Universidade de Lisboa

Faculdade de Farmácia

Neuroblastoma

Approaches for the Optimisation of the Dose Rationale for BGA002 and Clinical Trial Design in Rare Paediatric Diseases

Constança Matias de Oliveira

Mestrado Integrado em Ciências Farmacêuticas

2020

Universidade de Lisboa

Faculdade de Farmácia

Neuroblastoma

Approaches for the Optimisation of the Dose Rationale for BGA002 and Clinical Trial Design in Rare Paediatric Diseases

Constança Matias de Oliveira

Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

Orientador: Professor Associado com Agregação, Bruno Miguel Nogueira Sepodes

2020

Final Report

Erasmus programme

University College London, School of Pharmacy

Department of Clinical Pharmacology and Therapeutics

Neuroblastoma

Approaches for the Optimisation of the Dose Rationale for BGA002 and Clinical Trial Design in Rare Paediatric Diseases

Constança Matias de Oliveira

Supervisors:

Professor Oscar Della Pasqua

Sean Oosterholt

2020

The work presented in this thesis was developed in the Erasmus+ Programme, in the Clinical Pharmacology and Therapeutics Department of University College London, School of Pharmacy, under the supervision of Mr. Sean Oosterholt and Professor Oscar Della Pasqua

Resumo

O neuroblastoma é o tumor extracraniano mais comum na população pediátrica. Para além de ser um cancro que afeta a população pediátrica de forma quase exclusiva, o neuroblastoma é considerado uma doença rara, visto que afeta menos de 5 em 10 000 pessoas na União Europeia (UE). Estas características especiais tornam o desenvolvimento de fármacos para o tratamento do neuroblastoma um desafio. Esta patologia é classificada em quatro categorias de risco distintas, tendo em conta vários fatores, nomeadamente a histologia tumoral e características genéticas. Devido às reduzidas taxas de sobrevivência dos doentes de alto risco, esta categoria continua a ser uma das mais desafiantes, mesmo após terapêutica com fármacos citotóxicos.

O fármaco BGA002 está atualmente a ser desenvolvido para o tratamento do neuroblastoma de elevado risco, sendo constituído por péptidos e ácidos nucleicos. Cerca de metade dos casos de neuroblastoma de elevado risco apresenta uma amplificação do gene *MYCN*. Este gene é essencial para a produção da proteína N-Myc a qual, por sua vez, está envolvida no crescimento e progressão do neuroblastoma. Deste modo, o fármaco BGA002 foi desenvolvido com o intuito de inibir seletivamente o gene *MYCN*, levando assim a uma menor produção da proteína N-Myc. Consequentemente, ocorre um aumento da apoptose das células do neuroblastoma.

O objetivo deste estudo é determinar uma dose segura e eficaz do fármaco BGA002 num ensaio clínico pediátrico. Pretende-se ainda elaborar um protocolo do ensaio clínico, o qual suporta o planeamento do ensaio de fase I. O uso de uma escala alométrica permite fazer uma estimativa da farmacocinética em humanos, baseando-se apenas em dados pré-clínicos. Além disso, a implementação de uma estratégia baseada em modelos e simulações é extremamente útil no sentido de determinar dados adicionais de farmacocinética e farmacodinâmica em humanos. A utilização de um modelo matemático em ensaios clínicos proporciona inúmeras vantagens, tais como a investigação de várias doses, e diferentes durações de tratamento e desenhos do estudo, antes de se iniciar o próprio ensaio clínico em crianças. Um ensaio clínico pediátrico de fase I deve ser planeado de forma a garantir não só a maior segurança possível, mas também a possibilidade de os doentes receberem um tratamento farmacologicamente ativo.

Palavras-chave: neuroblastoma, escala alométrica, modelos e simulações, ensaios clínicos

Abstract

Neuroblastoma is the most common extracranial tumour in children. Besides being a cancer that almost exclusively affects children, neuroblastoma is considered a rare disease, since it affects less than 5 in 10,000 people in the European Union (EU). These unique characteristics make the development of drugs for the treatment of neuroblastoma a challenging task. This disease is classified into four risk categories, according to several factors such as tumour histology and genetic features. High-risk neuroblastoma patients remain one of the most challenging groups, with a low survival rate, despite treatment with chemotherapy drugs.

BGA002 is a peptide nucleic acid drug that is being developed for the treatment of paediatric high-risk neuroblastoma. In about half of high-risk neuroblastoma cases, *MYCN* amplification is present. The *MYCN* gene is essential for the production of the protein N-Myc, which is involved in the growth and progression of neuroblastoma. Therefore, BGA002 is a drug that was developed to selectively inhibit the *MYCN* gene, leading to a lower production of the protein and consequently leading to a higher death rate of neuroblastoma cells.

The aim of this study is to determine a safe and effective dose of BGA002 for a first-in-children clinical trial, and to elaborate a clinical study protocol that supports the design of the phase I trial. An allometric scaling approach enables the prediction of human pharmacokinetics based only on preclinical data. Moreover, modelling and simulations (M&S) are extremely valuable to further predict human data and provide complementary pharmacokinetics and pharmacodynamics information. A modelling approach is particularly useful in drug development because it allows the exploration of numerous doses and trial designs, before moving on to the actual clinical study in children. A phase I paediatric clinical trial should be developed in order to be as safe as possible, while giving the child a potentially active treatment.

Keywords: neuroblastoma, allometric scaling, modelling and simulations (M&S), clinical trials

Table of Contents

Table of Figures

List of Tables

List of abbreviations

ASCT	Autologous Stem Cell Transplant
BSA	Body Surface Area
CgA	Chromogranin A
COMT	Catechol-O-methyltransferase
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
EMT	Epithelial-Mesenchymal Transition
EU	European Union
FDA	Food and Drug Administration
HED	Human Equivalent Dose
HVA	Homovanillic Acid
IDRF	Image-Defined Risk Factors
IL-2	Interleukin-2
INRG	International Neuroblastoma Risk Group
INSS	International Neuroblastoma Staging System
LDH	Lactate Dehydrogenase
M&S	Modelling and Simulations
MABEL	Minimum Anticipated Biologic Effect Level
MAO	Monoamine Oxidase
mIBG	Meta-iodobenzylguanidine
miRNA	microRNA
MRSD	Maximum Recommended Starting Dose
MYCN	Neuroblastoma derived (avian) v-myc myelocytomatosis viral related oncogene
N-Myc	Protein encoded by the *MYCN* gene
NOAEL	No-observed-adverse-effect level
NONMem	NONlinear Mixed Effects Modelling
NSE	Neuron-Specific Enolase
PD	Pharmacodynamics
PK	Pharmacokinetics
PNA	Peptide Nucleic Acids
SIOPEN	International Society for Paediatric Oncology Europe Neuroblastoma Group
siRNA	Small interfering RNA
VMA	Vanillylmandelic Acid

1 – INTRODUCTION

According to the European Medicines Agency, a disease is defined as rare when it affects less than 5 in 10,000 people in the European Union (EU). The term "orphan medicinal product" refers to a medicine which is intended for the diagnosis, prevention or treatment of rare diseases that are life-threatening or chronically debilitating (1,2). While over 6000 rare diseases have been identified thus far, only 5 percent of them have an approved treatment. About 80 percent of rare diseases are genetic. Although individually rare, it is estimated that rare diseases affect 30 million people in Europe and 300 million worldwide (3,4).

About half of the people affected by rare diseases are children and, unfortunately, 30 percent of them will not live to see their 5th birthday (5). A rare paediatric disease is defined as a rare disease that primarily affects individuals aged from birth to 18 years, including age groups often called neonates, infants, children, and adolescents (6).

Neuroblastoma is a childhood tumour derived from neural crest cells and is the most common extracranial solid tumour in children (7). Neuroblastoma is considered a rare paediatric disease, since the number of patients affected by the condition is below the ceiling for orphan designation. In 2019, neuroblastoma affected approximately 1.1 in 10,000 people in the EU, on the basis of data from the EU 28, Norway, Iceland and Liechtenstein. This was equivalent to a total of around 57,000 patients, within a population of 518,400,000 (8).

