At the frontier of progress for paediatric oncology: the neuroblastoma paradigm

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Introduction: Neuroblastoma is one of the commonest and deadliest forms of childhood cancer and major initiatives are ongoing to improve the outcome of these patients.

Sources of data: Data for this review were obtained from PubMed and abstracts from the American Society of Clinical Oncology and Advances in Neuroblastoma Research.

Areas of agreement: Collaborative clinical trials have led to major improvements in treatment outcomes for low and intermediate risk neuroblastoma, and international initiatives such as the International Neuroblastoma Risk Group have produced a very refined risk stratification incorporating clinical and biological risk factors.

Areas of controversy: Despite many efforts, the outcome for high-risk neuroblastoma is still poor and the only new strategy incorporated into frontline treatment is anti-GD2 immunotherapy. It is unclear how new drugs targeting specific molecular aberrations will be incorporated.

Growing points: Genomic characterization and drug development have undergone major advances in the last 5 years leading to a much deeper understanding of tumour biology as well as active biomarker-driven preclinical and clinical research on new molecules that will hopefully progress faster and more efficiently into frontline combination treatment strategies.

Areas timely for developing research: Significant effort remains to be done in integrating the different new strategies, combining new molecularly targeted agents to maximize therapeutic benefit and incorporate immunotherapy together with targeted therapies.

Keywords: neuroblastoma/phase I/phase II/childhood cancer/drug development/biomarkers

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Introduction

Despite the major improvements in survival for childhood cancers seen over the past two decades, there is still a major unmet need to develop new drugs and strategies for high-risk paediatric malignancies. Cancer remains the main cause of death in children aged 1–14 years overall accounting for 18% of all childhood deaths.\(^1\) Even now, ~25% of children and adolescents diagnosed with cancer will die from their disease. Survivors face a significant burden of long-term disabling toxicities related to the intensity of multimodal treatment with >60% developing at least one chronic condition, >25% of them severe; the most common include major joint replacement, cardiac failure and coronary artery disease, cognitive dysfunction or second cancers, amongst others.\(^2\)

Moreover, improvements in survival have now plateaued after the maximal possible intensity for the therapy of many cancers has been achieved with the use of intensive chemotherapy, surgery, radiotherapy and myeloablative therapy (MAT) with autologous haematopoetic stem cell rescue (AHSCR), amongst others.\(^3\) Enhanced efforts aimed at addressing treatment-related morbidity and mortality by improved supportive care have contributed to better outcomes, but have probably reached a peak in the degree of further survival benefit they can offer. It is therefore clear that introducing novel drugs into frontline therapy for paediatric cancer patients is a major priority to further improve the number cured long term, and the quality of that cure.

Molecularly targeted agents directed at specific oncogenic pathways offer particular hope for the future and a number of successful examples have been seen in adult cancers.\(^4\) However, these targeted agents have not yet reached clinical trials in children in adequate numbers, due to a considerable number of bottlenecks in the paediatric drug development process.\(^5\)\(^–\)\(^7\) To date, only one targeted agent, imatinib, has been introduced into frontline therapy for a malignancy which occurs in children, chronic myeloid leukaemia, and this only because the condition is predominantly a disease of adulthood which happens to also occur, although rarely, in children.\(^8\) In this case, the childhood condition is biologically similar to the adult disease, and extrapolation from adults, with limited early phase trials in children, has thus been possible.

Neuroblastoma

Neuroblastoma provides an excellent paradigm of a poor prognosis cancer where new therapies are urgently needed, and serves well to
illustrate the challenges and achievements in the field of paediatric oncology drug development.

Neuroblastoma is the most common solid extracranial tumour of childhood and accounts for 7% of all childhood cancers, with an incidence of ∼100 new cases in the UK per year. It is the principal cause of death due to cancer in infancy and the third most common cause of death due to malignancy in children after central nervous system (CNS) tumours and leukaemias (11% of all deaths due to cancer).

