Профессор Торстен Симон закончил Медицинскую школу Университета Лейпцига (Германия) в 1990 г. и проходил ординатуру по педиатрии в Университете г. Эрфурт. В 2001 г. профессор Симон завершил переподготовку в области детской онкологии в Кельнском университете и приступил к работе в отделении детской онкологии и гематологии Университетской детской больницы, которое он возглавил в январе 2014 г.

Торстен Симон – активный участник профессиональных сообществ: SIOP, GPOH, ANRA и др., а также руководитель научных исследовательских групп по вопросам диагностики и лечения солидных опухолей, в частности нейробластомы. Научный и клинический интерес профессора Симона – оптимизация протоколов для ведения пациентов с нейробластомой и герминогенно-клеточными опухолями.

Individualized therapy in neuroblastoma*

T. Simon[1], M. Fischer[2], B. Hero[1]

[1]Department for Pediatric Oncology and Hematology, Children's Hospital, University of Cologne, Cologne, Germany;
[2]Department for Experimental Pediatric Oncology, Children's Hospital, University of Cologne, Cologne, Germany

Contact details: *Thorsten Simon thorsten.simon@uk-koeln.de*

Neuroblastoma, a malignant embryonal tumor of early childhood, has the unique feature of regression after mild or even no chemotherapy in low risk patients. In contrast, most high-risk patients die of disease despite intensive multimodality treatments. It is, therefore, an ideal model tumor for establishing individualized therapies. For many years, neuroblastoma patients have undergone risk adapted treatment according to clinical and molecular characteristics of the patient and the tumor, respectively, at the time of diagnosis. Recently, other approaches such as treatment modifications based on response to treatment as well as targeted molecular therapies directed against distinct abnormal pathways are becoming increasingly important. Every approach must rely on prospectively evaluated treatment strategies.

Key words: *children, neuroblastoma, treatment individualization*

Introduction

Neuroblastoma is one of the most common malignant diseases of early childhood. Depending on the individual risk profile of the patient the disease can either regress with minimal or even no treatment or can lead to death despite intensive multimodality high-risk treatment. Therefore neuroblastoma has been a model for risk adapted therapy for many years. The combination of multiple clinical and molecular variables increasingly allows adapting the treatment according to the individual needs of the patient. Individualized treatment aims to avoid inefficient treatments in refractory disease, to identify patients who benefit from alternative experimental treatments, and to de-escalate treatment in low risk patients. General strategies for treatment individualization in cancer treatment are (1) risk adapted treatment by precise pre-treatment risk prediction based on clinical and/or molecular characteristics, (2) structured treatment modifications based on the response to treatment, (3) and molecular targeted therapies based on individual molecular characteristic of the tumor (Table 1). It is important to note, that individualized treatment must follow structured guidelines which are either subject to prospective clinical trials or have already been evaluated by prospective clinical trials. Individualized therapy solely based on individual believes of physicians are very likely to impair the outcome of patients.

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Table1. *General approaches for individualization of neuroblastoma treatment*

Stratified treatment by precise initial risk groups assignment
Stratified treatment by molecular prediction of the individual risk
Treatment adaption by individual treatment response
Targeted treatment on individual molecular aberrations

Treatment individualization by precise risk prediction at diagnosis

Neuroblastoma risk groups

During the last decades clinical and molecular risk factors have been identified in order to describe the individual risk of relapse and, as a consequence, the individual need of treatment of each patient. Commonly accepted risk factors are stage of disease, age at diagnosis, and amplification of the proto-oncogene *MYCN* [1, 2]. Some clinical trials have also applied the copy number status of chromosome 1p [3-5] and Shimada histology classification [6-9] as additional criteria for the identification of high-risk patients. Other clinical and molecular factors such as genomic loss of chromosome 11q [10, 11], gain of chromosome 17q [12, 13], expression of Trk receptors [14-16], status of Akt phosphorylation [17], RNA expression profile [18-21], and genomic analyses [22, 23] also distinguish between patients with good and poor prognosis but have not been published as stratifying markers in prospective clinical trials, so far.

