Future directions in the pharmacology of anti-cancer agents in children

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In the 1990s, over two-thirds of children with malignancy are expected to be long term survivors. This success in therapy is predominantly due to the chemosensitivity of childhood tumours as well as co-ordinated multi-disciplinary care through the use of protocols and designated children’s cancer centres. In view of the pivotal importance of chemotherapy in childhood malignancies in the future therapy can be improved considerably by exploring ways to optimise treatment either by introducing new agents or utilising existing drugs better.

There has been a substantial increase in the knowledge of the pharmacology of anti-cancer agents in children over the last decade and this is now beginning to have an impact on the care of children with cancer. Especially major advances in understanding the molecular pharmacology of anti-cancer agents, including their intracellular metabolism and the interaction between the drug and target, are likely to affect clinical management. It is expected as a consequence of pharmacological research, therapy will be optimised which, in turn, will result in improvements in survival rates and reduction in late sequelae.

There are two main areas where pharmacological research is directly being applied to and benefiting paediatric oncology: optimisation of treatment by pharmacologically guided therapy according to either clinical or molecular pharmacology and new anti-cancer agents.

Pharmacologically guided therapy

The aim of pharmacologically guided therapy is to compensate for inter-patient variability in clinical and molecular pharmacology. This will result in the maximum anti-tumour activity and minimum toxicity of chemotherapy in individual patients. Thus, each child with malignancy will receive the optimum individualised treatment. The concept of pharmacologically guided therapy can be best exemplified by considering clinical pharmacology. If all children receive the same dose of an anti-cancer agent, serum concentrations will vary considerably. In clinical
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practice inter-patient variability is partially compensated for by modifying the dose according to the child's size. There are other strategies whereby inter-individual variability can be reduced.

Pharmacologically guided therapy is based upon the premise that differences in a specified pharmacological variable will result in different clinical effects, e.g. low serum concentrations will result in reduced efficacy and high serum concentrations increased toxicity. It is first important to document that there is variability in the pharmacological factor, be it serum concentration of anti-cancer agent or DNA adduct level. Secondly, it must be demonstrated that the clinically observed inter-patient variability relates to differences in clinical effect and outcome. Thirdly, methods to control inter-individual variability must be developed. It must then be shown that pharmacologically guided therapy using these methods does reduce inter-individual patient variability. The final goal is to demonstrate in a randomised study that pharmacologically guided therapy improves clinical effect, i.e. increases efficacy and decreases toxicity.

Clinical pharmacology

Clinical pharmacology is concerned with information regarding the concentration of a drug and any active or toxic metabolites within the body, which is, in turn, influenced by the processes of drug absorption, distribution, metabolism and excretion.

The area under the concentration time curve (AUC) after a given dose is a measured parameter used as a determinant of overall body exposure. Since drug levels are usually measured in blood or plasma, the AUC reflects the exposure of blood or plasma, not necessarily that of the site of action of the drug.

Nevertheless, plasma and drug concentrations are more likely to reflect the true concentration at the site of action than drug doses alone. The AUC is the integral of all drug concentrations after administration and is dependent upon the drug dose and the plasma clearance. The total plasma clearance is the sum of all individual organ clearances. The major organs involved with clearance are generally the liver (metabolism and excretion) and the kidneys (excretion mainly). The aim is to reduce inter-individual variability in AUC and facilitate predictable AUCs to be achieved in current therapeutic protocols.

Current clinical practice is to administer cytotoxic chemotherapy in fixed doses scaled to body size, normally surface area, i.e. mg/m². This practice is based on the concept that drug clearance, and hence the drug exposure obtained with a given absolute dose is positively related to body
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size and that for a larger body a greater dose will be needed to achieve the same exposure and hence biological effect. Studies in adults only indicate a weak relationship between clearance and size\(^1\), however, in children, the wide range in body sizes result in a greater need to adjust dose.

The convention is to reduce dosages in infants less than 10 kg, with calculations based on body weight rather than surface area. In some protocols the dose is empirically reduced further by two-thirds on the basis that tissue tolerance in infants is less than older children. Recently, detailed investigations have concluded that special dose calculation guidelines for infants receiving etoposide are not substantiated by age-dependent pharmacokinetics or tolerance\(^2\). Steady state serum concentrations and AUC in infants were significantly lower than those for older children using current dosing approaches. The conclusion from this investigation is that etoposide dose calculations in infants should be according to surface area determined from body weight alone. The previously accepted dosing strategy probably resulted in significant under-dosing in many infants with possible reduced efficacy. Further studies are needed to determine if dose calculations for all drugs in infants should be based on body weight.

A non patient source of inter-individual variability is differences in commercial preparations of the same agent. This is exemplified by L-asparaginase, an enzyme used to achieve the maximal possible reduction of blood concentrations of the amino acid asparagine which is essential for the growth of leukaemic blasts.

Preparations of asparaginase from different sources result in significantly different serum trough levels with asparaginase derived from *Erwinia chrysanthemi* being associated with lower enzyme activity than products from *Escherichia coli*\(^3\). These variations are reflected in the degree of reduction of asparagine, with greater depletion occurring with asparaginase derived from *E. coli*. Furthermore, different preparations of asparaginase from the same source result in different asparaginase levels, i.e. Asparaginase medac versus Crasnitin. Further clinical studies are required to confirm these findings and determine their clinical significance. Based on these findings, it is possible that administration of asparaginase in childhood acute lymphoblastic leukaemia can be optimised.

Very variable serum concentrations and resultant AUC have been documented for a large number of anti-cancer agents even when children receive the same dose based upon either surface area. For the majority of agents, including carboplatin, methotrexate and etoposide, a 3–4-fold variation in AUC has been described\(^4\,^6\). The clinical importance of this variation depends on whether these differences in AUC are responsible for differences in clinical effect. This depends on whether the agent itself is cytotoxic or requires metabolism to become active. For example, the
platinum complexes require no metabolic activation in contrast to the thiopurines which are reliant on metabolic transformation for their cytotoxicity. Based on these observations, the hypothesis has been made that these inter-individual variations in AUC are responsible for differences in clinical effects, i.e. a patient with an AUC higher than the mean will be more toxic, whilst a patient with a AUC lower than the average is more likely to be receiving ineffective therapy and their tumour will not respond to therapy. If this is correct, the delivery of a more predictable AUC will theoretically reduce toxicity and improve efficacy in the population as a whole.

The effect of variations in AUC on clinical outcome has been mostly demonstrated in adults receiving anti-cancer therapy, however, there are some examples in paediatric oncology. For example, the AUC of carboplatin when administered as a single agent or in combination with vincristine relates to the percentage reduction in platelet count. Similarly, the steady state serum concentrations of tenoposide relates to the response rate in relapsed tumours, mucositis corresponds with tenoposide serum concentrations and lung damage with busulphan levels.

