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Paediatric Update

Has chemotherapy reached its limits in pediatric cancers?

Gilles Vassal *

Department of Paediatric Oncology, France
UPRES EA3535, Pharmacology and New Treatments of Cancers, Institut Gustave Roussy, 39 Rue Camille Desmoulins, 94805 Villejuif Cedex, France

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1. Introduction

Over the last 50 years, chemotherapy has been used along with surgery and radiotherapy to treat malignancies in children.

Strictly speaking, chemotherapy is *''the prevention or treatment of disease by the use of chemical substances. The term is sometimes restricted to the treatment of infectious diseases with antibiotics and other drugs or to the control of cancer with antimetabolites and similar drugs (in contrast to radiotherapy)''* (Oxford concise Medical Dictionary 1998 New Edition). It is therefore not restricted to cancer. Physicians use antibacterial chemotherapy and antiviral chemotherapy daily. Since the first half of the 20th century, most medicines used as single agents or in combination to treat human cancers have been chemical compounds with cytotoxic properties. They may originate from plants (for example, vinca alkaloids, camptothecins), from the earth (anthracyclines) or from chemistry (for example, alkylating agents) and have been identified as anticancer drugs through massive screening of substances using *in vitro* and *in vivo* models. These models were murine tumour models before 1985, and human tumour models after 1985 [1]. The drugs were chosen for their capacity to kill cells or at least to inhibit their growth, whatever their mechanisms of action. Thousands of compounds were screened, but very few became anticancer drugs approved for use in humans. Most were known to be cytotoxic and were used in humans before their exact mechanism(s) of action was identified. For example, etoposide was identified as a topoisomerase II inhibitor years after it was introduced in humans.

Anticancer compounds are also toxic to normal tissues, especially to the bone marrow. The strategy was, and still is, to eradicate malignant disease by combining compounds administered at their maximum tolerated dose (MTD), i.e. the highest dose that can be used in humans, with toxicity considered tolerable by physicians. During the second half of the 20th century this has been the basis of cancer chemotherapy: ''the more, the better'' [2–4].

However, during the last 10 years, pharmaceutical companies have set up new strategies for anticancer drug development. They are based on the molecular pathology of cancer. Biology and genomic high-throughput technology platforms are used to identify genes, proteins and pathways, altered in malignant cells, that may be interesting therapeutic targets. Then, functional assays of these proteins are developed to screen large chemical ''libraries'' in order to identify at least one compound (the ''lead compound'') that is able to inhibit the target significantly. Optimising this lead compound eventually makes a derivative available for clinical development. The result is a putative anticancer drug, the so-called ''targeted compound'', which is believed to target malignant cells more that traditional drugs and to kill them (cytotoxic) or prevent them from proliferating (cytostatic). Among these new types of compound are (a) the recently approved imatinib mesylate (Glivec®) for the treatment of chronic myeloid leukaemias (CML) and gastrointestinal stromal tumours (GIST), (b) gefitinib (Iressa®) for the treatment of non-small cell lung carcinoma, and (c) trastuzumab (Herceptin®) (a monoclonal antibody) for the treatment of c-erbB2-positive breast cancers. They are believed to induce less toxic effects, especially haematological toxic-

* Tel.: +33 1 42 11 49 47; fax: +33 1 42 11 53 08.
E-mail address: gvassal@igr.fr.

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ity, than conventional anticancer compounds, or at least different types of toxicity. In addition, targeted compounds are believed to be active below their MTD and best used at their optimal biological dose (OBD). All of these compounds, including monoclonal antibodies, should nevertheless be considered as ''chemotherapy'' even though their mechanisms of action, their spectrum of activity and toxicity differ from that of conventional cytotoxic agents. With these specific targeted anticancer agents, the strategy may be to control tumour cell proliferation rather than eradicate every last cell, although some of these agents are able to induce apoptosis.

Thus, when one asks the question ''Has chemotherapy reached its limits?'' in paediatric cancers, one is unconsciously referring to ''conventional cytotoxic anticancer compounds'' that have been used for the last 50 years in children with cancer, as opposed to the new ''targeted'' treatments based on tumour biology.