Neuroblastoma arises from neural crest cells within the sympathetic nervous system; most commonly intra-abdominally in an adrenal gland, although it may also arise in other intra-abdominal sites, or anywhere along the sympathetic chain including in the neck, chest or pelvis. It is a fascinating disease with a plethora of prognostic biological features resulting in phenotypes, which range from tumours which regress or differentiate spontaneously into ganglioneuromas to a highly aggressive form which is frequently fatal. High-risk neuroblastoma is characterized by metastatic disease (most frequently metastasizing to liver, bone, bone marrow, skin and occasionally central nervous system [CNS]) and/or amplification of the MYCN oncogene.

Tumours have historically been staged according to the International Neuroblastoma Staging System (INSS), a staging system based on anatomical location and surgical resectability of the tumour, then additionally classified as low, intermediate or high risk based on features such as patient age and tumour biology. MYCN amplification was initially described in the 1980s and remains the most powerful prognostic factor. The biological behaviour of the tumours, and therefore the treatment priorities for patients in these different risk groups are very different.

Patients with low and intermediate risk disease have an excellent prognosis and in these subgroups, efforts are being made to reduce therapy-related toxicities. Tumours in patients diagnosed before the age of 18 months can spontaneously regress or undergo tumour differentiation to the benign end of the disease spectrum (ganglioneuroma). This also includes those tumours in infants exhibiting a particular metastatic pattern designated stage MS (previously called stage 4S). However, patients with high-risk disease, defined as metastatic (stage M) or harbouring amplification of the MYCN oncogene have a poor prognosis despite the implementation of intensive multimodal therapy.

With the use of intensive chemotherapy, surgery, myeloablative chemotherapy with AHSCCR, radiotherapy and differentiation therapy using 13-cis-retinoic acid, overall survival is still below 50%. Recently, immunotherapy with anti-GD2 monoclonal antibodies in combination with cytokines has been introduced into frontline therapy and shown promising improvements in 2-year survival but the long-term benefits are yet to
be established.\textsuperscript{20,21} After relapse, 5-year overall survival (OS) was 8\% for children with relapsed metastatic neuroblastoma and 4\% for those with \textit{MYCN} amplification.\textsuperscript{22}

Thus, not only is there a pressing need for new drugs, but also a requirement to integrate these into new therapeutic strategies and existing gold standard regimens, in order to develop better risk stratification, thereby facilitating opportunities for personalizing therapy: – intensifying treatment for those who need it but reducing the burden of late toxicities for those with lower risk disease and therefore more likely to survive to adulthood.

**Improving risk stratification: the International Neuroblastoma Risk Group collaboration**

Major advances have been achieved in the field thanks to the collaboration between academic investigators. There are established networks such as the International Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN), the German Paediatric Oncology and Haematology Group (GPOH) and the US Children’s Oncology Group (COG) amongst others.

To develop a consensus approach to risk stratification, a task force of global leading investigators was convened in 2004 and established The International Neuroblastoma Risk Group (INRG) classification system, which brought together experts from European, American, Australian and Japanese cooperative groups. This initiative gathered data from 8800 cases of neuroblastoma diagnosed between 1990 and 2002.\textsuperscript{15} A key output was a new staging system, called the INRG Staging System (INRGSS), based on radiological features\textsuperscript{2,3} and a comprehensive risk stratification based on clinical (patient age, tumour stage), pathological (histology, differentiation) and biological (\textit{MYCN} amplification, 11q aberrations, ploidy) variables (Tables 1 and 2).\textsuperscript{15} This system identifies 4 rather than 3 risk groups: very low, low, intermediate and high risk. The task force output has also included consensus documents on molecular diagnostics, imaging, nuclear medicine imaging and detection of minimal neuroblastoma cells, thereby facilitating comparability between different research studies worldwide.