The situation is more complex with anti-cancer agents which require metabolism before they are cytotoxic, since variations in AUC may not relate to clinical effect. This is exemplified with the 6-thiopurines, mercaptopurine and 6-thioguanine, as they require metabolic activation for clinical effect, no direct relationship has been demonstrated between serum concentrations and clinical outcome. In contrast, however, variations in intracellular metabolite levels have related to outcome. However, a relationship has been demonstrated between the clinical pharmacology of cyclophosphamide, an inactive pro-drug, and clinical outcome. There is an inverse relationship between AUC and both cardiac toxicity and efficacy. In this case, the variation AUC and clearance of cyclophosphamide relates to the rate of metabolism and indicates patients who have a different metabolic profile.

Methotrexate undergoes polyglutamation, i.e. the addition of gamma-linked glutamyl residues within the cell, to form methotrexate polyglutamates. With methotrexate some of its cytotoxic action is by virtue of the parent compound, whilst additional effects are due to methotrexate polyglutamates. It has been demonstrated for over 20 years that elevated serum concentrations for prolonged periods of time cause toxicity. Hence the current therapeutic approach is to modify folinic acid rescue according to methotrexate serum concentrations at 72 h after administration. Two papers published in the 1980s suggested that differences in clearance and steady state serum concentrations, after high dose methotrexate, related to outcome in childhood acute lymphoblastic leukaemia (ALL). However, subsequent analysis showed that the
survival advantage disappeared with time, apart from in the poor prognosis sub-group\(^{18}\). In childhood ALL, when methotrexate is administered at a lower dose (20 mg/m\(^2\)), there is considerable intra-individual variability in serum concentrations and AUC and neither can be related to outcome\(^{19}\).

In view of the different effects of methotrexate and its polyglutamates, it can be expected that differences in AUC may not simply relate to clinical effect. Therefore, pharmacologically guided therapy is simpler and less complicated if the compound is not converted to cytotoxically active metabolites.

If for a certain agent a relationship between AUC and clinical effect is established, then the next step in the development of pharmacologically guided treatment is to develop methods to control inter-individual variations in AUC. Three approaches have been used: (i) adaptive dosing; (ii) adaptive dosing with feedback; and (iii) test dosing.

Adaptive dosing on the basis of pretreatment patient characteristics allows an improved estimate of drug clearance in each patient to be made prior to therapy. This is possible when an accurate prediction of clearance can be made from a physiological parameter. For example, the plasma clearance of carboplatin is predominantly renal, by glomerular filtration, with only limited non-renal elimination in patients with normal renal function\(^{20}\). This small non-renal component is due to tissue binding and is proportional to differences in body size. As a close correlation between the plasma clearance of carboplatin and glomerular filtration is seen, the glomerular filtration rate (GFR) can be used to predict carboplatin clearance. Based upon this observation, dosing equations have been developed. The first one used in adults relates differences in carboplatin clearance to GFR plus a fixed constant for non-renal clearance, on the assumption that the majority of adults do not vary greatly in body weight\(^{20}\):

\[
\text{Dose (mg)} = \text{Target AUC} \times \left[ \text{GFR (ml/min)} + 25 \right]
\]

For children, it was found that this was not an appropriate model, firstly because there are significant differences in children’s body size: therefore a version of the adult carboplatin formula was developed\(^4\):

\[
\text{Dose (mg)} = \text{Target AUC} \times \left[ \text{GFR (ml/min)} + \left( 0.3 \times \text{body weight (kg)} \right) \right]
\]

A further equation was developed to reduce errors resulting from the measurement of GFR using the plasma clearance of \(^{51}\)Cr-EDTA. These errors were found to originate from inaccuracies in the measurement of the volume of distribution of \(^{51}\)Cr-EDTA, hence this latter equation employs the \(^{51}\)Cr-EDTA half life to calculate the GFR.

These paediatric carboplatin dosing formulas have been shown to be both more precise and less biased than the initial adult formula and to
predict carboplatin clearance accurately. Recently, a dosing formula has been proposed in which the GFR is determined from serum creatinine concentrations, body size and the number of kidneys. Detailed validation of this method and comparison with other formulas are required to determine its role in therapy.

An alternative strategy is adaptive dosing with feedback. In this approach, the dose is based on post-treatment plasma concentrations in order to achieve a desired target plasma AUC or steady state concentration. The anti-cancer agent is first administered and then subsequent dosing is modified. Usually drug levels during, or at a limited number of time points after the end of, the infusion are determined, and based upon previously described data (i.e. a population model), an estimate of the AUC in the patient is made. Limited sampling models of this type have been developed; however, they are only valid if parameters such as infusion or sampling times do not differ.

As etoposide is normally eliminated both by renal (40%) and hepatic (60%) mechanisms, adaptive dosing based on a single physiological measurement is more complex. Therefore, adaptive dosing with feedback would seem to be the technique of choice for pharmacologically guided etoposide therapy. An accurate limited sampling model for the estimation of etoposide AUC after a single intravenous dose has been developed. The model is based on a single etoposide sample taken at the end of the infusion and the elimination constant of $^{51}$Cr-EDTA as a surrogate for the etoposide elimination rate constant.

A further alternative strategy is test dosing, where a small dose of the anti-cancer agent is administered, a full pharmacokinetic study is performed to predict the plasma clearance of the drug and subsequent doses are based on this known clearance. Test dose studies have been carried out with melphalan and methotrexate. The limitations of the test dose method are that the length of hospital admission is increased, the measured plasma clearance on one occasion may not predict subsequent clearance, the approach involves administration of a sub-therapeutic dose before administration of a full therapeutic dose and analytical methods may be not adequate to detect the relatively low plasma concentrations following the test dose.

Once a method has been established to control inter-individual variations in AUC, this technique needs to be employed to demonstrate that pharmacologically guided therapy does reduce variability in AUC or steady serum state concentrations.

As with conventional dosing strategies, there is consistently a 3–4-fold variation in AUC when the same dose based on surface area is administered; an objective for pharmacologically guided therapy is to reduce this variability to 2-fold or less. This would effectively result in all patients receiving between 75–125% of their target AUC. The detailed
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Pediatric carboplatin dosing formula described above is currently being validated in children with normal renal function in an UK Children's Cancer Study Group investigation. The proportion of children to achieve an AUC within 25% of the target is being compared when the same patient is dosed according to surface area or the formula. The results of this study are awaited with great interest. Pharmacokinetically guided therapy with etoposide has been shown to be feasible.