2. Cytotoxic chemotherapy has significantly improved cure rates

Dramatic progress has been achieved with conventional cytotoxic chemotherapy in the treatment of several paediatric malignancies. It is now well established that children with acute lymphoblastic leukaemia (ALL) can be cured with combination chemotherapy, without the use of central nervous system (CNS)-directed radiotherapy that was previously thought to be essential. Short, intensive chemotherapy regimens achieved cure rates exceeding 90% in children with Burkitt-type non-Hodgkin's lymphoma (NHL) [5]. These results were obtained through successive prospective clinical trials, many of them aimed at increasing the dose intensity and developing effective doses and schedules to prevent CNS relapses. Thus, the concept of eradicating all malignant cells has been successfully applied in these diseases using cytotoxic compounds at their MTD. Other paediatric tumours, such as nephroblastoma, proved to be highly sensitive to radiation therapy and then to cytotoxic chemotherapy; cure rates exceeding 90% are now being achieved. Based on this difference in chemosensitivity in childhood tumours compared with adult tumours, cytotoxic chemotherapy has been developed in almost all paediatric malignancies and has significantly improved cure rates to 70%, overall [6].

In paediatric oncology, conventional cytotoxic chemotherapy proved to be an effective systemic treatment:

- to improve significantly overall survival of patients with the most chemosensitive paediatric malignancies,
- to eradicate distant micrometastasis from solid tumours, e.g. sarcomas,
- to enable safe reduction of the dose of radiation therapy or to avoid using it in several diseases, e.g. sarcomas, brain tumours, nephroblastoma, acute lymphoblastic leukaemias, Hodgkin's disease,
- to increase tumour operability and allow the use of conservative treatments, e.g. osteosarcoma, rhabdomyosarcoma, hepatoblastoma.

In parallel, huge efforts have been expended to identify, analyse and control acute toxic side-effects, along with long-term side-effects (''late effects'') that may occur after cure of a childhood cancer. For those patients with a ''good prognosis'' malignant tumour, the goal is to improve the benefit/risk ratio and significantly increase their quality of life both during treatment and when cure is achieved.

In the 1980's, high-dose chemotherapy (HDCT) with autologous haematopoietic stem cell transplantation was proposed for the treatment of high-risk paediatric malignancies. In an effort to eradicate the disease, the strategy was to use higher doses of chemotherapy than those usually tolerated in order to kill residual tumour cells in children in complete remission of a paediatric malignancy with a high risk of relapse. At that time, HDCT proved to be effective in children with non-Hodgkin's lymphomas, confirming the validity of the dose-effect concept in these lymphoid diseases and arguing in favour of more intensive first-line chemotherapy. This strategy was further developed so successfully that current curative treatment of B-cell or T-cell non-Hodgkin's lymphomas no longer contains HDCT [5]. Neuroblastoma is a complex and heterogeneous disease that is usually sensitive to cytotoxic chemotherapy, but children with metastatic disease are at a very high risk of relapse, even after a complete remission. HDCT has been studied extensively in high-risk neuroblastoma, using different doses and schedules of different anticancer drug combinations [7–9]. HDCT can significantly improved the prognosis for some children with high-risk neuroblastoma as shown in a large randomised study [10]. HDCT is a standard treatment for children in complete or a very good partial remission of a high-risk neuroblastoma. However, the overall cure rate of 40% is still unsatisfactory and new therapeutic strategies are urgently needed.

HDCT proved to be effective in other chemosensitive paediatric tumours such as medulloblastoma [11], but was ineffective in chemo-resistant tumours such as malignant glial tumours and ependymomas [12].

In general, conventional chemotherapy has succeeded in greatly improving overall cure rates. for children with malignancies. In addition, high-dose chemotherapy proved to be effective in some high-risk chemosensitive tumours, but reached its limits in chemoresistant tumours. Even though more that 3 out of 4 children can now be cured of their cancer with chemotherapy —

containing multi-modality treatment, chemotherapy is still an aggressive treatment with acute, chronic and long-term side-effects that need to be continuously and prospectively evaluated.

The challenges of the next decades are:

- to continue to improve the quality of life yet sustain cure rate for children with a good prognosis malignancy,
- to improve cure rates for children with a poor prognosis malignancy.

3. New cytotoxic compounds

During the last 15 years of the 20th century, pharmaceutical companies continued to develop conventional anticancer compounds for the treatment of adult cancers and several agents have been registered for use against adult cancers or conditions related to cancer (Table 1). Most of these compounds have also been evaluated in phase I and II trials in children, mainly in the United States (US), since it is extremely difficult to obtain new drugs for their evaluation in children in Europe. As a result, the paediatric information available in the summary of product characteristics (SPC) is still poor [13].