The INRG project has also provided a powerful tool for the validation of prognostic variables in a large dataset. For example, the presence of any segmental chromosomal aberrations (defined as any chromosomal gain or deletion) has recently been proven to have a significant negative impact on patient survival\textsuperscript{24} and this knowledge has now been incorporated into prospective clinical trials such as the current European trial for localized neuroblastoma (EudraCT 2010-021396-81).
Moreover, prognostic tools are being developed involving the monitoring of minimal residual disease in blood or bone marrow using technologies such as QRT-PCR, flow cytometry or immunocytology together with new generation platforms such as mRNA expression profiles, genomic DNA profiles or epigenetics/miRNA profiles.

INRG2 is a new database that will double the number of patients included and incorporate extensive genomic data, developing a more precise classification system.

Of particular interest is the identification of the patients with the worst prognosis, the so-called ‘ultra-high-risk’ group. Identifying patients with invariably fatal outcome robustly at diagnosis would facilitate offering them newer therapeutic strategies at a much earlier stage, potentially sparing them toxicity from intensive therapies which will probably be

<table>
<thead>
<tr>
<th>INSS 1993</th>
<th>Definition</th>
<th>INRGSS 2009</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Localized tumour with complete gross excision, with or without microscopic residual disease; ipsilateral lymph nodes negative for tumour microscopically</td>
<td>L1</td>
<td>Localized tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment</td>
</tr>
<tr>
<td>2A</td>
<td>Localized tumour with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumor microscopically</td>
<td>L2</td>
<td>Locoregional tumour with the presence of one or more image-defined risk factors</td>
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<tr>
<td>2B</td>
<td>Localized tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. Enlarged contralateral lymph nodes must be negative microscopically</td>
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<tr>
<td>3</td>
<td>Unresectable unilateral tumour infiltrating across the midline, or localized unilateral tumour with contralateral regional lymph node involvement; or midline tumour with bilateral extension by infiltration or by lymph node involvement</td>
<td>M</td>
<td>Distant metastatic disease (except stage M5)</td>
</tr>
<tr>
<td>4</td>
<td>Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for stage 4S)</td>
<td>Ms</td>
<td>Metastatic disease in children younger than 18 months with metastases confined to skin, liver and/or bone marrow</td>
</tr>
<tr>
<td>4S</td>
<td>Localized primary tumour (as defined for stage 1, 2A or 2B), with dissemination limited to skin, liver and/or bone marrow (limited to infants &lt;1 year of age)</td>
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Check original references for full details. Note that the distinction between L1 and L2 relates to the presence of image-defined risk factors and does not necessarily correlate with Stages 2 and 3 from the INSS.
<table>
<thead>
<tr>
<th>INRG stage</th>
<th>Age (months)</th>
<th>Histologic category</th>
<th>Grade of tumour differentiation</th>
<th>MYCN</th>
<th>11q aberration</th>
<th>Ploidy</th>
<th>Pretreatment risk group</th>
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<tr>
<td>L1/L2</td>
<td></td>
<td>GN maturing; GNB intermixed</td>
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<td>NA</td>
<td></td>
<td></td>
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<tr>
<td>L1</td>
<td></td>
<td>Any, except GN maturing or GNB intermixed</td>
<td></td>
<td>NA</td>
<td>Amp</td>
<td>No</td>
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<tr>
<td>L2</td>
<td>&lt;18</td>
<td>Any, except GN maturing or GNB intermixed</td>
<td></td>
<td>Amp</td>
<td>No</td>
<td>Yes</td>
<td>K: high</td>
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<tr>
<td></td>
<td>≥18</td>
<td>GNB nodular; neuroblastoma</td>
<td>Differentiating</td>
<td>NA</td>
<td>No</td>
<td>Yes</td>
<td>D: low</td>
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<td></td>
<td></td>
<td>Poorly differentiating or undifferentiated</td>
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<td>NA</td>
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<td>G: intermediate</td>
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<td>Amp</td>
<td>No</td>
<td>Yes</td>
<td>P: high</td>
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unsuccessful, given the failure of current intensive multimodal treatments for these particular children.