In the future it is expected that by using population modelling, with a relatively large data base, children can be identified who would benefit from pharmacologically guided therapy. The optimum dosing formula should not require any pharmacokinetic sampling and should be reliant on a physiological variable that can be easily and accurately measured. In this way pharmacologically guided therapy can be employed at all children's cancer centres.

Once dosing formulas have been validated and demonstrated to reduce variation in AUC, the ultimate goal are prospective randomised studies to show that guided therapy based on clinical pharmacology, will improve clinical effect, i.e. increase efficacy and reduce toxicity. Only one such study to date has been undertaken the St Jude's Hospital Total XII regimen for the treatment of childhood ALL. Pharmacologically guided therapy with methotrexate, cytosine arabinoside and 6-mercaptopurine was feasible even in this complex multi-agent combination setting. The published results are awaited with great interest.

The ability to deliver curative chemotherapy to children with malignant disease who have hepatic or renal failure poses major problems. Using pharmacologically guided therapy, it is possible to administer chemotherapy to these patients. If an anti-cancer agent, or its active metabolites, is excreted by the kidney, administration in conventional dosage can cause significant toxicity, so dose reduction is necessary. In contrast, drugs such as vincristine, doxorubicin, cyclophosphamide and actinomycin D, which are eliminated by other routes, can be given in standard doses in renal failure. Using pharmacokinetic or therapeutic drug monitoring drugs which are eliminated by the kidney without extensive prior metabolism can be administered to patients with renal failure.

Specifically, carboplatin and etoposide have been administered by pharmacologically guided therapy using adaptive dosing with feedback in patients with renal failure requiring dialysis. Target AUCs are defined based upon knowledge of those AUCs achieved routinely in patients receiving current protocols. If the dose is divided over 2 days, then pharmacological studies carried out on the first day can determine if the target AUC predicted has been achieved. Peritoneal dialysis does not remove carboplatin or etoposide from the plasma, however carboplatin, but not etoposide, is cleared by haemodialysis. Thus, it is possible to
obtain target AUCs for both drugs using this approach, so that effective
drug levels are achieved with acceptable toxicity. It is possible that in the
future similar approaches can be used with other anti-cancer agents.

By pharmacologically guided therapy, it is hoped and expected that a
group of children with malignancy, who at present are failing therapy,
can be long-term survivors.

Molecular pharmacology

Molecular pharmacology relates to the intracellular events including the
intracellular drug or metabolite concentrations, levels of proteins
involved in the metabolism or detoxification of the anti-cancer agent;
the drug target and drug target interaction. The same steps in establishing
the role of pharmacologically guided therapy relates to molecular as well
as clinical pharmacology.

6-Mercaptopurine: The relationships between intracellular metabolite
levels with outcome has perhaps best been elucidated with 6-mercaptopu-
purine. The thiopurine, 6-mercaptopurine itself is not cytotoxic and is a
pro-drug requiring metabolic transformation to be active for cytotoxic
activity\(^7\). 6-Mercaptopurine has three metabolic fates:

1. Activation by the enzyme hypoxanthine-guanine phosphoribosyl
transferase (HPRT), eventually 6-thioguanine nucleotides will be
produced. These are the cytotoxic metabolites which are incorporated
into DNA.
2. Oxidation by xanthine oxidase in the intestinal mucosa or liver into
the inactive 6-thiouric acid.
3. Inactivation by thiol methylation catalysed by the enzyme thiopurine
methyl transferase (TPMT).

Individuals lacking HPRT activity, e.g. Lesch Nyhan syndrome, show no
toxicity with 6-mercaptopurine. There is very little intra-individual
variation in the activity of xanthine oxidase, however, there are
significant differences in the activity of the enzyme TPMT.

These differences in enzyme activity are due to genetic polymorphism.
There are two alleles for TPMT activity, one for low and one for high
activity. 1 in 300 individuals have undetectable enzyme activity and have
two alleles for low activity, 11% of the population have intermediate
TPMT activity and have a high and low activity allele and 88% of the
population have high TPMT activity being homozygous for the high
activity alleles\(^{26}\).
Red cell 6-thioguanine nucleotide concentrations have been used as an indicator of an individual’s tissue level of these cytotoxic metabolites. In children with ALL receiving 75 mg/m² 6-mercaptopurine daily, there is wide variation in red blood cell 6-thioguanine nucleotide concentrations (126–832 pmol per $8 \times 10^8$ red cells)\textsuperscript{14}.

The red blood cell concentration of TPMT correlates with the levels in other tissues. Red blood cell TPMT activity in children treated for ALL also varied widely 7–25.1 U/ml of red blood cells\textsuperscript{27}. TPMT activity was significantly higher in children on therapy for ALL than normal children and those who had completed therapy for ALL suggesting that enzyme activity may be induced by chemotherapy. TPMT activity and 6-thioguanine nucleotide concentration are inversely related with patients having lower TPMT activity having higher nucleotide concentrations.

This suggests that if an individual has high TPMT activity, more 6-mercaptopurine will be detoxicated to 6-methyl-mercaptopurine and less will be available to be activated to the cytotoxic 6-thioguanine nucleotide.

6-Thioguanine nucleotide red cell concentrations have been related both to myelosuppression and leukaemia relapse. Patients who had higher concentrations of thioguanine nucleotides had lower neutrophil counts 2 weeks later\textsuperscript{28}. In addition, a red blood cell 6-thioguanine nucleotide concentration greater than 275 pmols per $8 \times 10^8$ red cells, was associated with a better event free survival in children with ALL ($P<0.001$)\textsuperscript{15}. These observations appear to be valid, although studies have indicated that there is a very substantial intra-individual variability in red blood cell 6-thioguanine nucleotide concentrations in any one patient receiving the same dose of 6-mercaptopurine.
Multivariate analysis showed that a low red blood cell 6-thioguanine nucleotide concentration, a high white blood cell count at diagnosis and male sex, were independently associated with a higher risk of relapse. Thus, those children who inherit high TPMT activity inactivate more 6-mercaptopurine, produce less active cytotoxic 6-thioguanine nucleotide and have a greater risk of relapse than those who have low TPMT activity.

Low red blood cell 6-thioguanine nucleotide concentrations may either be due to high TPMT activity or poor compliance with the prescribed therapy. Thus, during therapy, a low red blood cell thioguanine nucleotide concentration may predict children who have a higher risk of recurrence of leukaemia.

Therapy may be altered in these patients, either by ensuring that they comply with medication or altering therapy by using alternative thiopurine, i.e. 6-thioguanine. Although there was a very strong suggestion that low red blood cell 6-thioguanine nucleotide concentration relates to poor outcome, it has not yet been proved that increasing red blood cell 6-thioguanine nucleotide concentration will reduce rates of recurrence.