Recently, the International Neuroblastoma Risk Group (INRG) collected clinical and selected molecular data of more than 8,800 neuroblastoma patients which was the foundation for extended analysis of risk factors in neuroblastoma and the development of the International Neuroblastoma Risk Group (INRG) classification system (INRGSS). Different from the post-surgery INSS system [24], it distinguishes between localized L1 tumors without image defined risk factors, localized L2 tumors that have image defined risk factors making initial complete resection unlikely [25], stage M metastatic disease, and metastatic disease restricted to skin, liver, and bone marrow in children 18 months or younger at diagnosis referred as stage MS [25]. Considering the variables INRG stage, histology, grade of tumor differentiation, *MYCN* status, 11q status, and ploidy, finally 16 different patient subgroups were defined and assigned to one of the risk categories very low, low, intermediate, and high risk. [1]. The INRGSS high-risk category includes all patients with metastatic neuroblastoma ≥ 18 month at diagnosis (5 year event free survival [EFS] 23% ± 1%, 5 year overall survival [OS] 31% ± 1%), all patients with localized and MS neuroblastoma with *MYCN* amplification (5 year EFS 46% ± 4%, 5 year overall survival 53% ± 4%), and stage M patients < 18 months with *MYCN* amplification (5 year EFS 26% ± 4%, 5 year OS 29% ± 4%). Also, stage MS patients < 18 months with loss of 11q were included in the high-risk category, however, the risk classification of this very small subgroup is still a matter of debate. The INRGSS intermediate risk group included L2 patients <18 month with aberrations of chromosome 11q excluding ganglioneuroma and ganglioneuroblastoma intermixed, L2 tumors ≥ 18 months either with differentiating neuroblastoma with aberrations of chromosome 11q or poorly and undifferentiated neuroblastoma regardless of the status of chromosome 11q, and stage M neuroblastoma < 18 months with diploidy. The INRGSS low risk group includes all patients with L2 tumors < 18 months excluding maturing ganglioneuroma and ganglioneuroblastoma intermixed with normal chromosome 11q, L2 patients Times New Roman 18 months with differentiating nodular ganglioneuroblastoma or neuroblastoma with normal status of chromosome 11q, , and stage M < 18 month with hyperdiploid tumors. The INRGSS very low category includes all L1 and L2 patients with maturing ganglioneuroblastoma and ganglioneuroblastoma intermixed, all L1 patients with neuroblastoma other than maturing ganglioneuroblastoma and ganglioneuroblastoma intermixed, and all stage MS patients <18 months without aberrations of chromosome 11q.

Molecular risk prediction

The majority of low and intermediate risk patients will achieve stable first remission with limited treatment [26-28]. Moreover, most of relapses or progressions are cured with moderate second-line treatment. However, some patients experience sequential relapses or progressions and finally succumb of disease. It is challenge to identify those risk patients early at initial diagnosis in order to apply the adequate first-line therapy. Retrospective studies indicate that molecular analyses of copy number aberrations by array CGH [29] and RNA expression analysis by microarray [18, 30] may allow the identification of these risk patients: In infants, the presence of segmental chromosomal aberrations in the tumor was associated with inferior outcome (5 year EFS 70.7% ± 6.6%) compared to children whose tumors lacked segmental chromosomal aberrations (5-year EFS 92.0% ± 2.1%). This impact of segmental chromosomal aberrations was even stronger in the subgroup of stage 4S patients who had not undergone frontline chemotherapy (5 year EFS 25.0% ± 15.3% vs. 95.3% ± 3.2%) [29]. Another approach is based on a gene expression signature which has been developed by the analysis of the RNA expression profiles in neuroblastomas obtained from patients with favorable outcome and unfavorable outcome. The gene expression classifier identified risk patients better than established risk groups definitions: Among patients classified as low risk by stage, age, and *MYCN*, the classifier identified a small group of risk patients with poor outcome (5 year

EFS 29% ± 10% vs. , 84% ± 2%, 5 year OS 76% ± 11% vs. 99% ± 1%). Similar results were found among patients classified as intermediate risk (5 year EFS 41% ± 10% vs. 88% ± 6%, 5 year OS 70% ± 9% vs. 100%) and high risk (5 year EFS 33% ± 3% vs. 63% ± 9%, 5 year OS 46±4% vs. 83±7%) [30]. Prospective clinical trials are open or will be open soon in order to assess whether the precise identification of risk patients based on molecular findings will improve the outcome. In these trials, treatment may be either intensified for patients with unfavorable risk profile or reduced for patients with favorable characteristics.