Firstly, the development of orphan medicinal products can be very challenging due to unique features of rare diseases. The most obvious challenge with clinical trials in small populations is the limited number of patients available to be recruited. Therefore, if researchers are not able to recruit an adequate number of patients, the clinical trial probably will not yield significant results. Also, the affected patients are generally geographically dispersed and the diseases usually have varying phenotypic presentations. Another obstacle is the limited interest of pharmaceutical companies associated with the high cost of drug research and development and the small orphan drug market. Furthermore, there is frequently a poor understanding of the disease natural course and a lack of previous clinical trials. Often there is uncertainty about appropriate and realistic endpoints. Possible biomarkers are frequently unknown or poorly validated (9–11).

Imagine a new non-rare disease for which there is insufficient knowledge of the pathophysiology and natural course. One reasonable approach that could be adopted in order to address the fact that the natural disease history is poorly understood and to produce accurate results in the clinical trial, would be increasing the number of patients recruited. However, if this was a rare disease, it would be easily recognized that this strategy could not be adopted. Alternatively, one possible way to address these problems in rare disease clinical trials is through modelling and simulations (M&S). M&S provides researchers with the opportunity to understand the disease better and, therefore, to design and conduct clinical trials that meet the legal requirements and yield significant results.

Secondly, about half of the patients affected by rare diseases are children. To determine the safety and efficacy of medications in children, it is essential to perform clinical trials in this special population. However, drug development in paediatrics presents many challenges, mainly due to practical and ethical concerns (12). Practical issues in paediatric drug development include the reduced number of eligible children for clinical research and, often, the limited number of blood samples available for the assessment of pharmacokinetics of a drug. As explained above, the availability of patients is essential to satisfy the statistical power of the clinical trial, which is further complicated in rare diseases (12,13). In what regards ethical

concerns, a fundamental principle in paediatric drug development requires that “children should not be enrolled in a clinical study unless necessary to achieve an important paediatric public health need” (14). The paediatric population represents a vulnerable subgroup. Therefore, in paediatric drug development, special measures are needed in addition to those provided for adult subjects, in order to protect the rights of children (10,14).

In the case of neuroblastoma, these two features are linked together: not only is neuroblastoma a rare disease, but it also happens almost exclusively in children. In fact, less than 10 percent of all neuroblastoma cases are diagnosed after the age of 10 years (7,15).

BGA002 is a peptide nucleic acid drug that is being developed by BIOGENERA SpA, a pharmaceutical company located in Bologna, Italy. BGA002 is intended for use in the treatment of paediatric high-risk neuroblastoma. Numerous preclinical studies including *in vitro* and *strict* experiments have been conducted. These studies yielded important preclinical data, such as *in vitro* efficacy on neuroblastoma cell lines, pharmacokinetic studies and safety profile in mice. Since BGA002 is a new drug being developed for a rare paediatric disease, there is a lack of information about its pharmacodynamics and pharmacokinetics in humans. Therefore, modelling and simulations (M&S) can be extremely useful to predict human data such as human exposure and exposure-response relationships, based on animal data. The implementation of M&S is encouraged by the European Medicine’s Agency (16,17), to provide complementary PK/PD information and guide the process of dose selection and dose escalation. Furthermore, a model can also be used to run simulations with numerous doses, different treatment durations and trial designs, in order to predict what the effects on the tumour would be. This procedure is essential to further plan and conduct first-in-children clinical trials. A model-based approach is not only desirable but also necessary to address such practical and ethical limitations.

2 – OBJECTIVES

As mentioned earlier, the development of drugs for neuroblastoma is a challenging task, mainly because neuroblastoma exhibits two unique features: it is a rare and a paediatric disease. In order to develop safe clinical trials in children with rare diseases, modelling and simulations (M&S) can be a great approach. The aim of this research project is to determine a safe and effective dose of BGA002 for a first-in-patient clinical trial for paediatric neuroblastoma, using M&S.

Additionally, a clinical study protocol was elaborated: “A Double-Blind Phase I Study To Evaluate The Safety And Efficacy Of BGA002 For The Treatment Of Paediatric High-Risk Neuroblastoma”, which includes important information such as eligibility criteria and the study design.

3 – BACKGROUND

3.1 – Definition of Neuroblastoma

Neuroblastoma is a childhood tumour derived from primordial neural crest cells and is the most common extracranial solid tumour in children (7). During embryonic development, neural crest cells migrate and differentiate (Figure 1) into several different lineages (Figure 2), such as sensory, enteric, melanocytic and sympathetic neurons (2). These developing neurons undergo a massive, programmed cell death; about a half of the neurons die by apoptosis (Figure 1). The surviving neurons complete their differentiation and become mature neuronal cells, like ganglion cells with proper function (19).

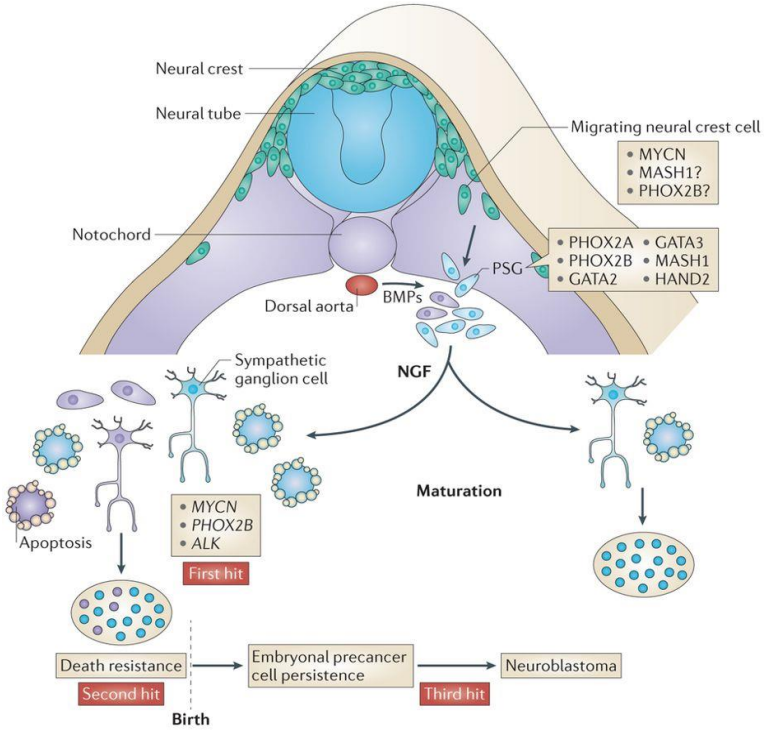

Figure 1 – The process of migration and differentiation of neural crest cells, and the development of neuroblastoma; Marshall GM, Carter DR, Cheung BB, Liu T, Mateos MK, Meyerowitz JG, et al. The prenatal origins of cancer. Nat Rev Cancer. 2014;14(4):277–89

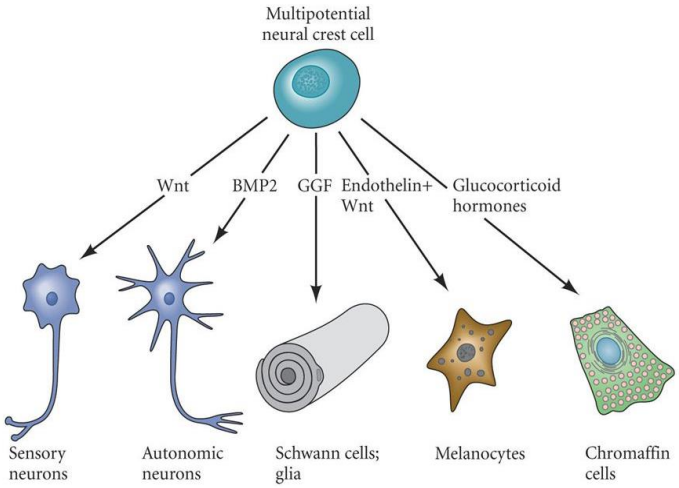

Figure 2 - Different lineages derived from neural crest cells; Gilbert SF. Developmental Biology. 8th ed. Sinauer Associates Inc.; 2006.

