]: Rodent MAD/MTD
Rogletimide	Single or twice daily oral	500 mg ×2	10	2	1000 mg	>1500	<0.4
CB7630	Single oral dose	10 mg	15	5	500 mg	>2400	<0.1
Idoxifene	Daily oral for 7 days	10 mg/d ×7	20	4	60 mg/d ×7	>300	<0.1
AG2034	Short iv infusion	1 mg/m^2	28	8	11 mg/m^2	1890	0.006
CA4P	Short iv infusion weekly	5 mg/m^2	34	8	114 mg/m^2	300	0.4
DMXAA	20 min iv infusion weekly	6 mg/m^2	46	15	4900 mg/m^2	60/>1200	82/<4
PaTrin 2	Short iv infusion	10 mg/m^2	38	3	40 mg/m^2	>60/>60	<0.7/<0.7
AMD-473	60 min iv infusion	12 mg/m^2	42	8	150 mg/m^2	120/>120	1.2/<1.2
PK2[a]	60 min iv infusion	20 mg/m^2	31	5	160 mg/m^2	135	1.2
CT2103[b]	30 min iv infusion	11 mg/m^2	19	7	266 mg/m^2	>243/246	<1.1/1.1
MAG-CPT[c]	30 min iv infusion	30 mg/m^2	23	6	240 mg/m^2	120/480	2/0.5
Limonene	Daily oral for 21 days as 3 divided doses	500 mg/m^2	32	8	12,000 mg/m^2	5000	2.4
SPAG	6 h iv infusion weekly	2 mg/m^2	24	14	400 mg/m^2	60/21	7/19
Biotransdox	Short iv infusion	5 mg/m^2	26	7	90 mg/m^2	45/45	2/2

[a] Doxorubicin equivalent dose.

[b] Paclitaxel equivalent dose.

[c] Camptothecin equivalent dose.

[d] For species see Table 2, where two values are given the sequence is mouse/rat.

[e] Where human doses were not based on surface area a value of 1.7 m^2/subject has been used.

Table 5
Qualitative human toxicology and predictive performance of rodent studies

Compound	Dose limiting toxicity	Predicted	Other toxicities observed (not dose limiting)	Predicted
Rogletimide	Not reached	–	None	–
CB7630	Not reached	–	Headache, hot flushes, and a mild increase in abdominal and testicular pain	All NE
Idoxifene	Not reached	–	N&V, anorexia, lethargy, weakness	All NE
AG2034	Gastrointestinal/diarrhoea	Y	Haematological, myalgia, neurosensory, anorexia	Y, NE, NE, NE
CA4P	Ataxia, cardiovascular, motor neuropathy	N, N, N	Dyspnoea, haematological, tumour pain, hyper/hypo-tension, visual, diarrhoea, vomiting	NE, Y, NE, NE, NE, Y, NE
DMXAA	Neurological, urinary incontinence	N, NE	Haematological, N&V, anorexia, hyper/hypo-tension, local dermatological	Y, NE, NE, NE, N
PaTrin 2	Not reached	–	Anorexia N&V, lethargy, phlebitis	NE, NE, NE, N
AMD-473	Haematological	Y	N&V, hypokalemia, malaise, fatigue, abnormal taste	NE, N, NE, NE, NE
PK2	Haematological, stomatitis, fatigue	Y, N, NE	Alopecia, fatigue, hepatic	N, NE, Y
CT2103	Haematological, motor neuropathy	Y, Y	Hypersensitivity	NE
MAG-CPT	Haematological, diarrhoea	Y, Y	N&V	NE
Limonene	Nausea, diarrhoea	NE, N	Vomiting	NE
SPAG	Not reached	–	Facial flushing	NE
Biotransdox	Haematological	Y	Cardiac, fatigue, alopecia, N&V, stomatitis	N, NE, N, NE, Y

Y-yes, N-no, N&V- nausea and vomiting, NE- not evaluated in rodent studies.

toxicities were haematological (5/17), gastrointestinal (4/17) and neurological (3/17), the remaining DLTs only being reported on one occasion each. Of the human DLTs that were evaluable in the rodent studies (i.e. 14/17), 8/14 were correctly predicted, i.e. haematological—5/5, neurological—1/3 and gastrointestinal—2/4. Non-dose limiting toxicities reported in clinical trials (Table 5) were largely those that cannot readily be evaluated in rodents.