Treatment individualization based on response to treatment

Low-and intermediate-risk neuroblastoma

Spontaneous regression of infant neuroblastoma is a well described phenomenon [27, 31-34]. Over-diagnosis of localized neuroblastoma found by screening programs also indicates a substantial number of spontaneous regressions during and after the 1st year of life [35]. In low risk neuroblastoma patients, individualization of treatment first of all aims to de-escalate chemotherapy and to limit operations in patients with favorable risk profile. It also aims to identify those few patients in the low risk group who are at risk for tumor progression finally leading to death despite multiple sequential treatments. The German NB95-S trial demonstrated a high rate of spontaneous regressions in patients <1 year of age at diagnosis with localized neuroblastoma. Some infants required chemotherapy in order to overcome symptomatic transient tumor progression [27]. It has been demonstrated in the trial that limited intensity chemotherapy can induce regression in low risk neuroblastoma. This approach has been further developed and is currently being tested in the NB2004 trial including an extended cohort of low risk neuroblastoma patients (stage 1 neuroblastoma patients aged 0-21 years diagnosis, stage 2 neuroblastoma aged 0-21 years at diagnosis without aberration of chromosome 1p, stage 3 neuroblastoma patients 0-2 years of age without aberrations of chromosome 1p, and stage 4S patients 0-1 year of age at diagnosis). Patients with tumor associated threatening symptoms or rapid progression, who are otherwise classified as observation patients, are scheduled for chemotherapy with vincristine, cyclophosphamide and doxorubicin. According to the protocol, the chemotherapy is stopped as soon as the tumor induced symptoms are relieved and/or tumor progression is stopped. Results of the trial NB2004 are not published yet, but interim analysis demonstrated that the burden of chemotherapy was reduced without increase of event and death rate.

High-risk neuroblastoma

The situation is very different in high-risk neuroblastoma. With high-intensive multimodality treatments, about 50% of patients survive at least 5 years after diagnosis [36]. Long term survivors of high-risk neuroblastoma face several late effects such as hearing impairment [37-40], hypothyroidism [40, 41], focal nodular hyperplasia of the liver [42], pulmonary and cardiac diseases [43], and second malignant disease [44-46]. Early response assessment aims to detect patients in whom conventional induction chemotherapy will most likely fail, and who therefore may require new experimental treatment strategies. Several approaches have been applied in order to identify these ultra-high risk patients: Studies on the prognostic value of residual bone marrow involvement using different techniques such as immuno-cytology and PCR analysis resulted in controversial results [47-49]. Prospective trials on the prognostic value of bone marrow clearance using standardized techniques are still ongoing, but final results are not available yet. Delayed clearance of MIBG positive lesions during induction chemotherapy was found predictive for poor outcome in retrospective analyses [50, 51]. The prognostic value of residual MIBG positive lesions during and after induction therapy has been further improved by the application of semi-quantitative scoring systems such as the Curie and the SIOPEN system [51-53]. In addition, response of the primary tumor was also found to be prognostic value on outcome in patients with stage 4 neuroblastoma as well as in patients with *MYCN* amplified neuroblastoma regardless of stage. However, only the reduction of the longest tumor diameter >30% but not the reduction of the tumor volume during induction chemotherapy was associated with better EFS and OS [54].