In studies of 6-mercaptopurine, red blood cell concentrations have been used as the principal indicator of molecular pharmacology. It is perhaps surprising a relationship has been determined between the constitutional phenotype and clinical outcome rather than that of the malignant cells. One study, however, has demonstrated a relationship between TPMT activity in leukaemic blasts and red blood cells in the same individual. This assumes the metabolic phenotype of the blast is the same as the metabolic phenotype of the patient, i.e. the constitutional phenotype.

Methotrexate: Within the cell, the enzyme folypolyglutamate synthetase (FPGS) can add up to 6 gamma-linked glutamyl residues to methotrexate to produce methotrexate polyglutamates (Fig. 2). Methotrexate is an inhibitor of dihydrofolate reductase (DHFR) and, therefore, intracellular reduced folate levels are depleted. Methotrexate polyglutamates, in addition to inhibiting DHFR, inhibit thymidylate synthase and the transformylases used in purine synthesis. Interestingly, methotrexate polyglutamates are retained within the cell for longer periods than methotrexate and it may well be that methotrexate polyglutamate is a more cytotoxic agent than methotrexate itself.

In vitro, various mechanisms by which the cell can become resistant to methotrexate have been shown. These include reduced methotrexate transport, increased levels of DHFR resulting from gene amplification, altered affinity of DHFR for methotrexate and decreased accumulation of methotrexate polyglutamates.
Wide variability in the production of methotrexate polyglutamates by malignant lymphoblasts has been documented. Lymphoblasts obtained at the time of diagnosis of ALL have been incubated in vitro with methotrexate and non-exchangeable methotrexate and methotrexate polyglutamates quantitated by HPLC. There was a 6-fold variation in unmetabolised intracellular methotrexate and an 18-fold variation in methotrexate polyglutamates. Reasons for differences in intracellular levels of methotrexate metabolites are at present unknown.

At present, there have been few investigations in this field. One study of 43 children with ALL demonstrated that those children whose lymphoblasts accumulated more methotrexate and methotrexate polyglutamates, experienced a better 5 year event-free survival than the remainder (65% versus 22%, P = 0.001). Patients who had either a high intracellular methotrexate or methotrexate polyglutamate concentration fared intermediately. Differences in event-free survival according to intracellular methotrexate and methotrexate polyglutamate concentrations were only seen in good prognosis patients, i.e. females, age less than 7 years, a white cell count of less than $20 \times 10^9/l$ at the time of diagnosis and non-T, non-B cell leukaemia. Therefore, differences in intracellular methotrexate polyglutamate concentration may be a factor which accounts for some relapses in children with good prognosis ALL.

**Platinum-DNA adducts:** The mechanism of anti-tumour action of the platinum complexes (carboplatin and cisplatin) is believed to involve the covalent binding of the diamino platinum radical to DNA. The platinum (Pt)–DNA adducts so formed account for the observed anti-tumour cytotoxicity by impairing the function of DNA to act as a template for further DNA replication. Four types of Pt–DNA adducts have been suggested: monofunctional adducts; inter-strand cross links; intra-strand cross links; and DNA protein cross links. Platinum appears to be linked to the N7 atom of the purine bases, guanine and adenine, either to produce guanine-guanine intra and inter-strand links or guanine-adenine...
inter-strand cross links. It is uncertain whether the guanine-guanine inter-strand or intra-strand cross links are the important lesions in determining cytotoxicity\textsuperscript{33}. Intra-strand cross links are more frequent. Studies have demonstrated the relationship between the levels of Pt–DNA adducts formed in peripheral blood mononuclear cells with the subsequent response of patients to platinum-based chemotherapy. The level of intra-strand adducts has been related to the response rate in patients with testicular and ovarian malignancies\textsuperscript{34–37}. It is interesting that a relationship has been demonstrated between Pt–DNA adducts in peripheral blood and clinical affect, as it would be expected that the determinants of the amount of Pt–DNA adducts formed differed between constitutional and tumour DNA.

Studies have indicated that the amount of Pt–DNA adducts formed are solely dependent on the exposure of the cell to the platinum complexes, as indicated by AUC\textsuperscript{38}. This strongly suggests that other factors are responsible for platinum DNA interaction\textsuperscript{38}. In cell lines, a relationship has been determined between Pt–DNA adduct levels and cytotoxicity\textsuperscript{38}. However, this has yet to be demonstrated in clinical tumour samples. Recently developed immunoassay techniques to detect Pt–DNA adduct levels in clinical tumour samples will be of major benefit\textsuperscript{39–42}. As the frequency of these adducts is low and the amount of clinical material available is small, alkaline elution methods, although sensitive, are not able to quantitate accurately the levels found following platinum treatment. Immunological methods, in which antibodies are used that specifically bind to Pt–DNA adducts have been developed.

In particular, the rat monoclonal antibody produced by Tilby \textit{et al.} to measure Pt–DNA adducts has been proved be useful, as has the sensitive competitive ELISA assay which has subsequently been developed\textsuperscript{42}.

It is now necessary to determine whether tumour cells and clinical biopsies vary in their Pt–DNA adduct levels. An ideal system would be the \textit{ex vivo} treatment of tumour biopsies with platinum complexes. However, it is first necessary to determine that \textit{ex vivo} treatment will result in similar levels as the \textit{in vivo} treatment. This should allow a relationship between Pt–DNA adduct formation with tumour effect to be elucidated. This will address the question whether variability in response to platinum anti-cancer agents is due to differences in level of Pt–DNA adduct formation or due to down-stream effects, e.g. those involving \textit{p53}.

\textit{Cytosine arabinoside:} In order to exert a cytotoxic effect, cytosine arabinoside (ara-C) must first be transported into the cell and undergo phosphorylation to ara-C triphosphate (ara-CTP). Extensive studies by Plunkett and colleagues have shown the persistence of intracellular ara-CTP is related to the inhibition of DNA synthesis in leukaemic cells \textit{in vitro} and subsequent response in patients with refractory leukaemia\textsuperscript{43}. 

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Complementary work by Wiley et al.\textsuperscript{44} has shown that in cells from patients with a variety of lymphomas and leukaemias, the formation of ara-CTP \textit{in vitro} was related to the number of nucleotide transporter molecules present on the cell surface, as measured by the binding of nitrobenzylthionosine. These latter data suggest that membrane transport may be the limiting factor in ara-CTP accumulation following conventional dose ara-C administration, a suggestion that is consistent with the lack of a relationship plasma ara-C levels and response\textsuperscript{45}. Thus intracellular ara-CTP concentrations are the predominant active metabolite of ara-C. To improve the efficacy of ara-C, various methods have been investigated to increase intracellular ara-CTP concentrations, including: modifying the dose and/or schedule of ara-C\textsuperscript{46,47} and also concurrent administration of ara-C with biochemical modifiers, either fludarabine or 6-MP\textsuperscript{48,49}.