Towards a full understanding of genomics and disease biology

The neuroblastoma community provides an excellent example of international collaboration and several collaborative groups have performed detailed characterization using different ‘omics’ platforms. This includes characterization by mRNA expression profiling, miRNAs, epigenetics, proteomics, metabolomics and importantly, whole genome sequencing. These unbiased approaches are providing very valuable insights into neuroblastoma biology, suggesting potential therapeutic targets and novel biomarkers. There are several key areas of recent progress in the field:

MYCN amplification is the most established genomic aberration in neuroblastoma, present in ~20% of patients and a major oncogenic driver. The role of MYCN expression at the mRNA and protein levels in MYCN amplified and particularly non-amplified neuroblastomas has not yet been elucidated. Recently, a MYCN 157-gene signature that could characterize patients with ‘MYCN-driven disease’ has been identified.\textsuperscript{29}

In 2008, mutations in the kinase domain of the anaplastic lymphoma kinase (\textit{ALK}) gene were described in most cases of hereditary and 10% of sporadic neuroblastomas.\textsuperscript{30} This led to intensive efforts clinically and preclinically in attempts to drive forward the rapid development of ALK inhibitors for children with neuroblastoma.

The ‘Therapeutically Applicable Research to Generate Effective Treatments’ (TARGET) initiative reported results of a combination of whole-exome, genome and transcriptome sequencing in a study of 240 cases to determine the spectrum of somatic mutations in high-risk neuroblastoma.\textsuperscript{31} In comparison with other cancers, particularly those in adults, the frequency of recurrent somatic mutations at presentation was low, and results of studies at relapse are awaited with great interest. The most frequent genomic aberrations found were ALK in 9.2%, \textit{PTPN11} in 2.9%, \textit{ATRX} in 2.5%, \textit{MYCN} in 1.7% and \textit{NRAS} in 0.83% of patients. Given this low frequency of recurrent mutations, it becomes crucial to understand the interactions at the mRNA, epigenetic and protein levels in order to identify suitable targets for potential therapeutics.

New drug development for neuroblastoma

Over the last decade there has been an exponential increase in groups dedicated to paediatric cancer drug development. This has led to greater knowledge of tumour biology and a rise in preclinical testing, therefore
making the selection of drugs taken forward for further development for paediatric cancers is much more rational.

The Innovative Therapies for Children with Cancer Consortium is a non-profit association created in 2003 gathering more than 40 European Paediatric Oncology centres with expertise in conducting early phase trials in children and adolescents and 9 European research laboratories, with the aim of developing novel therapies for the treatment of paediatric and adolescent cancers in cooperation with regulatory bodies, pharmaceutical enterprises, parents and patients.

Specific tasks include (i) linking research laboratories with academic drug developers to identify the most relevant targets and drugs for paediatric cancers, (ii) developing early clinical trials in collaboration with academics and industry, conducted in selected European sites to high-quality standards, (iii) partnering with pharmaceutical companies, regulatory bodies and patient–parent groups to increase collaboration and streamline efficiency of the drug development process and (iv) educating the paediatric oncology community in early drug development.

Currently, there are hundreds of molecularly targeted agents entering the clinic for adult cancers. Historically, the use of new drugs for childhood cancers has been preceded by their development in adult malignancies by some years. Only once late stage clinical studies had been completed in the adult setting would studies in children commence and usually only when the agent had secured a label for use in an adult cancer. This timeline meant that ‘new’ agents had often been in adult clinical development for many years before entering paediatric trials. For example, the paediatric clinical evaluation of carboplatin in the 1980s in the UK only commenced after the drug had been commercially launched. Additionally, drugs have been used off-label in children based on extrapolation from adult use, with robust data in children not being collected.