Treatment individualization by molecular targeted therapies

Another promising innovative treatment approach is currently the application of designed compounds which interact with the target of the tumor cells. Serious systemic off-target side effects may be attenuated or even avoided when the molecular target is not expressed or not mutated in normal cells. Examples of several molecular targets in neuroblastoma are found in Table 2. The ganglioside GD2 is the target for GD2-directed immunotherapy [55-58]. It is expressed by most of primary neuroblastoma. Therefore, anti GD2 directed drugs are targeted but not strictly individualized therapies. Angiogenesis is also up-regulated in all high-risk neuroblastoma patients. Pre-clinical data on the effects of anti-angiogenetic treatment are conflicting [59, 60] while phase I clinical trials have given encouraging results [61, 62]. The norepinephrine transporter system is the molecular basis for MIBG therapy [63-66]. High-dose MIBG therapy is commonly applied if MIBG positive lesions are present after induction chemotherapy and is, therefore, an example for response adapted individualized therapy particularly when applied during first-line therapy [65]. In addition, MIBG therapy has also been applied as frontline-therapy [66, 67] and in relapsed MIBG positive neuroblastoma [64, 68, 69]. Furthermore, the RAS/MAPK

Table 2. *Examples for molecular targets in neuroblastoma (NB, neuroblastoma)*

Target	Agent	Target status in neuroblastoma	Preclinical data	Clinical trials including neuroblastoma patients
Ganglioside GD2	Anti-GD2 antibodies	Expressed in >90% of NB patients	[93]	[55-58]
Vascular endothelial growth factor (VEGF)	Bevacizumab	Expression associated with tumor stage	[94, 95]	[61, 62]
norepinephrine transporter (NET)	MIBG	Expressed in 90% of NB patients	[63]	[64-66]
MEK	Binimetinib, Selumetinib	Active in all NB	[70, 72]	Not available yet
mTOR	Everolimus, Temsirolimus	Active in all NB	[73, 74]	[78, 79]
PI3 kinase	SF1101/LY294002	Overexpressed in 50% of NB patients	[75-77]	Not available yet
ALK	crizotinib, ceritinib, alectinib	Mutated or amplified in 20% of NB patients	[83]	[82, 84]
Aurora A kinase	alisertib	Amplified in 20% of NB patients	[90]	[91, 92]

pathway seems to play an important role in malignant growth particularly in relapsed neuroblastoma [17, 70, 71]. Treatment with selected inhibitors of downstream targets of the pathway is considered a promising approach of targeted treatment [70, 72-77]. However, clinical data on these treatments particularly in combination with chemotherapies with are very limited, so far [78, 79].

The mutation frequency in neuroblastoma is low, and the spectrum of mutations is heterogeneous, resulting in a limited number of potential targets for individualized molecular therapies [80]. More recent studies suggest that the molecular hallmark of high-risk neuroblastoma is activation of telomere maintenance mechanisms, separating it from tumors that will eventually regress [81]. Telomere maintenance may be mediated by either telomerase induction, caused by rearrangements of the telomerase reverse transcriptase encoding gene *TERT* or by *MYCN* amplification, or by activation of the alternative lengthening of telomeres pathway, facilitated by inactivating *ATRX* mutations. In general, targeting aberrant molecular structures is considered as an ideal example of individualized treatment strategies, because such therapies are based on individual molecular characteristics of the tumor. Most of the molecular aberrations observed in neuroblastoma are not specific for this malignancy, such as induction of telomerase, which is present in the vast majority of cancers, or activating *ALK* mutations, which may also occur in lung cancer or lymphomas. Furthermore, most of the molecular targeted treatments are only effective if the aberrant target or target pathway is present in the tumor tissue of the individual patients. Tumor biopsy and tumor tissue banking are, therefore, are mandatory at first diagnosis and at relapse.

One of the most promising targets in neuroblastoma are activating mutations of the tyrosine receptor kinase ALK, which are found in about 10% of neuroblastoma patients [82, 83]. One phase I trial on the ALK inhibitor crizotinib in refractory pediatric solid tumors demonstrated acceptable toxicity and promising response. Among 11 neuroblastoma patients with known *ALK* mutations one complete remission and two stable diseases were observed [82, 84]. New ALK inhibitors, such as ceritinib and alectinib, are expected to be more effective on different types of *ALK* mutations [85-87]. Phase 3 trials on primary neuroblastoma with *ALK* mutations are currently in preparation and will assess the value of ALK inhibition as an element in high-intensive multimodality treatment protocols.