However, in some cases, defects in neural crest cell migration, differentiation or maturation, many of which remain to be defined, can compromise the neurons' development. When these defects occur on precursor cells of the sympathoadrenal lineage, it can lead to the development of neuroblastoma (Figure 1). In fact, neuroblastoma does not occur from the other lineages derived from the neural crest cells, but only from the precursor cells of the sympathoadrenal lineage (19,20).

Neuroblastoma exhibits unique features, such as diverse clinical presentation and tumour biology, with some children having tumours that regress spontaneously, while other children have extensive metastatic tumours with poor outcomes despite multimodal therapy (19). The phenotype of the disease is highly associated with age. For instance, patients who are less than 18 months of age are much more likely to have a tumour that will spontaneously regress than older children. Indeed, attempts to detect neuroblastoma early by measuring urinary catecholamine metabolites revealed that about a half of the neuroblastomas that occur in the first year of life are never detected due to spontaneous regression (15). The mechanisms of tumour regression are not fully understood, perhaps because these tumours regress before they manifest clinically.

3.2 – Epidemiology

Neuroblastoma accounts for approximately 10 percent of all paediatric cancers and unfortunately also accounts for up to 15 percent of deaths in children from cancer (7). About 90 percent of neuroblastoma tumours arise in children who are less than 10 years old, with a median age at diagnosis of 19 months. It occurs rarely in adolescents and adults (7,15). These data show that this is a disease of infancy, with the highest rate of diagnosis in the first month of life.

The estimated incidence of neuroblastoma is 10.5 cases per year per million children under 15 years old in North America and Europe, with minimal ethnic or geographic variability. Neuroblastoma occurs slightly more often in boys than girls, by a ratio of 6:5. African American and Native American patients are more likely to have a more aggressive disease with lower survival rates, than individuals of European descent, although the aetiologies of these differences are unclear (7,15). As for environmental influences, no clearly documented factor has been shown to increase the risk of neuroblastoma. However, environmental exposures, such as maternal drug use and the use of hair dyes during pregnancy, might have a role in neuroblastoma, but at a much lower level than in adult malignancies (15).

3.3 – Pathophysiology

The neural crest is a structure that arises from the neural tube after tube closure, in the third to fourth week of embryonal development, and it is present only during embryogenesis (21). During development, neural crest cells lose polarity and have reduced adhesive properties, which enables them to migrate from the neural tube, by a process named epithelial-mesenchymal transition. These migrating neural crest progenitor cells then differentiate into several different lineages, by a complex process which involves histone modification, DNA methylation and the expression of transcription factors and bone morphogenetic proteins. Cell types derived from neural crest cells include, for instance, melanocytes, sensory, enteric and sympathetic neurons (15).

When neural crest cells migrate to the dorsal aorta in response to key transcription factors, they differentiate into sympathoadrenal precursor cells, which eventually give rise to cells of the sympathetic nervous system, including sympathetic ganglia and the adrenal medulla, which is composed of adrenal chromaffin cells (22). Actually, these are the main sites in which neuroblastoma arises. The fact that neuroblastoma occurs only from the precursor cells of the sympathoadrenal lineage suggests that the oncogenic events that lead to neuroblastoma occur after the point when neural crest cells have differentiated into sympathetic neurons (19).

One of the key transcription factors involved in the migration of neural crest cells that ultimately differentiate into sympathetic neurons is N-Myc, which is encoded by the *MYCN* gene. The *MYCN* gene belongs to the Myc family of proto-oncogenes that encode transcription factors important for the regulation of gene expression in numerous cellular processes, such as proliferation, growth, apoptosis, energy metabolism and differentiation. Other transcription factors belonging to this family are c-Myc and L-Myc (23).

Several genomic alterations have been shown in neuroblastoma cells, including amplification of *MYCN* (encoding N-Myc), mutations of ALK (encoding Anaplastic Lymphoma Kinase) and segmental chromosomal alterations. The most malignant neuroblastomas often harbour amplification of *MYCN*, and this is usually associated with poor survival outcomes (19). *MYCN* gene is considered amplified when more than four copies are present in the tumour; this is found in approximately 20 percent of tumours (24).

N-Myc is an important transcription factor that promotes cell-growth and inhibits cell differentiation, while maintaining a self-renewal capacity; its levels correlate with metastasis and the induction of angiogenesis. Furthermore, in order to support higher energy needs of neuroblastoma cells, *MYCN* overexpression affects metabolism, by increasing glycolysis and glutaminolysis. In addition, N-Myc alters the expression of proteins that are involved in mitochondrial dynamics; however, the mechanism of this effect is not fully understood (25).

To sum up, neuroblastomas can form anywhere in the sympathetic nervous system, but are found most frequently in the adrenal medulla (approximately 40 percent of tumours) or in lumbar sympathetic ganglia. Neuroblastomas may also arise in the thoracic, cervical or sacral sympathetic ganglia (21).

The adrenal medulla consists of chromaffin cells (26), which are modified postganglionic neurons, since they have lost their dendrites and axons. Preganglionic neurons connect directly to adrenal medulla cells and stimulate the release of catecholamines (adrenaline, noradrenaline and dopamine) (Figure 3). Hence, as a cluster of neuron cell bodies, the adrenal medulla is considered a specialized ganglion of the sympathetic nervous system (22).

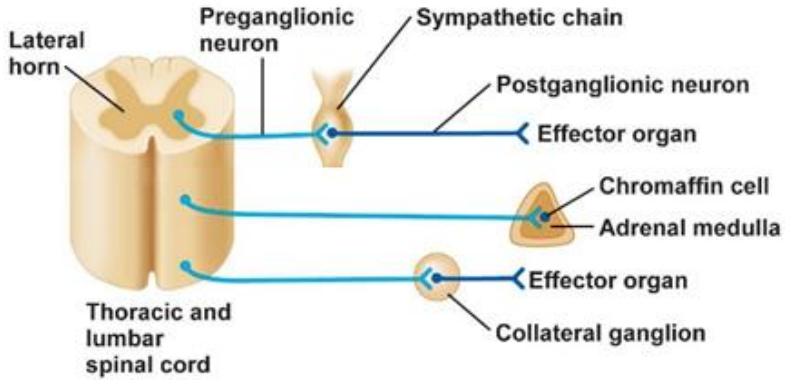

Figure 3 - Anatomy of the Sympathetic Nervous System and the three patterns in which pre- and postganglionic neurons may be arranged; G. Gardner D, M. Shoback D. Greenspan's Basic and Clinical Endocrinology. In: 10th ed. McGraw-Hill Education; 2017. p. 924.

Signs and symptoms

The clinical signs and symptoms of neuroblastoma vary based on the number and location of tumours. Metastatic disease is detected in approximately 50 percent of patients at diagnosis, frequently in the regional lymph nodes, bones and bone marrow, but liver and skin metastasis are more common in young infants who are less than 18 months of age (15).

Most neuroblastoma tumours arise in the abdomen, with the most common primary site being the adrenal gland. Abdominal masses may be asymptomatic or may lead to hypertension and abdominal pain or distension. Approximately 10 to 15 percent of patients present with paraspinal tumours, which originate in the paraspinal sympathetic ganglia, and these can extend into the neural foramina, which may lead to spinal cord compression and paralysis (7).

Primary tumours in the cervical region might cause damage to the stellate ganglion, leading to Horner syndrome, which is characterized by miosis, ptosis and anhidrosis. Patients with metastatic disease may also present with subcutaneous skin nodules, bone pain, and pancytopenia (15).

Diagnosis

Neuroblastoma is, like other cancers, diagnosed using a combination of laboratory tests, radiographic imaging and pathology. The findings of these tests, combined with other prognostic factors, will allow patients to be stratified for treatment according to the different risk groups.