\textit{Topoisomerase II}: Topoisomerase II (topo II) is an essential eukaryotic enzyme, it is a scaffold protein in metaphase chromosomes and is required for DNA replication and for the separation of daughter chromosomes during mitosis. Human DNA topo II is the target for etoposide, daunorubicin, doxorubicin, tenoposide, mitoxantrone and amsacrine.

Etoposide appears to exert its cytotoxic effect by promoting topoisomerase-associated DNA breakage. Drug-induced DNA damage by topo II is an unusual form of DNA damage. Topo II is a homodimer that acts as a ‘DNA gate’, passing one duplex DNA segment through a transient enzyme-bridge double strand break. Both the intercalative and non-intercalative anti-cancer agents interrupt enzymatic DNA breakage-reunion by topo II, promoting the formation of ‘cleavable complexes’, cytotoxic lesions that ultimately result in cell death\textsuperscript{50}.

Topo II activity in human cells is derived from two structurally different isoforms, topo II\textsubscript{α} and β\textsuperscript{51}. These isoenzymes are differentially expressed during the cell cycle and in transformed cells. They have different tissue distributions and appear to be located differentially in the nucleus. Altered levels and ratios of topo II\textsubscript{α} and β have been observed in drug resistant cells, suggesting they may play a role in drug resistance. Resistance to etoposide in a leukaemic cell line has been shown to be predicted by changes in topo II\textsuperscript{52}.

Despite its importance in chemotherapy, relatively little is known about the levels and variability of human topo II\textsubscript{α} and β \textit{in vivo} in normal or tumour tissue. It is also not known whether levels of topo II\textsubscript{α} or β relate to sensitivity to etoposide or whether levels of topo II-etoposide complex relates to clinical effect. Once these questions have been determined then the possibility of pharmacologically guided therapy with etoposide according to topo II levels can be addressed.
Oxazaphosphorines: The clinical and molecular pharmacology of the oxazaphosphorines are complicated. Cyclophosphamide and ifosfamide are themselves inactive and require metabolic transformation by hepatic cytochrome P450 enzymes to produce cytotoxic species. The metabolic pathways involved are shown in Figure 3. The pharmacological parameter which has been most extensively related to clinical outcome is parent drug clearance, which is a measure of metabolism to a cytotoxic...
species. The assumption that increased drug clearance and metabolism equates with greater anti-tumour effect is supported by the inverse relationship between AUC and response observed in women with breast tumours.

Cyclophosphamide’s metabolism to 4-hydrocyclophosphamide is catalysed in man by a number of cytochrome P450 enzymes—CYP2A6, CYP2B6, CYP2C8, CYP2C9 and CYP3A4. However, the expression and activity of CYP3A appears to be the major determinant of cyclophosphamide activation and cytotoxicity. Thus agents which regulate the expression and activity of this cytochrome P450 effect cyclophosphamide metabolism.

Part of the described high degree of inter-patient variation in the metabolism of cyclophosphamide can be related to prior treatment with dexamethasone and concurrent allopurinol or chloropromazine therapy, drugs which are known to affect CYP3A4 expression. Differences in drug scheduling may also affect metabolism, as cyclophosphamide clearance is greater during a 24 h compared to a 1 h infusion. This is consistent with enzyme saturation occurring during short administration schedules. Once a relationship between the metabolism and clinical effects of cyclophosphamide have been conclusively established, then a programme of pharmacologically guided therapy can developed. Manipulation of the metabolism of cyclophosphamide by altering the expression of the relevant cytochrome P450 and by modifying the schedule of administration are possible components of this programme.

Cytochrome P450s involved in the metabolism of cyclophosphamide have recently been identified in adult tumours using immunocytochemistry and Western blotting techniques. It seems possible that the expression of certain cytochrome P450 isoforms in tumour cells may influence their response to cyclophosphamide. There is some experimental evidence, including transfection studies, to support the hypothesis that expression of CYP3A4 leads to increased cyclophosphamide sensitivity as a result of drug activation within the tumour cell.

The extent of production of carboxyphosphamide from aldophosphamide, the major inactivation reaction, is closely related to the expression of aldehyde dehydrogenase (ALDH) which catalyse the reaction. Increased intracellular ALDH activity has been associated with cyclophosphamide resistance in tumour cell lines.

The hypotheses that tumour expression of CYP3A4 is associated with chemosensitivity and ALDH drug resistance requires testing in paediatric tumours. If a relationship is determined then therapy can be guided, at diagnosis, according to tumour phenotype for CYP3A4 and ALDH.
New anti-cancer agents and phase I/II studies

Overview

As not all children with malignancy are cured and a significant number have major late sequelae of treatment, there is a major need to identify new anti-cancer agents which are more effective and less toxic than existing drugs in the treatment of childhood malignancies. In view of the current high survival rates for childhood malignancies, an ideal new cancer agent is one which is predicted to be devoid of late side effects. As there are a large number of compounds which are being developed, in paediatric oncology it is particularly important to prioritise these agents for further evaluation in phase I and II studies. Identification of the most important agents is more challenging in paediatric than adult practice as there are a smaller number of eligible children than adults for phase I studies due to the relative rarity of childhood malignancies and the superior efficacy of initial therapy.

Each year in the UK there are 300–400 children with relapsed or recurrent malignancies in contrast to 120–150,000 adults. At present, essentially all anti-cancer agents are first developed for use in adults. The choice of which of the available compounds should be evaluated in children is guided by a number of features.

1. Preclinical studies in xenografts can indicate agents which should be evaluated further. For example, the efficacy of melphalan in rhabdomyosarcoma which was first demonstrated in xenografts and confirmed in clinical trials\(^5^9\). Xenograft models suggest strongly that topoisomerase I inhibitors will be active in rhabdomyosarcoma and neuroblastoma\(^6^0\).

2. Mechanism of action—compounds which have novel mechanisms of action warrant further study. Also, agents which are specific inhibitors of single drug targets, for example thymidylate synthase (TS) inhibitors which has only one loci of action in contrast to three with methotrexate\(^6^1\).

3. Drugs which have, or are likely to have, little acute and late toxicity are important, as the majority of children with malignancy will be long term survivors. Antimetabolites are particularly interesting compounds in this regard. A number of analogues have been developed over the last decade with the principal aim of reducing toxicity. Carboplatin was developed predominantly to overcome the significant non-haemopoietic toxicity introduced, e.g. vomiting, ototoxicity, renal damage and neurotoxicity observed with cisplatin. Newer anthracyclines which have potentially less cardiotoxicity are being considered\(^6^2\).
4. Agents which appear to be active in cell lines which are resistant to a range of conventional drugs.