According to the European Paediatric Regulation (2006), devised to improve access to novel drugs and information about these drugs for paediatric use, pharmaceutical firms developing drugs of potential interest for childhood illnesses are obliged to develop and comply with agreed Paediatric Investigational Plans (PIPs) in order to gain a marketing authorization even for use in adults, unless granted a waiver by the Paediatric Committee of the European Medicines Agency. In order to incentivise paediatric studies, successfully completed PIPs are rewarded with a 6-month patent extension, which is financially beneficial for the companies. Over the last 2–3 years, there has, therefore, been a significant increase in the number of early clinical trials driven by the Paediatric Regulation, as part of PIPs. However, these will take time to mature, and the deferrals granted for many will prolong this process.

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The paradigm now is that agents targeting known molecular aberrations relevant to paediatric cancers should undergo full preclinical development, including rigorous testing in in vitro and in vivo models, at a very early stage in their development. These drugs should then enter paediatric clinical trials immediately after adult phase I development has been completed when adult safety data and dosing information is available to help inform the paediatric phase I study. It has become clear that drugs should be explored in children whenever their mechanism of action is relevant for paediatric cancers, not necessarily for the disease for which they were initially developed in adults.6

Importantly, the preclinical data package required to effectively drive forward the clinical development of a given drug has expanded to include a number of key evaluations, not limited to in vitro or in vivo anti-tumour activity,33–35 and for paediatric tumours there are specific considerations. First, the target has to be identified in tumour samples. Secondly the target must be validated using pharmacologic (inhibitors) and non-pharmacologic approaches (such as siRNA). Thirdly, activity and efficacy data should include a wide representative range of preclinical models beyond conventional in vitro cytotoxicity assays and in vivo subcutaneous xenografts. New models such as patient-derived xenografts or genetically engineered murine models should be incorporated wherever possible. Since these models better recapitulate tumour biology, it is envisaged that they will be more predictive of anti-tumour activity in the clinical setting. Fourthly, it is crucial to develop both pharmacodynamic (PD) and predictive biomarkers so that they can be implemented in early clinical trials. PD biomarkers are able to show that the drug is modulating the target adequately and exerting the desired downstream effects. They may be particularly useful to help inform ‘go-no go’ decisions regarding future development of the agents at the end of phase I trials. Predictive biomarkers select those patients most likely to benefit from a given targeted drug based on the molecular characteristics of their tumour. Fifthly, potential toxicities in growth and development should be considered and perhaps, studies in juvenile animals should be conducted. This is particularly true where the drugs may be used in very young children, which is the case in neuroblastoma, with the majority of children being diagnosed in the first 5 years of life, and many under the age of 2 years.

The drug development process should therefore advance from the bench to the bedside and back again, so that the reasons behind both efficacy and lack of activity of new agents can be adequately understood.

A good example is the development of ALK inhibitors for ALK-driven neuroblastomas. Shortly after ALK mutations were found in neuroblastoma, the Children’s Oncology Group (COG) in the USA launched a paediatric phase I trial of crizotinib, an ALK/MET inhibitor. This study was a major success: thanks to the COG’s intensive efforts, the paediatric
study was initiated only 1 year after the adult phase I study in NSCLC with ALK rearrangements, one of the primary adult conditions for which the drug was being developed. Crizotinib was shown to be very efficacious in paediatric diseases harbouring ALK translocations, such as anaplastic large cell lymphoma, where 61% of these patients responded and 100% of them achieved clinical benefit (disease stabilization or better). However, despite achieving doses doubling the equivalent adult recommended phase II dose, responses in ALK-mutated neuroblastomas were not optimal, with only 1 out of 11 patients responding.\textsuperscript{36,37}

It seems, therefore, that sensitivity to ALK inhibitors is different for ALK-translocated and ALK-mutated tumours. The F1174L ALK mutation, commonly present in ALK-mutated neuroblastomas confers resistance to crizotinib.\textsuperscript{38} A paediatric phase I study of the novel and more potent second generation ALK inhibitor LDK378, which is able to overcome resistance to crizotinib in patients with ALK-translocated NSCLC, has recently opened. Additionally, Berry \textit{et al}.\textsuperscript{39} have suggested that the addition of an mTOR complex inhibitor could overcome resistance to single agent crizotinib in preclinical transgenic models. This is driving academic efforts to translate this preclinical hypothesis into phase I/II clinical trials of ALK inhibitors in combination studies.