Laboratory testing

Neuroblastoma is a neuroendocrine tumour that secretes various regulatory peptides, including the catecholamines noradrenaline, adrenaline and dopamine. Increased levels of plasma catecholamines as well as their metabolites in urine, such as vanillylmandelic acid (VMA), homovanillic acid (HVA) and 3-methoxytyramine, can be used in clinical diagnosis and for early detection in screening programs of neuroblastoma (Figure 4). In fact, these metabolites, along with dopamine (27), can be detected in the urine of 90 percent of all patients with neuroblastoma (15,19). Their relative amounts in the urine are related to the degree of maturation of neuroblastoma's cells, with increased dopamine levels or HVA/VMA ratio associated with biologically unfavourable disease (28).

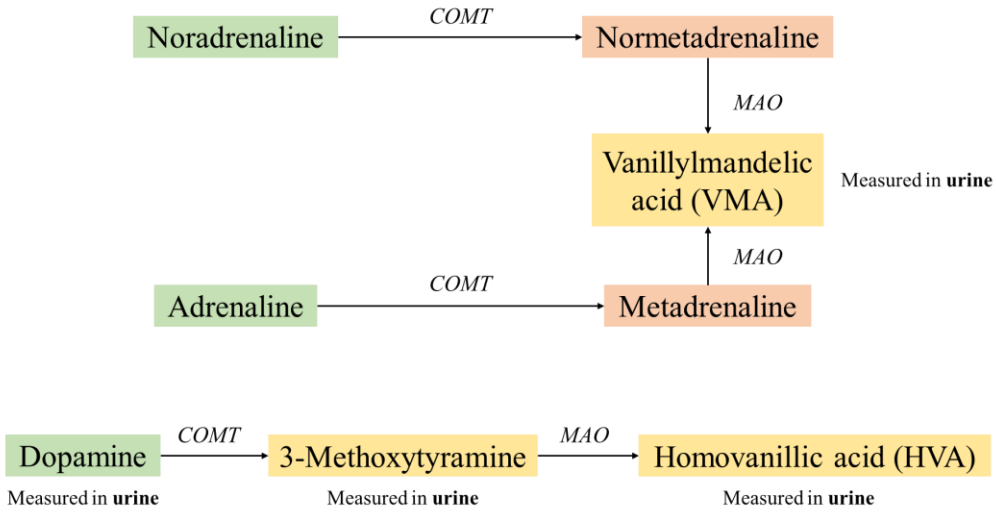

Figure 4 - Catecholamines (*green*) produced by the organism and its metabolites (*red* and *yellow*)

Since obtaining adequate urine samples in infants may sometimes be a difficult task, a convenient alternative to urine markers is the measurement of plasma-free and total catecholamine metabolites. These metabolites include normetadrenaline (derived from noradrenaline), metadrenaline (derived from adrenaline) and 3-methoxytyramine (derived from dopamine) (15).

Other biological tumour markers tested in clinical practice include chromogranin A (CgA), neuron-specific enolase (NSE), lactate dehydrogenase (LDH) and ferritin. Increased serum levels of these markers have been described as poor prognostic markers in neuroblastoma; however, they are not specific to neuroblastoma (19).

Radiographic imaging and metastatic evaluation

Tumour imaging is determined by radiological assessment of the primary tumour, either with CT or MRI to determine primary tumour size and regional invasion. Additional imaging of the chest, abdomen and pelvis is performed to identify spread to other distant sites (7).

The extent of metastatic disease is evaluated by a meta-iodobenzylguanidine (mIBG) scan, which uses radiolabelled mIBG, a molecule with a similar structure to noradrenaline. mIBG scan has an estimated sensitivity of 90 percent and a specificity of 99 percent and enables the assessment of both local and metastatic soft tissue and bone marrow disease (15). Approximately 90 percent of neuroblastomas are mIBG-avid, due to the expression of the noradrenaline transporter, which enables mIBG uptake into tumour cells. The radiolabelling of mIBG is preferably performed with Iodine-123 (^{123}I). As for tumours that are not mIBG-avid, the extent of disease can be assessed using techniques that are independent of mIBG uptake, such as ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) scans (7,15).

Pathology

Neuroblastoma pathology is an important determinant of prognosis. In order to obtain all the biological data required for risk-group assignment, a tumour biopsy needs to be performed. Some procedures may be done on the tissue that is removed, such as immunohistochemistry, fluorescence *in situ* hybridization for *MYCN*, flow cytometry to determine DNA index (ploidy),

cytogenetic analysis to determine the presence of chromosomal alterations, and bone marrow aspiration and biopsy with immunohistochemistry (7,15,19).

Classification System: Stages and Risk Groups

Once a diagnosis of neuroblastoma is confirmed, the following step is the disease staging. The patient is then stratified as very low-risk, low-risk, intermediate-risk and high-risk, based on clinical and molecular risk factors. The stratification of neuroblastoma in different risk groups will help clinicians to decide the best course of treatment (15).

International Neuroblastoma Staging System (INSS)

The International Neuroblastoma Staging System (INSS) was developed in 1986, and it was the first internationally accepted staging system for neuroblastoma. The INSS is a postsurgical staging system, which is based on the degree of surgical resection. Therefore, it is not suitable to be applied before treatment (29).

The INSS categorises neuroblastoma in six different stages (30): 1, 2A, 2B, 3, 4 and 4S (Table 1).

INSS Stage	Description
1	Localised tumour with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour could be positive)
2A	Localised tumour with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically
2B	Localised tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. Enlarged contralateral lymph nodes should be negative microscopically
3	Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement; or localised unilateral tumour with contralateral regional lymph node involvement; or midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement
4	Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, or other organs (except as defined by stage 4S)
4S	Localised primary tumour in infants younger than 1 year (as defined for stage 1, 2A or 2B), with dissemination limited to skin, liver, or bone marrow (<10% malignant cells)

Table 1 - International Neuroblastoma Staging System, a surgery-based system; Adapted from Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. Lancet. 2007;369(9579):2106–20.

International Neuroblastoma Risk Group (INRG) Staging System

As explained earlier, the INSS is a postsurgical staging system that is inappropriate to establish different risk groups before treatment. Hence, in 2009, the International Neuroblastoma Risk Group (INRG) Task Force, consisting of neuroblastoma experts from Australia/New Zealand, China, Europe, Japan, and North America, developed a new INRG Staging System, which is based on tumour imaging and clinical criteria (29).

Unlike the INSS, the INRG Staging System uses only results of imaging tests taken before surgery. It does not include surgical results or spread to lymph nodes to determine the stage; however, knowledge regarding the presence or absence of image-defined risk factors (IDRF) are required for this staging system. IDRFs describe local extension of the primary tumour, infiltration of adjacent soft tissues and organs and infiltration of the neural foramina and epidural space of the spinal canal (15).

Furthermore, the INRG Staging System enables the definition of homogenous pre-treatment patient cohorts, which facilitates the comparison of risk-based clinical trials conducted in different regions of the world.

The INRG Staging System classifies neuroblastoma into four different stages (29): L1, L2, M and MS (Table 2).

INRG Stage	Description
L1	Localised tumour not involving vital structures and confined to one body compartment
L2	Locoregional tumour with presence of one or more IDRFs
M	Distant metastatic disease (except stage MS)
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow

Table 2 - International Neuroblastoma Risk Group (INRG) Staging System, which is based on tumour imaging and clinical criteria; Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging

The INRG Classification System uses a combination of clinical, pathologic and genetic markers to assess the clinical behaviour of the tumour and to define the risk (Table 3). Then, treatment will be tailored according to the assigned risk group. By using seven prognostic factors (stage of the disease according to the INRG Staging System, age at the time of diagnosis, histological category, grade of tumour differentiation, *MYCN* gene status, chromosome 11q status, DNA ploidy), neuroblastoma is classified into one of four categories: very low-risk, low-risk, intermediate-risk, or high-risk (7,15,29,31).