When these new cytotoxic drugs, usually used in adults, were evaluated in children, some interesting antitumour activities against paediatric malignancies were demonstrated but no major breakthrough was observed, unlike the results observed with imatinib mesylate (Glivec®) in CML in both adults and children. In fact, no new cytotoxic anticancer drugs have been introduced for treatment of paediatric malignancies since carboplatin.

Taxoids are important anticancer drugs in adult oncology. Paclitaxel (Taxol®) is available for the treatment of ovarian cancer and breast cancer, whilst Docetaxel (Taxotere®) is a major drug for breast cancer and has recently showed activity in other tumours such as carcinomas of the head and neck and prostate cancer. Taxoids stabilise microtubules during mitosis in contrast to the action of vinca alkaloids such as vincristine and vinblastine, which inhibit tubulin polymerisation – the initial step in mitotic spindle formation. In phase I studies, paclitaxel and docetaxel doses recommended in children were shown to be equivalent to those applicable to adults, with the same toxicity profile [14–17]. Toxicity of both paclitaxel and the solvents required for its intravenous (i.v.) formulation – ethanol and Cremophor EL has been a limiting factor with the 3 h infusion [18]. Antitumour activity has been observed in phase I and II studies [19] in rhabdomyosarcoma, leukaemia and medulloblastoma, but no clear phase II response rate has been demonstrated and so far these taxoid compounds have not proved to be of major importance for paediatric malignancies. The resistance mechanisms acquired during first-line treatments, through activation of the multi-drug resistance phenotype are considered responsible for the lack of response to these taxoid compounds when evaluated in phase II in relapsed or refractory paediatric malignant diseases. Thus, for the time being, taxoids are not recommended for use in children.

Temozolomide (Temodal®, Temodar®) a prodrug of mitozolomide, has been registered for the treatment of both malignant gliomas and melanoma in adults. Temozolomide was the first anticancer compound approved in Europe for which paediatric data, from phase I study, were available at the time of submission to the European Medicines Evaluation Agency (EMEA). The recommended dose and schedule of temozolomide proved to be identical in children and in adults in phase I studies conducted separately in the US and the United Kingdom (UK) [20,21]. Paediatric phase II trials revealed disappointing activity in brain stem glioma and a low response rate in hemispheric malignant gliomas [22]. Although no major response rate was observed, prolonged disease ''stabilisation'' did occur with a clear improvement of the clinical status in some patients with incurable brain tumours [23]. More recently, temozolomide was suggested to have activity in medulloblastoma [24] and low grade gliomas [25]. New schedules of administration are under development. Therefore, temozomolide seems to have an interesting potential in paediatric malignancies, but its place has yet to be defined. It should be borne in mind that temozolomide is a methylating agent with, thus, a theoretical risk of secondary leukaemia [26].

Camptothecins were identified in the late 1980's as a new category of anticancer compounds, namely DNA topoisomerase 1 inhibitors. Two are currently available worldwide: topotecan (Hycamtin®) for the treatment of ovarian cancer and irinotecan (Campto®, Camptosar®) for the treatment of colorectal cancer. Other topoisomerase 1 inhibitors, analogues of camptothecin or original chemical compounds, are currently being developed. A large topotecan and irinotecan development programme has been conducted in the US, Europe and Japan, both at the preclinical and clinical levels in children. Indeed, topoisomerase 1 is expressed in paediatric malignancies, especially in neuroblastoma [27], and preclinical evaluation of both topotecan and irinotecan in paediatric tumour xenografts showed activity in neuroblastoma, rhabdomyosarcoma, hepatoblastoma, medulloblastoma and primitive neuroectodermal tumours (PNET) [28–37]. Topotecan was the first agent used in a paediatric drug development programme and more than 15 paediatric phase I studies of topotecan have been conducted using it as a single agent or in combination, and given intravenously or orally using

Table 1
Medicinal products for the treatment of cancer or a cancer-related conditions having received EU marketing authorisation