**Early clinical trials**

The number of agents being tested in paediatric phase I trials is undoubtedly increasing with the most promising agents now transitioning to phase II studies. Numerous agents have been tested in the last 5 years and recently reported; further results of later studies are eagerly awaited.

Moreover, phase I and II studies in children are increasingly being designed to be more efficient. An analysis of paediatric dose finding phase I trials for molecularly targeted anti-cancer drugs showed how toxicities found in children are virtually always the same as those already known in adults and that the paediatric recommended phase II dose (RP2D) ranges from 80 to 120\% of the equivalent adult dose in most cases.\textsuperscript{40} This proves that paediatric phase I trials are necessary to identify the correct dose for children, but that they should be conducted efficiently, avoiding excessive numbers of dose levels or designs that remain closed most of the time.

In neuroblastoma, the number of druggable targets is constantly increasing while the number of patients with similar molecular characteristics is becoming smaller due to the identification of new molecular sub-types according to molecular profile. As a consequence, new strategies of clinical trials are required because small groups of patients on different personalized therapies cannot be subject to conventional randomized clinical trials.
The increased use of novel adaptive or Bayesian designs will be helpful in addressing these issues, and are already becoming the favoured approach for paediatric early phase studies, with appositive effect on time taken to complete informative studies.  

To date, only one randomized clinical trial has been conducted for neuroblastoma in the relapse setting and demonstrated the superiority of topotecan-cyclophosphamide to topotecan alone. This served to bring this regimen forward to the frontline setting in COG studies. Currently, more than 10 different second-line regimens have been reported for relapsed neuroblastoma in separate single-arm studies with heterogeneous populations and response criteria, and it is thus difficult to establish the best relapse regimen to take forward in future studies is. The BEACON-Neuroblastoma phase II trial is now open across Europe and will test in a randomized fashion which of the two most frequently used regimens (temozolomide or temozolomide-irinotecan) is superior in the relapsed/refractory disease setting, and should be taken forward as the backbone regimen of choice in future studies [EudraCT 2012-000072-42].

**Advances in the frontline setting**

Cooperative trials conducted on both sides of the Atlantic for >30 years have established frontline therapy, which has become increasingly intensive and now incorporates multiple modalities. First-line therapy for high-risk neuroblastoma is, by necessity, one of the most intensive, toxic and prolonged schedules administered for paediatric solid tumours. Three randomized trials have shown unequivocally that consolidation therapy with MAT with AHSCR is superior to continuation chemotherapy alone. Only one targeted therapy has been introduced into frontline treatment of neuroblastoma over the last 15 years: immunotherapy with anti-GD2 monoclonal antibody, plus cytokines. In a seminal study, Yu et al. showed in a randomized trial how the addition of 14.18 anti-GD2 monoclonal antibody with IL2 and GM-CSF during the last phase of differentiating therapy with retinoic acid increased survival compared with 13-cis-retinoic acid alone. A German study has shown how late relapses can occur after anti-GD2 immunotherapy. The significant effect demonstrated on 2-year event-free survival will need to be confirmed when longer follow-up data are available to show whether the effect on overall survival is maintained beyond 5 and 10 years.

Other major advances as a result of randomized studies in frontline therapy have also been reported. The European HRNBL1 study compared two conditioning regimens for MAT with AHSCR and showed the clear superiority of the busulfan-melphalan conditioning regimen versus the carboplatin-etoposide-melphalan regimen, with a 12% benefit in
3-year overall survival.\textsuperscript{47} Again, long-term follow-up of this study has not yet been reported and will be important.