INRG Stage	Patient age (months)	Tumour histology	Tumour differentiation	*MYCN* Status	Chromosome 11q aberration	DNA ploidy	Pre-treatment risk group
L1 or L2	Any	GN maturing or GNB intermixed	Any	Any	Any	Any	Very Low
L1	Any	GNB nodular or NB	Any	Not amplified	Any	Any	
MS	<18	Any	Any	Not amplified	No	Any	
L2	<18	GNB nodular or NB	Any	Not amplified	No	Any	Low
L2	≥18	GNB nodular or NB	Differentiating	Not amplified	No	Any	
M	<18	Any	Any	Not amplified	Any	Hyperdiploid	
L2	<18	GNB nodular or NB	Any	Not amplified	Yes	Any	Intermediate
L2	≥18	GNB nodular or NB	Differentiating	Not amplified	Yes	Any	
L2	≥18	GNB nodular or NB	Poorly Differentiated or Undifferentiated	Not amplified	Any	Any	
M	<18	Any	Any	Not amplified	Any	Diploid	
L1	Any	GNB nodular or NB	Any	Amplified	Any	Any	High
L2	Any	Any	Any	Amplified	Any	Any	
M	<18	Any	Any	Amplified	Any	Any	
M	≥18	Any	Any	Any	Any	Any	
MS	<18	Any	Any	Amplified	Any	Any	
MS	<18	Any	Any	Any	Yes	Any	

Table 3 - International Neuroblastoma Risk Group (INRG) Classification System and its seven prognostic factors; GN: ganglioneuroma; GNB: ganglioneuroblastoma; NB: neuroblastoma; This table was adapted from three different sources: Reference number 1: Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE. Overview and recent advances in the treatment of neuroblastoma. Expert Rev Anticancer Ther [Internet]. 2017;17(4):369–86. Available from: http://dx.doi.org/10.1080/14737140.2017.1285230; Reference number 5: Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. Nat Rev Dis Prim. 2016;2.; Reference number 19: Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33(27):3008–17.

3.4 – Therapeutic approaches

Very low and low-risk neuroblastoma

In very low and low-risk neuroblastomas, treatment decisions aim to deliver the minimum therapy while maintaining excellent patient survival (15).

Infants who are less than 3 months old with localised adrenal masses suspicious of neuroblastoma do not necessarily require biopsy or surgical resection and may be appropriately managed with observation alone. This observational approach avoids potential complications of surgery in young infants (32).

In other patients with very low or low-risk disease, the approach is either observation or surgical resection, with chemotherapy restricted to symptomatic patients with low-risk neuroblastoma (32).

Intermediate-risk neuroblastoma

For children with intermediate-risk neuroblastoma, the overall treatment strategy depends on clinical and tumour biological risk factors. Based on these parameters, the possible approaches are chemotherapy, surgery, radiotherapy and administration of *cis*-retinoic acid (32).

High-risk neuroblastoma

Children with high-risk neuroblastoma account for approximately half of all patients (7). While patients with a very low, low or intermediate-risk have a favourable outcome, the high-risk group has a survival rate below 50 percent (25). In fact, even though the treatment strategies have evolved over the years, this group remains one of the most challenging to treat.

Current standard of care

The current treatment regimens used for children with high-risk neuroblastoma have three main components: induction, consolidation and maintenance therapy (Figure 5). This treatment regimen lasts for approximately 18 months (24).

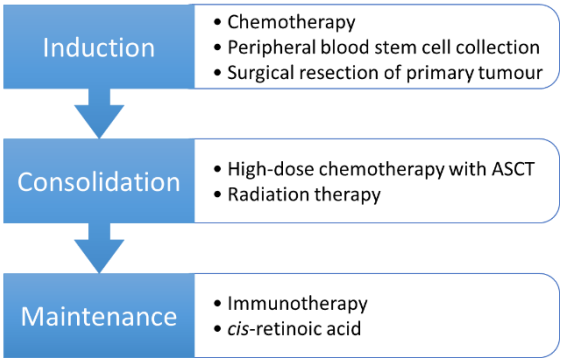

Figure 5 - Current standard of care for children with high-risk neuroblastoma, recommended by The International Society for Paediatric Oncology Europe Neuroblastoma Group (SIOPEN); Adapted from Smith V, Foster J. High-Risk Neuroblastoma Treatment Review. Children. 2018;5(9):114.

Induction

Even though the induction regimens currently in use vary between different cooperative groups, most regimens consist of 5-8 cycles of intensive chemotherapy including platinum, alkylating and topoisomerase agents (24). The International Society for Paediatric Oncology Europe Neuroblastoma Group (SIOPEN) recommends a Rapid COJEC regimen (33,34), comprising the chemotherapeutic agents cisplatin (represented by the first C of the acronym, otherwise abbreviated P), vincristine (O), carboplatin (J), etoposide (E), and cyclophosphamide (C). The schedule is administered in eight cycles separated by intervals of ten days (24,35,36).

Peripheral blood stem cells should be collected at the end of the eighth cycle, unless there is evidence of progressive disease. Following Rapid COJEC and ideally after peripheral stem cell harvest, patients should proceed to surgical resection of the primary tumour, in order to achieve as complete resection as possible (24,36).

Consolidation

The consolidation phase follows induction with the goal of eliminating remaining minimal disease. This phase is divided into two parts: high-dose chemotherapy followed by autologous stem cell transplant (ASCT) and radiation therapy (24).

The SIOPEN recommends the administration of high-dose chemotherapy with busulfan and melphalan, with the aim of being a myeloablative therapy. Then, peripheral blood stem cells re-infusion is performed (36).

Radiation therapy typically occurs once the patient has recovered from ASCT and is associated with a high rate of local control. The standard amount of radiation administered is 21 Gray (Gy) to the site of primary tumour, based on the pre-operative imaging (24,36).

Maintenance

The post consolidation or maintenance phase of therapy was developed to treat residual disease that remains despite intensive induction and consolidation treatment regimens (24).

The SIOPEN recommends that patients who have achieved at least partial response to chemotherapy and proceeded to myeloablative chemotherapy and radiotherapy, should receive anti-GD2 immunotherapy. The recommended anti-GD2 antibody is dinutuximab beta, given without IL-2 to these patients. Furthermore, in an effort to decrease the chance of relapse, differentiation therapy with isotretinoin (also known as 13-*cis*-retinoic acid) should be delivered (24,36).

BGA002 as an investigational therapy for neuroblastoma

About half of high-risk neuroblastomas present with *MYCN* amplification. Interestingly, *MYCN* is expressed during embryonic development and is virtually absent during adulthood. These factors make the N-Myc pathway a promising target for neuroblastoma therapy (25).

One of the possible approaches that has been proposed is the knockdown of *MYCN* expression which may be, in theory, achieved through one of three possible ways: inhibition of DNA transcription (with antigene strategies), inhibition of mRNA translation (with antisense strategies), or inhibition of *MYCN* protein. However, the latter approach has been challenging as *MYCN* proteins are composed of two extended alpha-helices with no obvious surfaces for

small molecule binding. Antigene oligonucleotides, on the other hand, showed high efficacy in blocking *MYCN* expression, by binding to chromosomal DNA and persistently inhibiting transcription. In fact, comparing antigene oligonucleotides with antisense oligonucleotides, the former demonstrated potent and specific antigene activity and higher therapeutic potential, due to their resistance to nuclease degradation (25,37).

Peptide Nucleic Acids (PNAs) are an example of oligonucleotide analogues. For instance, BGA002 is a single-strand PNA which was recently designed to act through an anti-gene strategy (25). PNAs are synthetic DNA/RNA analogues in which the sugar-phosphate backbone is replaced by an amino acid backbone. The amino acid backbone consists of repeating units of N-(2-aminoethyl)glycine, to which the purine and pyrimidine bases are attached, via a methyl carbonyl linker (Figure 6) (38).