Product brand name (Inn)	Company	Therapeutic area indication	Date of notification	Paediatric data in the SPC
Taxotere (docetaxel)	Aventis	Second-line treatment of breast tumours	29/11/1995	No
Fareston (toremifene)	Orion Corporation	Treatment of certain breast tumours	16/02/1996	No
Caelyx (doxorubicin)	SP Europe	AIDS-related Kaposi's Sarcoma	25/06/1996	No
Destara; Bondronat (ibandronic acid)	Roche Registration Ltd.	Hypercalcaemia of malignancy	27/06/1996	No
Hycamtin (topotecan)	GlaxoSmithKline	Treatment of metastatic ovary carcinoma	13/11/1996	No
Neorecormon (epoetin beta)	Roche Registration Ltd.	Treatment of anaemia	29/08/1997	Yes
Mabthera (rituximab)	Roche Registration Ltd.	Treatment of stage III–IV follicular lymphoma	26/06/1998	No
Temodal (temozolomide)	SP Europe US	Treatment of patients with recurrent malignant glioma	28/01/1999	Yes
Beromun (tasonermin)	Boehringer Ingelheim International GmbH	Adjunct therapy to surgery for unresectable soft tissue sarcoma of the limbs to prevent or delay amputation	15/04/1999	No
Paxene (paclitaxel)	Norton Health Care Ltd.	Treatment of advanced AIDS-related Kaposi's sarcoma	21/07/1999	No
Intron A (interferon alfa-2b)	SP Europe	Treatment of chronic hepatitis B and C, Hairy Cell Leukaemia, Chronic Myelogenous Leukaemia, Multiple Myeloma, Follicular Lymphoma, Carcinoid Tumours and Malignant Melanoma	13/03/2000	No
Myocet (doxorubicin)	Elan Pharma Internatioal Ltd.	Treatment of metastatic breast cancer	26/07/2000	No
Herceptin (trastuzumab)	Roche Registration Ltd.	Treatment of patients with metastatic breast cancer whose tumour overexpress HER2	04/09/2000	No
Panretin (alitretinoin)	Ligand Pharmaceuticals Ltd.	Topical treatment of cutaneous lesions in AIDS-related Kaposi's sarcoma	18/10/2000	No
Xeloda (capecitabine)	Roche Registration Ltd.	Treatment of metastatic colorectal cancer	06/02/2001	No
Fasturtec (rasburicase)	Sanofi	Treatment of tumour-induced hyperuricaemia	02/02/2001	Yes
Zometa (zoledronic acid)	Novartis Europharm Ltd.	Treatment of tumour-induced hypercalcaemia	20/03/2001	No
Nespo (darbepoetin alpha)	Dompé Biotec S.p.A.	Treatment of anaemia associated with chronic renal failure in adults and paediatric subjects $\geqslant$11 years. Treatment of anaemia in adult cancer patients with non-myeloid malignancies receiving chemotherapy	26/06/2001	Yes
Aranesp (darbepoetin alpha)	Amgen Europe	Treatment of anaemia associated with chronic renal failure in adults and paediatric subjects $\geqslant$11 years. Treatment of anaemia in adult cancer patients with non-myeloid malignancies receiving chemotherapy	26/06/2001	Yes
MabCampath (alemtuzumab)	Millenium & Ilex UK Ltd.	Second-line treatment of chronic lymphocytic leukaemia	10/07/2001	No
Foscan (temporfin)	Scotia Pharmaceuticals	Treatment of squamous cell carcinoma of the head and neck	26/10/2001	No
Glivec (imatinib mesylate)	Novartis Europharm Ltd.	Treatment of patients with chronic myeloid leukemia (CML)	12/11/2001	Yes
Trisenox (arsenic trioxide)	Cell Therapeutics (UK) Ltd.	Indicated for induction of remission and consolidation in adult patients with relapsed/refractory acute promyelocytic leukaemia (APL)	07/03/2002	Yes

(*continued on next page*)

Table 1 (*continued*)

Product brand name (Inn)	Company	Therapeutic area indication	Date of notification	Paediatric data in the SPC
Neulasta (pegfilgrastim)	Amgen Europe	Reduction in the duration of neutropenia	22/08/2002	No
Neupopeg (pegfilgrastim)	Amgen Europe	Reduction in the duration of neutropenia	22/08/2002	No
Busilvex (Busulfan)	Pierre Fabre Medicament	Treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients	25/07/2003	No
Emend (aprepitant)	Merck Sharp & Dome	Prevention of acute and delayed nausea and vomiting associated with highly emetogenic cisplatin-based cancer chemotherapy	28/11/2003	No
Zevalin (Ibirtumomab tiuxetan)	Schering AG	Treatment of adult patients with rituximab relapsed or refractory CD20+ follicular B-cell non-Hodgkin's lymphoma (NHL)	27/02/2004	No

Source: List of medicinal products with European Union marketing authorisation – status March 2004 http://www.emea.eu.int/pdfs/general/direct/listprod/868604.pdf Compounds authorised for the diagnosis of cancer or cancer-related conditions were not considered. Other anticancer compounds, such as Camtpo® (irinotecan) or Navelbine® (navelbine) received marketing authorisation in Europe during the same period. However, authorisation was obtained through mutual recognition, and they do not appear in the EMEA list of approved drugs.
SPC, summary of product characteristics.

different schedules. These studies showed that the recommended dose in children is schedule-dependent [38–44]. Extending the administration of topotecan, which has a short half-life, was shown to increase its activity at the preclinical level [30]. Few single-agent phase II trials of topotecan were performed in children [45–48], and it was soon combined with cyclophosphamide [43,49] and used to treat several tumours (Ewing's tumours, neuroblastoma and rhabdomyosarcoma).