5. Experience with adult phase I/II studies. If encouraging activity has been observed, as with taxol\textsuperscript{63}, topoisomerase I inhibitors\textsuperscript{64} and temozolomide\textsuperscript{65}, the development of the agent in children is prioritised. Conversely, if significant toxicity has been observed, the drug's development in children is delayed, for example, bryostatin which has severe myalgia as its major toxicity requiring opiates\textsuperscript{66}. Unfortunately, a number of the newer anti-agents, perhaps due to their lipophilic nature, have neurological dose limiting toxicity.

The direction of the rational development of new anti-cancer agents in children can, in addition, be based on:

1. Studies of molecular pharmacology, which identify metabolic pathways of anti-cancer agents which can be exploited. For example, if it has been determined in ALL blasts in patients with relapse did not have increased levels of TS but have changes in the levels of DHFR and FPGS conferring resistance and impaired transport, this would support the development of inhibitors of TS.

2. Drug development programmes, using molecular pathological features involved in drug resistant tumours, as targets. Examples could include the \textit{p53} pathway and the \textit{n-myc} oncogene which is amplified in poor prognosis neuroblastomas.

\textbf{New anti-cancer agents}

Many of the new anti-cancer agents which are about to, or have recently entered, phase I/II studies in children are not conventional cytotoxic agents and are specific for selected intracellular targets. Some of these are new classes with distinctive mechanisms of action, others target specific cellular proteins.

\textit{Analogue}s: A number of compounds have been specifically developed as analogues of established successful cytotoxic drugs which have activity in a large number of paediatric malignancies. Carboplatin has consistently been demonstrated to cause less ototoxicity, renal damage, neurotoxicity and vomiting than cisplatin\textsuperscript{67}. However, there have been no randomised studies comparing the efficacy of the two agents. Ifosfamide, although now widely used, has not been shown to have superior efficacy to high dose cyclophosphamide, although very significant renal damage caused by ifosfamide has been demonstrated\textsuperscript{68}. 

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Anthracycline analogues with no cardiotoxicity would be of interest. The benzothiopyranindazoles are active in preclinical and clinical studies in adults and preliminary studies suggest that they have less cardiotoxicity\textsuperscript{62}.

**Paclitaxel and docetaxel:** In view of the activity of these agents, demonstrated in adult malignancies and their mechanisms of action, i.e. stabilisation of tubular polymerases, there has been a great interest in the evaluation of these compounds in children\textsuperscript{63,69}.

**Topoisomerase I inhibitors:** Topoisomerase I has been identified as a potential target for anti-cancer agents and specific inhibitors have been developed, i.e. topotecan, irinotecan and 9-amino-camptothecin. Xenograft studies have suggested significant activity in neuroblastoma and rhabdomyosarcoma\textsuperscript{60}, which is being confirmed in very early phase II studies\textsuperscript{64}.

**Temozolomide:** Temozolomide is a cytotoxic alkylating agent which at physiological pH undergoes spontaneous degradation to its active metabolite monomethyl triazenoimidazole carboxamide (MTIC). MTIC is thought to be cytotoxic by reactive methylation of guanine, primarily at O\textsuperscript{6} and to a lesser degree at the N\textsuperscript{7} position\textsuperscript{76}. A specific DNA repair protein O\textsuperscript{6} alkyl-guanine DNA alkyl-transferase (AGT) removes the O\textsuperscript{6} methyl-guanine adducts by self-inactivation. The tissue level of AGT has been shown to be an important determinant of the cytotoxicity of temozolomide\textsuperscript{71}.

Preclinical studies and early clinical evaluation in adults demonstrated schedule dependency with administration of the same dose over 5 days demonstrating greater anti-tumour activity than a single dose administration\textsuperscript{65,72}. In phase I and II studies in adults, efficacy has been shown in high grade astrocytomas and malignant melanomas\textsuperscript{65,73,74}.

A phase I study in children suggested activity in high grade astrocytomas and diffuse intrinsic brain stem gliomas\textsuperscript{75}. Therefore, further evaluation of the compound is indicated in these tumours, where there is a paucity of effective agents.

Further studies are required to confirm whether the level of the DNA repair protein AGT in the tumour relates to the response to temozolomide. Specifically, the hypothesis is that the 22% of primary brain tumours in which the repair protein cannot be detected are sensitive to temozolomide. There are a number of AGT inhibitors, including O\textsuperscript{6} benzylguanine, being developed and these may potentiate the cytotoxicity of temozolomide. Alternatively, as twice daily administration of temozolomide causes rapid depletion of AGT levels, the agent may...
potentiate its own activity and cause maximal depletion of AGT itself. Thus the optimal scheduling of temozolomide needs to be determined.

Thymidylate synthase inhibitors: The classical anti-folate in clinical use in paediatric oncology is methotrexate. Methotrexate inhibits dihydrofolate reductase (DHFR), the enzyme which catalyses the reduction of dihydrofolate to tetrahydrofolate, thereby maintaining the reduced intracellular folate pools. Reduced folates are essential cofactors for de novo purine and pyrimidine biosynthesis. Methotrexate enters the cell by specific transport mechanisms, primarily the reduced folate carrier. Methotrexate is polyglutamated within the cell by the enzyme folypolyglutamyl synthetase (FPGS), thereby increasing its intracellular retention.

Polyglutamates of methotrexate can act at other cytotoxic loci, namely glycinamide ribonucleotide transformylase (GARPT), an early enzyme in de novo purine synthesis, or thymidylate synthase (TS), the terminal step in de novo thymidylate synthesis. It is unknown whether inhibition of these enzymes contributes to anti-tumour selectivity.

Resistance mechanisms to methotrexate are well defined including increases in level of the target enzyme (DHFR), mutation in the target enzyme, impaired uptake of drug due to alterations in the membrane carrier and reduced polyglutamation.

TS inhibitors may circumvent these resistance mechanisms and have only one locus of action. A number of TS inhibitors have recently been developed: CB3717, (which has renal and hepatic toxicity)\textsuperscript{77}, Tomudex (ZD1694)\textsuperscript{78}, AG337\textsuperscript{61} and LY231514\textsuperscript{79}. AG337 was designed using X-ray crystallographic techniques and molecular modelling. AG337 does not possess a terminal glutamate residue and, therefore, does not require specialised membrane transport and is not polyglutamated. The agent should, therefore, circumvent some of the mechanisms by which malignant cells become resistant to the classical anti-folates and also penetrate the blood brain barrier more effectively. The major acute toxicities are myelosuppression and mucositis, however, as AG337 is an anti-metabolite, it should be expected to be devoid of late side effects. Phase I evaluation in children is at present ongoing under the auspices of the UKCCSG.