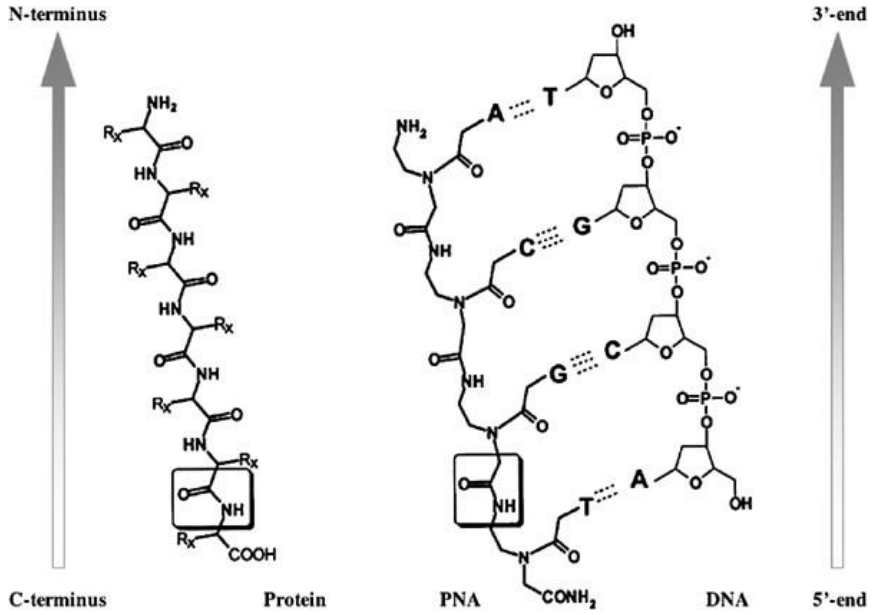

Figure 6 - Chemical structures of PNA as compared to DNA and protein; by convention, PNAs are depicted like peptides, with the N-terminus at the top position and the c-terminus at the bottom position; hydrogen bonds are indicated by "…."; in Pellestor F, Paulasova P. The peptide nucleic acids (PNAs), powerful tools for molecular genetics and cytogenetics. Eur J Hum Genet. 2004;694–700.

One unique characteristic of a PNA is that its backbone has a neutral charge. Consequently, unlike DNA and RNA, the neutral backbone provides PNAs with high flexibility. Therefore, PNAs hybridize with complementary DNAs or RNAs with remarkably high affinity and specificity, and the hybrids have high stability due to the absence of electrostatic repulsion (38,39). Furthermore, the absence of phosphodiester bonds turns PNAs into nuclease-resistant molecules, making them even more stable than antisense oligonucleotides, such as miRNA and siRNA (40).

All these features turn PNAs into promising tools, due to their applications in antisense and antigene therapies by inhibiting translation and transcription, respectively. However, PNAs may have a disadvantage regarding cellular uptake. For PNA-based gene targeting, there are two barriers that must be overcome: the cellular membrane and the nuclear membrane. In order to improve the transport of PNAs across membranes, it may be necessary to couple PNAs with cell penetrating peptides (41).

BGA002 is a PNA that has the ability of specifically targeting a unique sequence in the *MYCN* gene, which is responsible for the production of the protein N-Myc. BGA002 is

conjugated to a Nuclear Localization Signal (NLS) peptide, which promotes the penetration of the drug into cells and delivery to the nucleus (25). Inside the nucleus, BGA002 selectively inhibits the *MYCN* gene, leading to decreased transcription. Consequently, the protein N-Myc, which is involved in the growth, progression and spread of neuroblastomas, has a lowered production, leading to reduced cell viability and apoptosis in neuroblastoma cells (42).

As for the route of administration, BGA002 would be administered by parenteral route, based on preclinical data (40). In most cases, intravenous administration, when feasible, is advisable for first use in man studies since it eliminates variability related to bioavailability (17).

3.5 – Clinical Trials

Definition of Clinical Trial

According to the Clinical Trial Regulation (EU No 536/2014), a clinical trial is defined as any investigation in relation to humans intended to study one or more investigational medicines, mainly to discover or verify their clinical, pharmacological or other pharmacodynamic effects, to identify any adverse reactions or to study their absorption, distribution, metabolism and excretion. Overall, a clinical study aims to determine the safety and/or efficacy of those medicinal products (43). The regulation of clinical trials aims to ensure that clinical trials yield significant and reliable results, while protecting the rights, safety and well-being of trial subjects.

Main Phases of a Clinical Trial

Clinical drug development is often described as consisting of four temporal phases (Phase I-IV).

Phase I – Evaluation of Safety

Studies in Phase I usually have non-therapeutic objectives and may be conducted in healthy volunteer subjects. However, as for drugs with significant potential toxicity, e.g. cytotoxic drugs, they are usually studied in patients. Studies in this phase can be open, baseline controlled or may use randomisation and blinding, to improve the validity of observations (44).

In phase I trials, the main focus is studying safety concerns. For instance, it is important to determine the tolerability of the dose range expected to be needed for later clinical studies, and to determine the nature of adverse reactions that can be expected. The evaluation of adverse effects typically includes assessment of symptoms, physical examination, electrocardiogram and blood and urine laboratory analyses. Local toxicity at the site of administration should also be assessed (17).

Additionally, the pharmacokinetics of the drug is usually studied in phase I trials, namely its absorption, distribution, metabolism and excretion; also, it is important to assess its clearance and anticipate possible accumulation of parent drug or metabolites. Performing drug-drug interaction studies is equally valuable, as well as studying the effects of food on bioavailability, in the case of orally administered drugs.

Phase I trials may also involve pharmacodynamic studies and PK/PD studies, which relate drug blood levels to response. These studies can provide early estimates of drug activity and potential efficacy and further guide the dosage and dose regimen in later studies (44).

Phase II – Evaluation of Safety and Dosing

An important goal for Phase II studies is to determine the dosage and dose regimen for Phase III trials. Studies in Phase II are typically conducted in a group of patients who are selected by relatively narrow criteria, leading to a relatively homogeneous population and are closely monitored.

Early studies in this phase often utilise dose escalation designs to give an early estimate of dose response and later studies may confirm the dose response relationship for the indication in question by using recognised parallel dose-response designs (44). In case of minimal toxicity, intra-patient dose escalation may be appropriate in order to reduce the number of patients exposed to non-active doses (17).

Phase III – Evaluation of Safety and Efficacy

Studies in Phase III are designed to confirm the preliminary evidence accumulated in Phase II that a drug is safe and effective for use in the intended indication and population.

Studies in Phase III may also further explore the dose-response relationship, or explore the drug's use in wider populations, in different stages of disease, or in combination with another drug. These studies complete the information needed to support adequate instructions for use of the drug (official product information).

Phase IV – Post-approval studies

Studies in Phase IV are all studies, other than routine surveillance, performed after drug approval and related to the approved indication. They are studies that were not considered necessary for approval but are often important for optimising the drug's use. Commonly conducted studies include additional drug-drug interaction, dose-response or safety studies and studies designed to support use under the approved indication, e.g. mortality/morbidity studies, and epidemiological studies.

Special populations – children

Some groups in the general population may require special study because they have particular risk/benefit considerations that need to be taken into account during drug development. One example is the paediatric population, which is a vulnerable subgroup. Nevertheless, it is essential to perform clinical trials in this special population, in order to determine the safety and efficacy of drugs in children. The extent of the studies needed depends on the current knowledge of the drug and the possibility of extrapolation from adults and children of other age groups (44).

For a drug expected to be used in children, the evaluation should be made in the appropriate age group. One possible classification of the different age categories is the following: preterm newborn infants, term newborn infants (0 to 27 days), infants and toddlers (28 days to 23 months), children (2 to 11 years) and adolescents (12 to 18 years). It is usually appropriate to begin with older children before extending the trial to younger children and then infants (14,44).

When a medicinal product is being investigated for a disease that predominantly or exclusively affects paediatric patients, phase I studies in the paediatric population should start

as soon as clinical development can be initiated, especially when the disease is included in the field of oncology (45). This is the case with neuroblastoma, which is a paediatric tumour.

According to the EMA Guidelines, clinical trials in children should be designed in order to be as safe as possible. Hence, eligibility criteria for phase I trials in children are even stricter than for the general population. For instance, patients should have an adequate performance status, measured with an appropriate paediatric scale (e.g., Lansky-play performance) (45). This and other eligibility criteria, recommended by the EMA, were considered when elaborating the clinical study protocol.