Experience of the use of topotecan in rhabdomyosarcoma was recently reviewed by Smith [50]. In phase I single-agent studies, tumour responses were reported in patients with relapsed rhabdomyosarcoma. Phase II evaluation of single-agent topotecan in these patients yielded a poor response rate, but the response rate was nearly 40% in newly-diagnosed patients [48], and up to 60% of responses were reported when topotecan was combined with cyclophosphamide, both in relapsed and newly-diagnosed patients with rhabdomyosarcoma [51]. This regimen, with vincristine, is currently being evaluated in a randomised trial conducted by the Children's Oncology Group (COG). This trial will provide an answer to the question: does topotecan, added to a standard chemotherapy regimen, improve the survival of patients with non-metastatic rhabdomyosarcoma?

Topotecan has also been evaluated in combination with doxorubicin and cyclophosphamide in metastatic neuroblastoma with a 64% response rate [9]. Whether the addition of topotecan to first-line chemotherapy will significantly increase the number of complete remissions is an open question. More recently, topotecan whose dose-limiting toxicity is to the bone marrow was studied within high-dose combination regimens in children with high-risk neuroblastoma [52].

The story of topotecan shows how difficult comprehensive drug development is in paediatric solid malignancies. It raises a crucial question: what pre-requisites need to be generated in early phase I and II trials so that a new compound can be tested in a large randomised trial to evaluate whether or not this new anticancer drug, when added to standard treatment, can improve the survival of children with cancer.

Irinotecan was developed in phase I trials in children in the mid-1990's and four different schedules were evaluated in the US, Europe and Japan [53–56]. Interestingly, only one schedule – administration every 3 weeks – was the same as that used in adults, while the other three schedules were never evaluated in adults before being used in children. Irinotecan is a complex drug which is metabolised through different pathways, including carboxylesterase, UGT1A1 and cytochrome P450. Interesting responses have been observed in phase I trials in neuroblastoma, rhabdomyosarcoma, medulloblastoma and hepatoblastoma. Irinotecan was then evaluated in phase II studies, either through trials open to any tumour types, or focused on one disease. The

development of irinotecan in paediatric malignancies is less advanced than that of topotecan, in part due to the gastrointestinal toxicity (diarrhoea) regularly observed, whichever schedule is used.

Irinotecan has been explored in adults with malignant glial brain tumours and partial response rates up to 15% have been reported [57–60]. In addition, extensive pharmacokinetic and metabolic studies have been performed to define the interactions between irinotecan and anticonvulsant drugs, as well as the optimal/recommended dose of irinotecan when it is administered in this setting [61,62]. Using preclinical data, a phase I study of irinotecan in combination with temozolomide was carried out and recently reported [63].

Thus, topoisomerase 1 inhibitors are attractive drugs, but definitive confirmation is needed to evaluate their impact in the first-line therapy of paediatric malignancies. Protracted administration seems to be particularly relevant for this family of anticancer agents. Oral administration of the i.v. forms of topotecan has been evaluated, especially in children, although the i.v. form did not appear to be suitable for oral administration because of the wide inter-patient variability [39,64,65]. This is an important area to explore further, and especially when new topoisomerase 1 inhibitors for oral use become available.

4. Lessons for new drug development in children with cancer

When we look back at the last two decades, several summary statements can be made and lessons learned.

The first concerns ease of access to interesting new agents. Access to new compounds developed by pharmaceutical companies has been relatively straightforward in the US, mainly through National Cancer Institute (NCI) sponsored programmes, while it has been dramatically limited in Europe. One hopes that future European Regulation – (*European Parliament and Council Regulation (EC) on Medicinal Products for Paediatric Use*) – will significantly change this situation. Despite the difficulty in obtaining new compounds, Europe has developed expertise in the field [66]. There are research labs and clinical teams, with experience in preclinical evaluation and early drug development in children. International cooperation should be sought to avoid unnecessary duplication of early drug trials in children with cancer.