4. Discussion

The data presented in the current paper on 14 novel cancer therapeutics supports the conclusions of our previous studies on 25 separate agents [2], namely, that toxicology studies in a non-rodent species are not routinely needed for the identification of a safe Phase I trial starting dose for human trials, when the dose is normalised to body surface area and the proposed clinical schedule and route of administration are used. Thus, with the current 14 compounds there was only 1 case (i.e. only 1/39 compounds overall), the antipurine antifolate AG2034, where dosing based on studies in mice fed a standard diet would have resulted in an unsafe Phase I trial starting dose. However, the specific issue of studying the safety of antifolates in mice are now well recognised and can be taken into account in designing toxicology studies. The current study also shows that more common dose limiting toxicities, in particular haematological effects, can be predicted by studies in rodents.

For the 14 agents investigated here, 7 were studied in both mice and rats and for these compounds a comparison of the two species is possible. Overall, the two rodent species gave similar quantitative and qualitative results, although for DMXAA the rat was considerably more predictive of the human MTD than the mouse. Had the DMXAA Phase I trial starting dose been based on the rat data the number of dose escalation steps in the Phase I study would have been substantially reduced. Further comparative studies are needed; however, the possibility exists that the rat alone may be sufficient for studies with novel antitumour agents.

Four of the agents described in this paper, i.e. rogletimide, CB7630, idoxifene and Patrin 2; 3 anti-endocrine agents and one drug resistance modifier, used PD endpoints in both pre-clinical toxicology and Phase I trials, thereby avoiding the need to define an MTD and expediting early clinical trials. As cancer chemotherapy becomes increasingly target-based, PD and not toxicology can be used to define doses and endpoints for first-in-human studies, further reducing the need for detailed pre-clinical toxicology experiments.

In considering the type and extent of pre-clinical toxicology that is undertaken prior to a clinical trial it is worthwhile revisiting the reasons these studies are performed. Toxicological studies should first and foremost

predict a Phase I trial starting dose that is safe, but not one that is so low that it is orders of magnitude away from therapeutic doses. Most physicians, ethics committees, review boards and regulatory authorities appear to accept a starting dose that is 1/10th of the predicted active or toxic dose as a reasonable level at which to start clinical trials, and the results in Table 4 are further evidence of the basis for this practice. Once the Phase I trial starting dose has been shown to be safe, a variety of pharmacologically- and statistically-guided dose escalation strategies are employed to minimise the number of patients treated at levels that are not even potentially effective. The second major reason for performing pre-clinical toxicology is to define potential toxicities and their reversibility. Knowledge of the most likely toxicities is important for individual patients and their families, as well as healthcare professionals, in answering the question "What are the possible risks of taking the drug in relation to the potential benefits?" Also, a knowledge of the most likely toxicities can inform the drug development process; is inhibition of the drug target accompanied by acceptable side effects or not? In both respects, pre-clinical toxicology studies help to inform risk benefit decisions, and hence the precision to which the risk needs to be defined is influenced by the degree to which benefit can be predicted. For example, if a new treatment is very active in well-validated (i.e. predictive) pre-clinical models, and hence highly likely to proceed to Phase III trials, then extensive pre-clinical toxicology could be justified. Conversely, where a drug acts on a clinically un-validated target, or where pre-clinical models are known to have major limitations, then early proof-of-principle Phase I trials with pharmacological endpoints are a higher priority, with more extensive toxicology reserved until after preliminary (positive) clinical data are available. As discussed elsewhere in this issue, the predictive power of pre-clinical antitumour efficacy models is at best limited, and hence pre-clinical toxicology models should in general expedite, not delay, early clinical trials.

To inform further the debate relating to the extent and type of toxicology that is needed prior to early clinical trials it is instructive to consider the outcome of such studies, in particular how many agents are withdrawn from clinical trials because of unacceptable toxicity. Cancer Research UK have recently analysed data from the 1st 22 years of the operation of the Phase I/II Committee which relates to 89 cancer therapies: 28 molecules with novel or unknown mechanisms of action, 26 antibody targeted or immunotherapies, 25 cytotoxic drugs, 5 anti-endocrine agents and 5 polymer-targeted compounds [21]. Excluding antibody-based and immunotherapies, at the time of the review (2002) Phase I trials were planned or ongoing on 12 agents, 18 drugs had proceeded to Phase II/III trials or the market, and 33 compounds had been withdrawn from clinical trials. For the 51 drugs that had completed Phase I trials, only 12 (22%) had been withdrawn due to unacceptable toxicity (MAG-CPT was withdrawn for pharmacokinetic and safety reasons), i.e. neurotoxicity (4/12), nausea, vomiting and asthenia (4/12), haematological (3/12) or hepatic (1/12) toxicity. The only DLT that could have been, but was not, detected by pre-clinical studies in mice was the hepatic toxicity of the ribonucleotide reductase inhibitor didox [2]. Furthermore, had modern anti-emetic therapy been available at the time, dose limiting nausea and vomiting may well have been alleviated. In summary, the experience of Cancer Research UK is that unacceptable toxicity accounts for the withdrawal of only 1 in 5 agents from early clinical trials, and toxicology studies in mice can predict approximately 50% of the events that subsequently become dose limiting in patients.