Even though phase I studies generally have non-therapeutic objectives, as explained above, the same is not true when it comes to a paediatric tumour. In fact, phase I trials in children with cancer have to provide them a potentially active treatment, not only an evaluation of safety (45).

As for the evaluation of adverse effects, besides the typical assessment explained above in the phase II trials' section, it is also important to report any observed effects on organ maturation, growth and development, including fertility (17). These aspects will probably require further monitoring after the drug is authorized.

In addition, it may be relevant to assess whether the toxicity profile differs between distinct age categories. However, the availability of patients is essential to satisfy the statistical power of the studies, which is further complicated when categorizing different age groups. Once more, modelling and simulations are extremely useful tools to provide complementary information about the paediatric population, where data is difficult to obtain (17).

In what regards the starting dose and subsequent dose levels, the common practice is to start with a dose that is 80% of the maximum tolerated dose (MTD) determined in adult patients, and generally intra-patient dose escalation does not occur. However, using an adult MTD is not possible with neuroblastoma since it almost exclusively affects children. In this case, since the adult MTD is unknown, EMA recommends an intra-patient dose escalation (45). With this strategy, there is a higher chance that the child will receive an effective dose.

Small populations – rare diseases

The development of clinical trials in small populations poses another challenge for drug developers: there is simply not an adequate number of patients to be enrolled in the studies. Consequently, it is more difficult to conduct accurate and reasonably powered, randomised studies.

In rare disease clinical trials, it is essential that each patient provides as much information related to safety and efficacy as possible (46). Again, M&S prove to be extremely helpful tools for the development of clinical trials in rare diseases, because they make it possible to explore different study designs. Consequently, with M&S, drug developers generate valuable data that allows them to shape their study design according to the information they obtain. Otherwise, without M&S, the only data drug developers would have before planning clinical trials, would be animal data.

4 – METHODS

4.1 – Allometric Scaling

Definition

Determining a suitable starting dose is extremely important in drug development. One of the methods that can be used for the selection of a safe starting dose is allometric scaling.

The term "allometric scaling" is derived from "allometry", which is the study of size and its consequences; and "scaling", which is an engineering term meaning to adjust dimensions (or other parameters) with size. In other words, allometry means "by a different measure", and is the opposite of isometry (Figure 7), which in its turn means "by the same measure"(47).

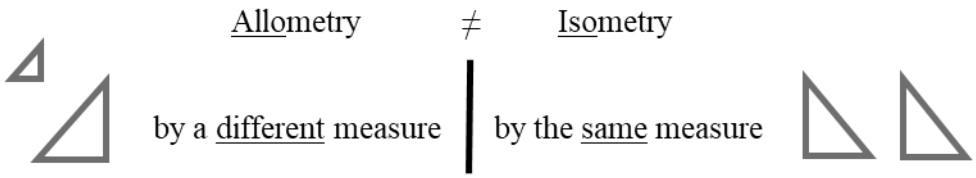

Figure 7 - Comparing Allometry and Isometry

Allometric scaling is one of the approaches that is frequently used in drug development, because it enables the prediction of human pharmacokinetics based upon animal data. Prediction methods, like allometric scaling, let us predict how a drug might behave in humans before any clinical studies are conducted. This is important information for both drug developers and regulators because it provides a data-driven foundation for establishing a safe starting dose in humans (48).

The basis of allometric scaling is that physiological parameters are proportional to body weight. The allometric approach is based on the power function, as the body weight from several species is plotted against a parameter of interest on a log-log scale. The power function can be written as follows (49):

$$P = a(W)^m \qquad (1)$$

where *P* is the parameter of interest, *W* is the body weight, *a* is an empirical coefficient and *m* is the exponent of the allometric equation. The exponents of allometry widely vary and are data dependent.

The previous equation can be linearized using log-transformation as follows:

$$\log P = \log a + m \log W \qquad (2)$$

where *a* is the intercept and *m* is the slope (49).

As revealed by the power function, if *m* equals to 1, then the physiological parameter (*P*) is directly proportional to body weight (*W*), a common approximation when considering tissue or organ mass, such as heart weight (Figure 8) (48).

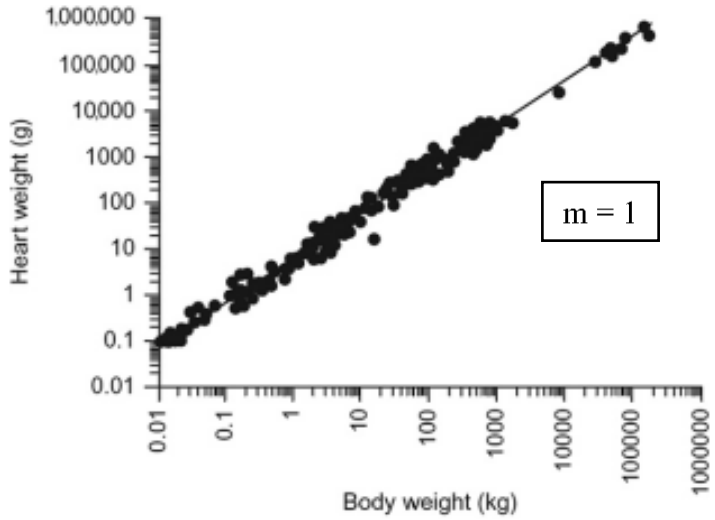

Figure 8 - Allometric relationship between body weight of mammals and heart weight (m = 1). The data are displayed as a log-log plot. Adapted from Huang S-M, Lertora J, Atkinson Jr. A, Markey S. Principles of Clinical Pharmacology. 3rd ed. Academic Press; 2012.

On the other hand, if *m* is less than 1, then the physiological parameter (*P*) does not increase proportionally with body weight (*W*). In other words, the physiological parameter (*P*) increases slower than body weight (*W*). This is frequently found with physiologic functions (Figure 9), such as glomerular filtration rate, tissue blood-flow rate, and daily heat production, with a value of *m* centred on 0.75 (16,48).

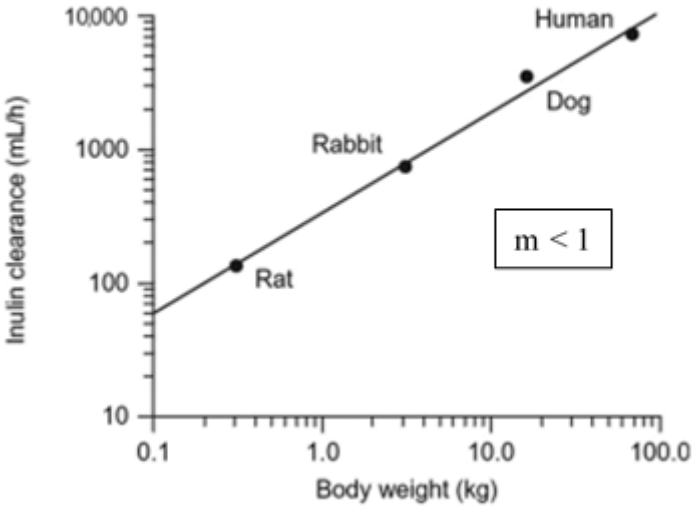

Figure 9 - Allometric relationship between body weight of mammals and inulin clearance (m = 0.75). The data are displayed as a log-log plot. Adapted from Huang S-M, Lertora J, Atkinson Jr. A, Markey S. Principles of Clinical Pharmacology. 3rd ed. Academic Press

To sum up, allometry has been widely used to predict pharmacokinetic parameters between species, including human pharmacokinetic parameters. Given that body composition tends to vary relatively little among mammalian species, it is expected that the volume of distribution of a drug is directly proportional to body weight (i.e., *m* = 1).