The second point concerns the measurement of "response". Paediatric oncologists are used to measuring tumour response after two cycles of treatment. Such was certainly the case in Burkitt's lymphoma and nephroblastoma. Considering stable disease with no objective shrinkage as a "failure", they are eager to switch to other treatment options. Expecting a rapid decline in tumour volume at the time of diagnosis may be valid for standard treatments, but the situation is different when new drugs are being evaluated. Indeed, disease found to be stable after two cycles of treatment with a new drug has been shown to respond after further cycles of the same treatment when the drug is continued. Therefore, there is a novel proposal not to measure response after two cycles in phase II trials, but rather to continue the treatment until the best response is observed – without imposing a time limit – and treatment only discontinued in the event of disease progression. Criteria used to "response" and "non-response" in paediatric oncology should be re-addressed.

The third topical issue the consequence of the fact that most children participating in phase I and II trials have already received prolonged and aggressive cytotoxic chemotherapy regimens. The question is "What must a new drug demonstrate for it to be considered potentially interesting and for it to be legitimately combined with standard treatments"? There are those who still advocate conventional evaluation of new agents in relapsed disease after initial treatment, whilst others favour its evaluation in newly-diagnosed patients before standard treatments are administered (the so-called "upfront window phase II"). Those holding the former view may consider that a drug exhibiting activity in relapsed disease after multiple treatments is potentially active even if it fails to yield at least a 20% response rate – a threshold regularly used in phase II trial – arguing that this may indicate that cells exhibiting resistance to standard compounds are nevertheless sensitive to the new agent. When the new drug is added to the standard treatment, it may be therefore capable of killing cells that are refractory to conventional drugs.

Conversely, if the new treatment is administered in newly-diagnosed patients when the tumour is chemotherapy-naive, high response rates (40–50%) may be observed. Here the question is "Will the new agent be capable of killing cells that other conventional drugs fail to eradicate or will it simply demonstrate activity upon cells that conventional drugs are already capable of eliminating"?

Finally, it is clearly difficult to design and conduct a development plan for an anticancer compound in paediatric cancers. Indeed, randomised trials comparing a new drug to standard treatment is neither feasible nor ethical in most paediatric tumours except, perhaps, brain stem tumours for which no curative treatment option currently exists. Adding the new compound to standard treatment and comparing this combination to the standard treatment should help to determine the impact of the new drug on survival. However, paediatric malignancies are rare cancers; when a phase III trial is conducted to answer a question, hundreds of patients are needed in large cooperative trials requiring 5–10 years of accrual. Thus, the rationale for undertaking such a

phase III trial to evaluate a new compound should be carefully assessed and extremely stringent criteria are mandatory. Further, a new drug can be considered as "salvage" or "palliative" treatment for patients whose disease has relapsed.

In conclusion, unless a compound exhibits outstanding activity, such as imatinib mesylate (Glivec®) in CML developing an anticancer compound from phase I to standard care in children is a long and difficult process that requires strong networking activities and steady international cooperation. Clearly, the know-how that is serially acquired with cytotoxic compounds will assist the development of the new anticancer agents of the future.

5. New therapeutic strategies using "old" cytotoxic compounds

During the last 20 years, new technologies for haematopoietic cell rescue, including peripheral stem cell collection, haematopoietic growth factors along with the improvement of supportive care through effective antimicrobial therapies, optimised transfusions and pain relief have allowed clinicians to explore the dose-effect concept, (i.e. increasing the dose beyond the conventional recommended dose), for several anticancer compounds in a variety of paediatric malignancies. The strategy was to use HDCT in minimal residual disease – as defined by a complete remisson – in order to try to eradicate the remaining malignant cells with one "shot" of therapy.

More recently, a new way to administer cytotoxic chemotherapy called "metronomic chemotherapy" has been developed. The concept is based on the use of protracted and continuous administration of cytotoxic compounds at non-toxic doses. Such schedules were proven highly effective *in vivo* in paediatric tumour xenografts [67]. The hypothesis that cytotoxic agents could act like anti-angiogenic agents was recently reviewed by Miller in Ref. [68]. This effect was presumed to be related to the presence of dividing endothelial cells, which may be sensitive to conventional cytotoxic compounds, in newly-forming tumour blood vessels. More recently, Bocci showed that the anti-angiogenic effect of the protracted administration of low-dose cyclophosphamide was abolished in thrombospondin-1 (TSP1)-deficient mice [69]. TSP1, a highly specific and potent endogenous inhibitor of angiogenesis, may therefore be a mediator of the anti-angiogenic effects of protracted low-dose chemotherapy.