In a recent series of discussion papers on pre-clinical toxicological studies with cancer drugs a number of important issues were raised. Barras [22] noted that the conduct of comprehensive studies that conform to the most extensive regulatory requirements prior to first-in-human studies would avoid a "regulatory hiatus" later on, should the drug be successful in Phase I/II trials. Conversely of course, given the continuing high attrition rate for drugs in clinical trials, such an approach means that many animals will be used needlessly, and hence this contravenes the requirement placed on all biologists to reduce animal experimentation. Importantly, Barnard [22] reported that of 198 new drugs marketed in the USA from 1976–1985, 102 had serious post-approval risks identified that were not predicted by pre-marketing tests. Of the 198 agents 9 were oncology drugs, and 5 of these had unanticipated risks identified after approval. Two drugs well-up in the top 10 cancer treatments, namely cisplatin and tamoxifen, were amongst these 5 agents, and this study makes two important points: there will always be toxic effects that are not predicted by pre-clinical models or even pre-marketing trials, and in cancer only unpredictable or frequent life-threatening toxicities will put patients off taking, or physicians off using, an active drug. Lastly, a major focus of this series of articles concerned the relative ethics of using rodent *versus* non-rodent species, and toxicologists were reminded by Navaratnam that the use of non-rodent species requires special justification on grounds of science, and not custom or practice [22]. Law is simply a compulsory form of "custom or practice" and if the use of a particular model or test required by law is not scientifically justified, the law should be changed.

In European Member States, from May 2004, the law governing early clinical trials will be the new European Clinical Trials Directive [1], which is currently being integrated in to national legislation. After this date all clinical trials undertaken in the UK will need to be

authorized by the Medicines and Healthcare products Regulatory Agency (MHRA)—there will no longer be any exemption (i.e. a Doctors and Dentists Exemption, DDX) for academic, non-commercial trials. The authorization procedure will require the submission of all relevant supporting data, including pharmacy, pharmacology, toxicology and any available clinical information. For early phase academic trials in the UK, which previously would have been conducted under a DDX, this change has the potential to significantly reduce the number of new agents being investigated in the academic arena.

Of direct relevance to the current article, a major concern is the amount of toxicology data required to support an early phase clinical trial with a new investigational medicinal product (IMP) under the new regulations. For example, according to the EMEA guidelines [4], a new oncology agent with a novel mechanism of action should be tested in both a rodent and non-rodent species prior to the initiation of a Phase I trial. In addition, an evaluation of safety pharmacology studies is required. These new requirements raise the concern that study designs, and hence the number of animals used, for academic projects will be forced to expand simply to satisfy a change in the legislation and not necessarily because of any scientific rationale. Another issue arising from the expanded nature of these studies is also the cost. Early feedback from the MHRA has been encouraging, with the agency keen to assure groups that this will not simply be a box-ticking exercise, and that good scientific justifications for not following the guidelines will be considered. It is clear, however, that any deviations will have to be well justified and clearly based on a sound scientific rationale. It should be remembered that the MHRA assessors will see many different agents and classes of products on a daily basis so nothing should be left open to interpretation or assumption in the application. Clear, well-balanced reasoning and presentation of all the available data will be essential to ensure that the changes in the legislation have the minimum impact on early phase academic studies. However, ultimately, the decision on whether to accept the justifications rests with the MHRA in the UK. The concern remains however that, even with the body of data that now supports the use of rodent-only toxicology studies, for first-time in human studies of new oncology agents there will be a move towards increased requirements as a result of the new European Clinical Trials Directive.

In summary, data for the 14 compounds described in the current paper, in conjunction with that for 25 agents previously published [2], demonstrates that rodents alone can predict a safe Phase I trial starting dose and the majority of the toxicities that subsequently become dose limiting with cancer chemotherapeutics. The only exception is an antifolate drug where the relative insensitivity of mice is due to a difference in folate status compared to humans when mice are fed the standard diet routinely used in toxicology studies. PD experiments with targeted agents, illustrated here by anti-endocrine drugs and a drug resistance modifier, can provide Phase I trial endpoints that substitute for toxicity and avoid the need to define MTDs in pre-clinical and clinical studies. Overall, these results further demonstrate that a non-rodent species is not routinely needed for pre-clinical toxicology studies with novel anticancer agents.

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