Multiple drug resistance reversal agents: Many in vitro studies employing cell lines have demonstrated that resistance to chemotherapy is modulated by a multi-drug resistance gene (MDR-1). This gene causes an increased expression of a membrane glycoprotein (P-170) which decreases intracellular accumulation of cytotoxic agents via an energy dependent efflux mechanism. Cells with a MDR phenotype have a decreased accumulation of certain cytotoxic drugs, including etoposide,
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tenoposide, daunorubicin, doxorubicin, vincristine and amsacrine, as compared to a cell with a normal phenotype.

The importance of MDR in paediatric tumours, particularly rhabdomyosarcoma and neuroblastoma, has been strongly suggested in some reports. In view of these findings, a therapeutic strategy is to attempt to inhibit the function of P-glycoprotein in drug resistant cancers. To date, verapamil and cyclosporin have been evaluated in paediatric malignancies. With both agents, non-haemopoietic toxicity prevented dose escalations.

PSC 833 is the most promising MDR reversal agent currently available. This compound is a non-immunosuppressive cyclosporin analogue, which is approximately 10-fold more potent than cyclosporin in its ability to modulate MDR in vitro. In adults, the drug limiting toxicity (DLT) was found to be neurotoxicity, predominantly cerebellar ataxia and PSC 833 effectively doubled the AUC of etoposide when administered concurrently. This effect was presumed to be due to interference with etoposide clearance. Phase I studies of PSC 833 and etoposide are ongoing in children with malignancy in the UK and France.

Protein kinase C inhibitors: Protein phosphorylation catalysed by protein kinases represents a critical pathway of intracellular signalling and, therefore, is a potential target for anti-cancer agents. Protein kinase C (calcium/phospholipid dependent kinases) catalyse the phosphorylation of serine and threonine residues. Protein kinase C is believed to be important in tumour promotion and in the regulation of cellular proliferation and differentiation. Bryostatin, a macrocyclic lactone, is a protein kinase C inhibitor and was isolated from a marine invertebrate. It has a wide spectrum of actions including growth inhibition, induction of apoptosis and differentiation and potentiation of the effects of IL2 and colony stimulating factors, to the degree that it stimulates haemopoiesis. The DLT in adult phase I studies, when the agent has been administered either as a 1 h or a 24 h infusion, is severe myalgia often requiring opiates. Activity has been observed in non-Hodgkin’s lymphomas and melanomas but in view of the toxicity further evaluation in children awaits better control of the dose limiting toxicity.

Tyrosine kinase inhibitors: Transmembrane receptors for a number of ligands, including platelet derived growth factor (PDGF), epidermal growth factor receptor (EGF), fibroblast growth factor (FGF) and neurotrophins have tyrosine kinase activity and are pivotal in intracellular signalling. Ligand binding promotes receptor dimerisation, autophosphorylation at tyrosine residues and a cascade of intracellular signalling. Inhibitors of tyrosine kinases are now becoming available and potentially they are of value in childhood tumours in which the expression of growth factors has been implicated in their malignant
behaviour, for example, neuroblastomas and intracranial primitive neuroectodermal tumours.

Inhibitors of cyclin dependent kinases: Cyclin dependent kinases (CDK) play a critical role in cellular proliferation by regulating the traversal of cell cycle checkpoints. The orderly activation and inactivation of CDKs may be dysregulated in malignant cells, for example a cyclin protein may be over-expressed resulting in abnormal activation of the CDK to which it binds. Alternatively, tumour cells may express homozygous deletions or a mutation of the genes encoding endogenous protein inhibitors of CDKs. Specific pharmacological inhibitors of CDK may be important novel anti-cancer agents.

Inhibitors of ras isoprenylation: The ras oncogene belongs to a family of membrane-bound molecular switches. The oncogenic activation of ras protein appears to be dependent on its association to the cell membrane via a 15 carbon isoprenyl (farnesyl) group which is covalently added to the cystine residues in a post-transitional modification. Certain agents are inhibitors of this ras isoprenylation and, potentially, these may have anti-tumour activity.

Differentiation agents: The first differentiation agents in clinical trials were all-trans and 13-cis retinoic acid. Subsequent studies have identified 9-cis retinoic acid which has affinity for both retinoic acid receptor (RAR) and retinoic x-receptor (RXR). In vitro studies indicate that this retinoid has a greater potential for gene induction, inhibition of proliferation and differentiation.

In addition, 9-cis retinoic acid may induce apoptosis on withdrawal of the agent and the pharmacological profile is more favourable. The DLT appears in adult studies to be headache. The relative values of 9-cis and 13-cis retinoic acid await randomised studies and the benefits of other RXR specific analogues require further evaluation.

Sodium phenylacetate and sodium phenylbutyrate induce maturation and growth arrest in cell lines including neuroblastoma and primitive neuroectodermal tumour lines. However, their anti-tumour action may also be via reduction in transforming growth factor β2 mRNA expression and inhibition of ras isoprenylation. Tributyrin is a prodrug of sodium butyrate and is also an inducer of leukemic blast differentiation.
The introduction of new anti-cancer agents in children is totally dependent upon phase I studies—identifying the toxicity and optimal dose, phase II studies—indicating the range of activity and, ultimately and phase III studies—determining the efficacy in terms of improvements in survival. Well designed, carefully conducted phase I studies are mandatory in the process of evaluating new anti-cancer agents in children. Recently, there has been substantial international collaboration to ensure that these studies are being carried out optimally.

Phase I studies have a number of objectives. These are primarily to determine the maximal tolerated dose (MTD), i.e. the dose to be used for the subsequent evaluation of the agent and also the nature and frequency of significant toxicities. The DLT is the major toxicity determined in the phase I studies. Parallel pharmacokinetic studies are essential. The other important objectives are to establish the supportive care that may be required during use of the agent, toxicity monitoring guidelines, cumulative toxicity and to help select the optimal safe dose schedule. The appropriate dose and toxicities for compounds in children cannot be predicted from phase I/II studies in adults and, therefore, separate phase I studies are necessary. The tolerance to anti-cancer agents varies between adults and children, either due to differences in drug disposition for physiological reasons, i.e. differences in renal or hepatic function and body composition, or cellular effect. In the past, the MTD of an agent for children has been shown to be 1.2 times higher than the adult MTD\textsuperscript{92,93}. However, this may be different in the future with the majority of children entering phase I studies having received more extensive prior therapy than adults.

It is imperative that the best estimate of the MTD in children is established, otherwise inappropriately low doses will be employed for phase II studies, potentially preventing the identification of important activity of an agent. Recently, the MTD for paclitaxel has been shown to be significantly higher in children than in adults\textsuperscript{94} and a different DLT has been demonstrated for \textit{trans}-retinoic acid in children\textsuperscript{95}. In addition, certain agents may be very effective in childhood tumours but less so in adults and, therefore, higher priority would be allocated to their development in the paediatric setting, for example, topotecan.