Preliminary experience in children is encouraging and "metronomic therapy" might be a new way to consider treating residual disease. Indeed, experience with prolonged treatments in paediatric malignancies is worth analysing. So-called "maintenance therapy" is well known to play a critical role in the cure of children with acute lymphoblastic leukaemias. However, to date, no evidence has been generated about the use of prolonged treatment, like this, in solid tumours. Recently, prolonged courses of non-intensive chemotherapy have proved to be effective in low-grade gliomas [70]. With respect to the growth of solid tumours, it is well established that a blood supply and new vessels are essential for the growth of a solid tumour that exceed 2 mm in size. This is also a strong rationale for considering anti-angiogenic therapy for high-risk solid tumours in complete remission in order to eradicate or control the growth of residual malignant cells. "Metronomic therapy" might be a way of evaluating the role of this form of prolonged therapy in malignant paediatric solid tumours.

Finally, efforts are being expended to improve drug delivery to the tumour rather than to normal tissues. This may lead to better-controlled toxicity and to an increase in the antitumour effects. For example, the liposomal formulation of amphotericin has significantly improved its therapeutic/toxic ratio. On the other hand, liposomal formulations of cytotoxic compounds have so far not resulted in compounds which are significantly more active and/or less toxic. Liposome-encapsulated daunorubicin, for example, has not significantly reduced the risk of cardiac toxicity. New "vectorisation" processes are being explored extensively, especially those using "nanospheres" [71]. This is a particularly interesting approach, considering the possible immunotargeting of these drug vehicles in order to modify drug distribution, and thus tolerance and efficacy. This is an active domain of drug research that may provide paediatric oncology with more specific cytotoxic compounds in the future.

6. Compounds with new mechanisms of action

Whilst the concept of tumour eradication through intensive chemotherapy may have reached its limits in poorly chemosensitive high-risk tumours in children, new strategies are worth considering. The challenge is either to control or to eradicate minimal residual disease, once complete remission has been achieved. There are two ways to attain these goals: either killing the cells or preventing them from entering the cell cycle and from proliferating.

Differentiation is a physiological status during which the cell is, out of the cell cycle, in G0. Terminal differentiation is physiologically characteristic of normal tissues. In other circumstances, differentiation is not terminal and cells can re-enter the cell cycle when needed. Inducing differentiation of minimal residual disease might prove effective therapy in children in complete remission. The first significant example of this type of drugs

was provided by retinoic acid in high-risk neuroblastoma [72]. All *trans*-retinoic acid proved to be highly effective in promyelocytic leukaemias by inducing differentiation of promyelocyte blasts, although relapses were observed in adults. Retinoids are also in use in adult head and neck cancer for chemoprevention of relapses.

Immature malignant neuroblasts can be made to differentiate *in vitro* under the action of several chemicals including retinoic acid (RA). This was the rationale for the development of retinoic acid for the treatment of neuroblastoma. A phase I study defined a dose of 13-*cis*-RA, which was tolerable in patients after myeloablative therapy [73], but it failed to achieve partial and complete remission in patients with a measurable tumour. Then, a phase III trial showed that post-consolidation therapy with 13-*cis*-RA improved event-free survival (EFS) for patients with high-risk neuroblastoma [10]. Several other differentiating therapies, including fenretinide, are being investigated as more effective therapy, especially in patients whose tumour is resistant to RA [74]. In addition, the fact that first-line cytotoxic chemotherapy induces differentiation of neuroblastoma, as observed on surgical specimens, is worth considering. Recently, we showed that irinotecan induces reversible differentiation in neuroblastoma xenografts [75], suggesting that cytotoxic chemotherapy may also exhibit new cytostatic properties when used at low doses on protracted schedules.

This example shows that strong preclinical evaluation may lead to short-track clinical development from phase I directly to phase III trials. This is an example to bear in mind when considering how to evaluate new compounds with new mechanisms of action in children with malignancies. Indeed, many new compounds that interfere with angiogenesis or signal transduction or apoptosis will be available in the future. It is unlikely that most of them will yield such an outstanding tumour response as that observed with imatinib mesylate (Glivec®) in CML or GIST [76], but some may prove to be potent anticancer compounds when combined with "conventional" cytotoxic agents. For example, imatinib mesylate (Glivec®) was recently shown to enhance the effect of ionising radiation, to sensitise resistant cells to topotecan and SN38 [77], and to inhibit the growth of human neuroblastoma xenografts with a reduction of vascular endothelial growth factor (VEGF) expression [78].