The SIOPEN INES studies and others by COG and GPOH have been pivotal in defining specific therapies for four sub-groups of infants with the following:

- Localized and unresectable neuroblastoma without MYCN amplification. These achieved a 5-year OS of 99\% after receiving a short course of chemotherapy with vincristine and cyclophosphamide.\textsuperscript{48} The German group GPOH showed how for infants with localised non-amplified neuroblastoma without life-threatening symptoms, approximately half of neuroblastomas showed spontaneous regression without any treatment.\textsuperscript{49}

- Stage M or stage MS without radiologically detected metastases to the skeleton, lung or CNS and without MYCN amplification. These achieved a 5-year OS of 97.6\% receiving chemotherapy only in the setting of life-threatening symptoms.\textsuperscript{50}

- Stage M tumours with radiologically detected metastases to skeleton, lung or CNS and without MYCN amplification. These had a 5-year OS of 95\% with four courses of chemotherapy\textsuperscript{50}

- MYCN amplified tumours had a very poor prognosis even in infants receiving four courses of chemotherapy, surgery and myeloablation and now receive high-risk therapy\textsuperscript{51}

\textbf{Reducing the burden of late effects}

Very importantly, as improvements in frontline therapy for high-risk neuroblastoma occur, survivors must be followed up to identify and treat long-term toxicities in order to achieve a good quality of life long-term.

Because of the intensity of neuroblastoma therapy, survivors show increased incidence of a significant list of long-term complications: (1) high-frequency hearing loss related to the use of platinum compounds and ototoxic antibiotics required for neutropaenic infections, possibly leading to learning difficulties and speech problems. (2) Renal toxicity related to the use of platinum agents, MAT with AHSCR, radiotherapy to the retroperitoneal area, nephrectomy or use of nephrotoxic antibiotics. Although decreases in glomerular filtration rate have been described, end-stage renal failure or need for renal transplantation has rarely been reported.\textsuperscript{52–54} (3) Second malignancies related to alkylating agents or radiotherapy. An increased frequency of leukaemias and myelodysplastic syndromes was related to high doses of etoposide and cyclophosphamide and a reduction of these was shown when the number of cycles was reduced from 7 to 5.\textsuperscript{55} Other studies have reported thyroid cancers and other solid tumours.\textsuperscript{52,54} (4) Endocrine complications have also been reported, such as hypothyroidism, growth impairment and reduced fertility.\textsuperscript{52,54,56}
Conclusions

In summary, major advances are currently being made in the field of neuroblastoma in four major areas. First, international expert collaboration is facilitating clinical and biological studies, improved risk stratification and better therapies tested in randomized clinical trials. Secondly, the biological and genomic understanding of neuroblastoma is increasing exponentially thanks to the incorporation of new technologies in the analysis of tumour samples (DNA, RNA and epigenetic levels). Thirdly, drug development is moving forward at the preclinical and clinical levels, and over the next 5 years many molecularly targeted drugs holding significant promise will be tested. Finally, academic clinical investigators, pharmaceutical companies, regulatory bodies and parent–patient representatives are increasingly working in partnership to drive forward more efficient drug development for children with cancer.

The best way to move new drugs and new strategies into frontline remains controversial. Treatment for neuroblastoma is currently very intensive but also not efficacious in a large number of cases. It is unclear how new molecularly targeted drugs and immunotherapeutic approaches should be integrated together in the existing multimodal treatment that already includes chemotherapy, surgery autologous stem cell transplant, radiotherapy, differentiation therapy and anti-GD2 immunotherapy.

Over the next decade we will see advances in the incorporation of genomic characterization into routine clinical practice and this will help moving towards a precision medicine approach where patients receive tailored therapies which are effective and less toxic in the long term.

Neuroblastoma will also serve as an excellent paradigm for similar strategies to be followed in other poor prognosis paediatric malignancies.

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