The major difference in phase I studies in children arises from the much smaller number of eligible children than adults. Based upon this, certain key features of phase I studies in children have been agreed.

These studies should be multi-institutional as no single institution is likely to be able to recruit adequate numbers of patients. Ideally, the paediatric phase I study should commence as soon as the adult phase I study is completed. In this way, all the experience gained during the adult study can
be applied in the design of the paediatric study and the drug can be rapidly evaluated. In the future it may be necessary to evaluate anti-cancer agents only in children and this will produce different requirements in the design of phase I studies. In view of the multi-institutional nature of paediatric phase I studies, the organisation is complex.

An efficient communication system is essential, such systems have been developed in Europe—United Kingdom Children’s Cancer Study Group (UKCSSG) and the French Society of Paediatric Oncology (SFOP) and in North America—Children’s Cancer Group (CCG) and the Paediatric Oncology Group (POG).

To reduce the number of patients entered, the starting dose of paediatric phase I studies is 80% of the adult MTD and the dose is escalated by 30% increments. Thus, a study may be completed in only 4–5 dose levels in contrast to over 10 in adults. Prioritisation of anti-cancer agents for phase I studies is of paramount importance. It is necessary to carefully define the population of patients, who are eligible for phase I studies, in order that the conclusions from these evaluations translate into a wider setting. Ensuring definite criteria for the level of organ function is important. Variation in renal function can significantly affect the MTD, as has been demonstrated with carboplatin and etoposide, as can differences in bone marrow reserve. Defining different levels of bone marrow reserve has not, as yet, been satisfactorily achieved.

Parallel pharmacokinetic studies permit the maximum knowledge to be learnt from phase I studies and recently multi-institutional pharmacokinetic studies have been possible in the UK. These investigations define inter-patient variation in AUC and identify sub-groups of patients with altered drug disposition which may affect toxicity. The relationship between dose and AUC; the drug’s half-life, which in turn affects drug scheduling and whether there is saturation of drug clearance (which assists in decisions regarding drug escalations) are all determined. It is also possible to develop limited sampling strategies which can then be taken forward into subsequent phase II and phase III studies, allowing the relationship between clinical pharmacology and clinical outcome to be determined. This will show whether differences in clinical pharmacology account for differences in the MTD of a drug between adults and children.

The importance of phase I studies in children has recently been fully appreciated and these studies are now the subject of much national and international attention. It is essential that phase I studies are efficiently and rapidly completed so that evaluation of the drug can progress. The concept of extending phase I studies to treat a larger number of patients with specific tumour types at the MTD is being used with some agents, thereby giving an early indicator of efficacy in the drugs developed.
Various strategies are being adopted to ensure the minimum number of patients are needed to define the objectives of a phase I study. A different dose escalation policy, guided by a pharmacological basis, has been proposed. Definition of different MTDs in patients with varying bone marrow reserve is a particularly vexed problem.

At one end of the spectrum, three patient groups could be studied to determine three independent MTDs, i.e. patients with no prior therapy; intensive prior therapy and those with bone marrow involvement. This would require a large number of patients to be recruited. An alternative approach is to carry out the phase I study in a group where the maximum number of patients are available. Once the MTD is determined, this can be modified with patients being entered at a higher or lower level in the corresponding sub-groups depending upon the extent of prior therapy.

The ability to undertake pharmacological studies is essential and, therefore, infrastructure at local centres and nationally have been developed in the UK. This requires considerable practical organisation and major interaction between the laboratory and the clinic. The designation of specific children's cancer centres which have developed experience and expertise in the phase I studies is a natural result of this process. The national organisation of phase I studies in the UK by efficient, rapid and reliable communication has been achieved in the last few years by the UKCCSG. In the future this will be further optimised and developed.

In the past, multi-institutional studies have been essential. In the future, progress can only be achieved through international collaboration. International guidelines for the conduct of phase I studies are about to be published which represent a consensus between the American and European investigators. This document provides a summary of accepted procedures for evaluating new agents in children with cancer and will be of value in interactions with the pharmaceutical industry and drug regulation agencies. Encouragement of pharmaceutical companies to be prepared to allow new anti-cancer agents to be evaluated in children is required. The development of anti-cancer agents in children is often, unfortunately, considered a lower priority than establishing the role of the agent in adults. This is predominantly because the smaller numbers of children with cancer limit the importance of the agent in the commercial market. Highlighting the importance of early evaluation of new anti-cancer agents in children can best be achieved through international collaboration.

Phase II studies determine the activity of anti-cancer agents. In order that phase II studies effectively define the anti-cancer activity of the agent, it is now agreed that they should target specific tumour types in order that an adequate number of patients with a single tumour type are entered. For this to be achieved, international collaboration is necessary.
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Recently established links between the UKCCSG and the SFOP pave the way in the future for many tumour-specific Phase II studies to be rapidly completed. This arrangement is similar to that already established in North America. The possibility of refining the MTD during Phase II studies needs to be considered in view of the problems of variability in bone marrow reserve in patients who have received prior therapy.

Conclusions

By a combination of these means, optimising the use of existing anti-cancer agents by pharmacological guidance according to clinical and molecular pharmacology and the introduction of new anti-cancer agents, ideally directed towards specific paediatric targets, substantial improvement can be made in the treatment of childhood malignancy.

References

35 Reed E, Ozols RF, Tarone R. Platinum-DNA adducts in leukocyte DNA correlate with disease response in cancer patients receiving platinum-based chemotherapy. *Proc Natl Acad Sci USA* 1987; 84: 5024-8
37 Reed E, Ostchega Y, Steinberg SM. Evaluation of platinum-DNA adduct levels relative to known prognostic variables in a cohort of ovarian cancer patients. *Cancer Res* 1990; 50: 2256–60
40 Fichtinger-Scheppmann AMJ, Dijt FJ, de Jong WH. In vivo cis-diaminedichloroplatinum (II)-DNA adduct formation and removal as measured with immunochemical techniques. *Proceeding of the Fifth International Symposium on Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, Padua, 1987
51 Austin CA, Sng JH, Patel S *et al.* Novel HeLa topo II is the topo II beta isoform: complete coding sequence and homology with other type II topoisomerases. *Biochim Biophys Acta* 1993; 1172: 283–91
67 Doz F, Pinkerton R. What is the place of carboplatin in paediatric oncology? Eur J Cancer 1994; 30A: 194-201
Cancer in children

85 Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 1995; 9: 484–96
90 Stockhammer G, Manley GT, Johnson R. Inhibition of proliferation and induction of differentiation in medulloblastoma and astrocytoma-derived cell lines with phenylacetate. J Neurosurg 1995; 83: 672–81