7. Improved understanding of inter-patient variability in tumour response and toxicity

Although, currently-established regimens often cure children with cancer, some patients fail initial therapies whose efficacy is proven or experience unpredicted severe toxicity that jeopardises further administration of an effective treatment. During the last 30 years or so, several teams have explored the relationship between exposure to the cytotoxic agents used and unexpected results like these. There are a few examples which suggest that systematic anticancer drug monitoring aimed at reducing inter-patient variability in systemic exposure may improve tolerance, although an impact on survival has not been demonstrated [79]. We now have evidence that variability in drug response may be genetically determined both in the tumour and by the patient's genetic heritage [80]. It is now established, for example, that children with a thiopurine *S*-methyltransferase (TPMT) deficiency are at risk of severe toxicity when they are given oral mercaptopurine for the "continuing treatment" of ALL [81]. Patients with CML carrying a methylenetetrahydrofolate reductase (MTHFR) mutated genotype are at risk of oral mucositis during high-dose chemotherapy and allogeneic haematopoietic stem cell transplantation [82]. Moreover, the MTHFR genotype may be associated with a poorer outcome than average in children with ALL [83]. More recently, the potential role of an MDR1 polymorphism in anticancer drug disposition in humans has been highlighted [84]. The development of high-throughput sequencing technologies will improve significantly the capacity to discover single nucleotide polymorphisms that may be involved in response to anticancer drugs. We will need to demonstrate whether adaptation of the chemotherapy schedule to the genetic information carried by patients is able or unable to improve their outcome.

If the constitutional genetic information in each individual can influence drug disposition and toxicity, the genetic information contained in the primary tumour and metastasis may also influence tumour response and patient outcome when chemotherapy is used. Breast cancer is already a case in point. Indeed, a tumour gene-expression profile at diagnosis may efficiently predict the outcome of women with a localised breast cancer treated by surgery and radiotherapy without chemotherapy [85]. If this observation is confirmed in a prospective study, anthracycline-containing adjuvant chemotherapy could be recommended only to patients at risk of metastasis, while patients with a "good prognosis" tumour genetic profile can be spared this chemotherapy. There are numerous circumstances in paediatric oncology where the identification of the gene-expression profile of a tumour at diagnosis may help to identify both those patients requiring the standard treatment and those in need of a different approach. Osteosarcoma, where the degree of tumour response to first-line chemotherapy has a prognostic value, is a case in point.

Thus, in children as well as in adults, structural and functional genomics will undoubtedly contribute to a better understanding of interpatient variability in response to drugs, and thus help us to predict toxicity, outcome and to adapt current treatments using anticancer chemotherapy. In this respect, genomics could be

viewed as a driving force pushing cytotoxic chemotherapy beyond its present limits.

8. Conclusions

During the last two decades of the 20th century, more intensive use of cytotoxic chemotherapy contributed to the striking increase in survival rates of several chemosensitive paediatric malignancies. However, progress has recently slowed down and several refractory diseases remain a difficult challenge for the coming years. This begs the question "Has chemotherapy reached its limits?". My belief is that it has not because there are now numerous new opportunities to improve the treatment of children with cancer even further. The use of genomics and protenomics will hopefully help to identify patients who are likely to benefit from a conventional treatment and those requiring innovative treatment options. The development of new anticancer drugs, to be used in combination with cytotoxic chemotherapy also has huge potential. Although 'targeted' compounds are very attractive to the entire oncology community, there is still a place for new 'conventional' cytotoxic agents. One may recall that the discovery and development of cisplatin has dramatically improve cure rates for patients with testicular and ovarian cancer, and for children with malignancies.

The central question is how to select from the wide range of new compounds whose development is in the offing – be they targeted or not, old-fashioned or not – those that deserve to be developed for paediatric cancers? In other words, how can we accelerate the drug evaluation process in children and how can we increase a child's chances of receiving a potentially active drug for his/her disease? Even though all pre-clinical models have their limits in predicting activity in humans, we will still need to rely on more straightforward and systematic preclinical evaluation to achieve this goal. Strong networking activities and cooperation on an international scale will also be needed in order to optimise the drug development process in children with cancer so that unnecessarily duplicated studies are strictly avoided and so that each child in Europe has a chance to have access to new treatments if he or she needs it.

Conflict of interest statement

None declared.

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