Amplification of the oncogene MYCN is found in about 20% of neuroblastoma. Effective inhibition of MYCN by BET bromodomain inhibitors has been demonstrated *in vitro*, but the compounds are not available for clinical trials, so far [88]. In an alternative approach, the MYCN pathway has been identified as a potential target for molecular therapies via inhibition of Aurora kinase A. Aurora kinase A plays a crucial role in stabilization and degradation of MYCN and, therefore, is considered as potential target [89, 90]. The Aurora kinase A inhibitor alisertib has recently successfully been tested as single agent [91] as well as in combination with irinotecan and temozolomide [92].

Summary

Neuroblastoma is an ideal model tumor for establishing individualized therapies because of its divergent courses of the disease. Individualization of cancer therapies can either focus on precise initial risk prediction of the patients at the time of diagnosis as prerequisite for risk adapted treatment, on

structured treatment modifications based on the response to treatment, on molecular targeted therapies for patients with distinct molecular aberrations, or combinations of all three strategies. With growing knowledge on the molecular and immunological mechanisms of neuroblastoma, more and more adaptations of treatment meeting the individual needs of the patient are developed. In the near future, patient derived xenograft systems may allow in vivo testing of several compounds in parallel to the treatment of the patient. However, any strategy on treatment individualization has to be evaluated in controlled prospective clinical trials of neuroblastoma patients. Since the number of patients is small, such clinical trials require international cooperation.

Conflict of interest

The authors declare no conflict of interest.

Индивидуализированная терапия нейробластомы*

Т. Симон[1], М. Фишер[2], Б. Херо[1]

[1]Отделение детской онкологии и гематологии, Детская больница, Кельнский университет, Кельн, Германия;
[2]Отделение экспериментальной детской онкологии, Кельнский университет, Кельн, Германия

Контактные данные: *Торстен Симон thorsten.simon@uk-koeln.de*
Авторы перевода: *К.И. Киргизов, Т.В. Шаманская*

Нейробластома (НБ) – злокачественная эмбриональная опухоль детского возраста, характеризующаяся возможностью развития спонтанной регрессии у пациентов группы низкого риска или регрессии опухоли после проведения низкодозовой полихимиотерапии. В отличие от больных указанной группы большинство пациентов группы высокого риска имеют крайне неблагоприятный прогноз, несмотря на проведение интенсивной мультимодальной терапии. Поэтому НБ является идеальной моделью для внедрения индивидуализированных терапевтических подходов. В течение многих лет пациентам с НБ проводилось риск-адаптированное лечение в соответствии с клиническими характеристиками заболевания и молекулярными особенностями опухоли на момент постановки диагноза.

В последнее время все большее значение приобретает внедрение подходов терапии, основанных на модификации лечения при проведении оценки ответа на проводимую терапию, а также использование таргетной молекулярной терапии, направленной против определенных молекулярно-генетических аномалий. Однако каждый терапевтический подход должен основываться на проспективных клинических исследованиях.

Ключевые слова: *дети, онкология, нейробластома, индивидуализация лечения*

Введение

Нейробластома (НБ) является одной из наиболее частых злокачественных опухолей у детей раннего возраста. В зависимости от индивидуальных характеристик опухоли НБ может либо регрессировать после минимальной полихимиотерапии или даже без лечения, либо приводить к смерти, несмотря на проведение комбинированной высокоинтенсивной терапии. Поэтому НБ на протяжении многих лет рассматривалась как модель для риск-адаптированной терапии. Комбинация определенных клинических и молекулярных данных позволяет адаптировать терапию в соответствии с индивидуальными потребностями пациента. Индивидуализированное лечение направлено на то, чтобы избежать неэффективных методов при рефрактерном течении заболевания, выделяя группу пациентов для проведения альтернативных экспериментальных методов лечения, а также для деэскалации терапии для пациентов группы низкого риска. Основная стратегия индивидуализированной терапии злокачественных новообразований основана на риск-адаптированном лечении [1] путем определения факторов риска до начала терапии, основанных на клинических и/или молекулярных характеристиках опухоли [2], модификации терапии в зависимости от ответа на лечение [3] и молекулярной таргетной терапии, основанной на индивидуальных молекулярных характеристиках опухоли (табл. 1). Важно отметить, что индивидуализация терапия должна проводиться на основе четко структуированных рекомендаций, которые являются или предметом изучения в простпективных клинических иссле-

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