On the contrary, the value of *m* for drug clearance, being a measure of functional activity, like glomerular filtration rate, is expected to be about 0.75 (48). For instance, to predict human clearance based on animal clearance, the following formula should be applied:

$$P_{human} = P_{animal} \times \left(\frac{W_{human}}{W_{animal}}\right)^{m} \quad (3)$$

$$Cl_{human} = Cl_{animal} \times \left(\frac{W_{human}}{W_{animal}}\right)^{0.75} \quad (4)$$

Allometric Scaling vs. Human Equivalent Dose (HED)

Human Equivalent Dose (HED) is a calculation method used to determine the maximum safe starting dose in first-in-human clinical trials. This method is the one recommended by the Food and Drug Administration (FDA), in the "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers". This guidance outlines an algorithm (Figure 10) for deriving the maximum recommended starting dose (MRSD) of new molecular entities, to ensure the safety of the human volunteers (50). This method was used as an alternative calculation method in order to double-check the results obtained when using allometric scaling.

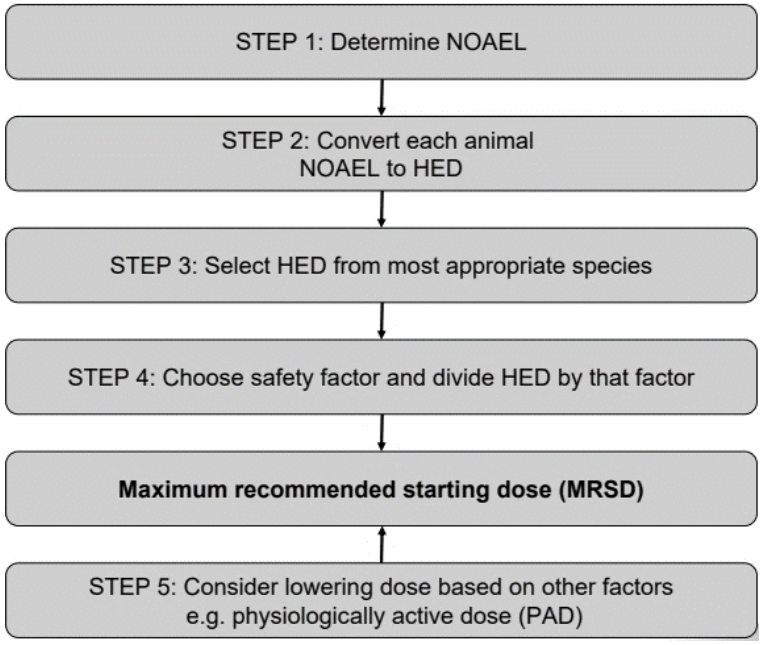

Figure 10 - Selection of Maximum Recommended Starting Dose for Drugs Administered Systemically to Normal Volunteers. Adapted from Appendix E, "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

These calculations use common conversion factors based on body surface area. The conversion factors and divisors shown in Table 4 are recommended as the standard values to be used for interspecies dose conversions, for weights inside the standard ranges (50).

Species	Reference Body Weight (kg)	Working Weight Range (kg)	To Convert Dose in mg/kg to Dose in mg/m² Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either	
				Divide Animal Dose By	Multiply Animal Dose By
Human	60	---	37	---	---
Child [b]	20	---	25	---	---
Mouse	0.020	0.011-0.034	3	12.3	0.081
Hamster	0.080	0.047-0.157	5	7.4	0.135
Rat	0.150	0.080-0.270	6	6.2	0.162
Ferret	0.300	0.160-0.540	7	5.3	0.189
Guinea pig	0.400	0.208-0.700	8	4.6	0.216
Rabbit	1.8	0.9-3.0	12	3.1	0.324
Dog	10	5-17	20	1.8	0.541
Primates:					
Monkeys [c]	3	1.4-4.9	12	3.1	0.324
Marmoset	0.350	0.140-0.720	6	6.2	0.162
Squirrel monkey	0.600	0.290-0.970	7	5.3	0.189
Baboon	12	7-23	20	1.8	0.541
Micro-pig	20	10-33	27	1.4	0.730
Mini-pig	40	25-64	35	1.1	0.946

Table 4 - Conversion of Animal Doses to Human Equivalent Doses (HED) based on Body Surface Area. a) For animal weights within the specified ranges, the HED for a 60 kg human calculated using the standard k_m value will not vary more than ± 20 percent from the HED calculated using a k_m value based on the exact animal weight. b) The k_m value is provided for reference only since healthy children will rarely be volunteers for phase I trials. c) For example, cynomolgus, rhesus, and stumptail. Adapted from Food and Drug Administration "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"

Alternatively, for species not listed or for weights outside the standard ranges, HED can be calculated from the following formula (50):

$$HED\ (mg/kg) = Animal\ dose\ (mg/kg) \times \left(\frac{W_{animal}\ (kg)}{W_{human}\ (kg)}\right)^{0.33} \quad (5)$$

However, it is worth noting that HED calculations do not directly scale pharmacokinetic parameters, since its calculations are based on body surface area, so there is no way to simulate concentration-time profiles. On the other hand, allometric scaling is based on body size and change in clearance, so it makes it possible to simulate concentration-time profiles, because it scales pharmacokinetic parameters directly (51).

Example of the difference between HED and Allometric Scaling

The following table shows an example of the difference between using HED calculations and allometric scaling in order to convert an animal dose (mg/kg) into a human dose (mg). Considering the average human weight to be 70 kg, and the average mouse weight to be 0.020 kg (Table 4), it is possible to calculate the human dose (mg), using the formulas seen before:

$$HED\ (mg) = Animal\ dose\ (mg/kg) \times \left(\frac{W_{animal}\ (kg)}{W_{human}\ (kg)}\right)^{0.33} \times W_{human}\ (kg) \quad (5)$$

for the HED calculations, and:

$$P_{human} = P_{animal} \times \left(\frac{W_{human}}{W_{animal}}\right)^{m} \quad (3)$$

$$Human\ dose\ (mg) = Animal\ dose\ (mg/kg) \times W_{animal}\ (kg) \times \left(\frac{W_{human}\ (kg)}{W_{animal}\ (kg)}\right)^{0.75} \quad (6)$$

for the allometric scaling calculations. Since these calculations are based on change in clearance, which is a physiologic function, *m* equals to 0.75.

Mouse dose (mg/kg)	Human dose (70 kg weight)	
	HED (mg)	Allometry (mg)
1	5	9
5	24	46
10	47	91

Table 5 - Conversion from a Mouse Dose (mg/kg) to a Human Dose (mg), obtained by two different methods: Human Equivalent Dose (HED) and Allometric Scaling

Table 5 shows an example of the different values for the human dose, obtained by two different methods: Human Equivalent Dose Calculations, which is recommended by the Food and Drug Administration (FDA) and Allometric Scaling, which is the method that is going to be used in the "Results" section.

4.2 – Modelling and Simulations (M&S)

Modelling and Simulations (M&S) can be defined as a range of quantitative approaches based on physiology, pathology and pharmacology, to characterize the interactions between a drug and an organ system which could predict quantitative outcomes of the drug and/or system's behaviour in future experiments (14).

In a modelling approach, a compartmental model is built with known PK, PD, and *in vitro* data and used to estimate PK parameters and how those parameters scale allometrically. Modelling can be conducted using a variety of software programs, such as NONlinear Mixed Effects Modelling (NONMEM). This model can also be used to run simulations with various doses to predict human exposure and exposure-response relationships. These methods can also predict variability in parameters and provide an estimate of how good the model is (40).

The data generated by NONMEM were processed using the program RStudio (software version 1.2.5033), which operates on the programming language R, usually used for statistical computing and graphics.

Figure 11 represents a PK/PD model that illustrates some of the pharmacokinetic and pharmacodynamic characteristics of BGA002, from previous work of Dr. Luca Ruotolo, "A rational-based PK/PD model for *MYCN* anti-gene PNA as a novel therapeutic strategy for the treatment of neuroblastoma in children". In Dr. Luca's work, the first step consisted in performing *in vitro* experiments on neuroblastoma cell lines and *in vivo* experiments in rabbits and mice. Secondly, using the preclinical data obtained, the software NONMEM was used to estimate parameter values such as clearance and volume of distribution. Then, a PK/PD model was built, based on preclinical data, parameter values and differential equations (40).

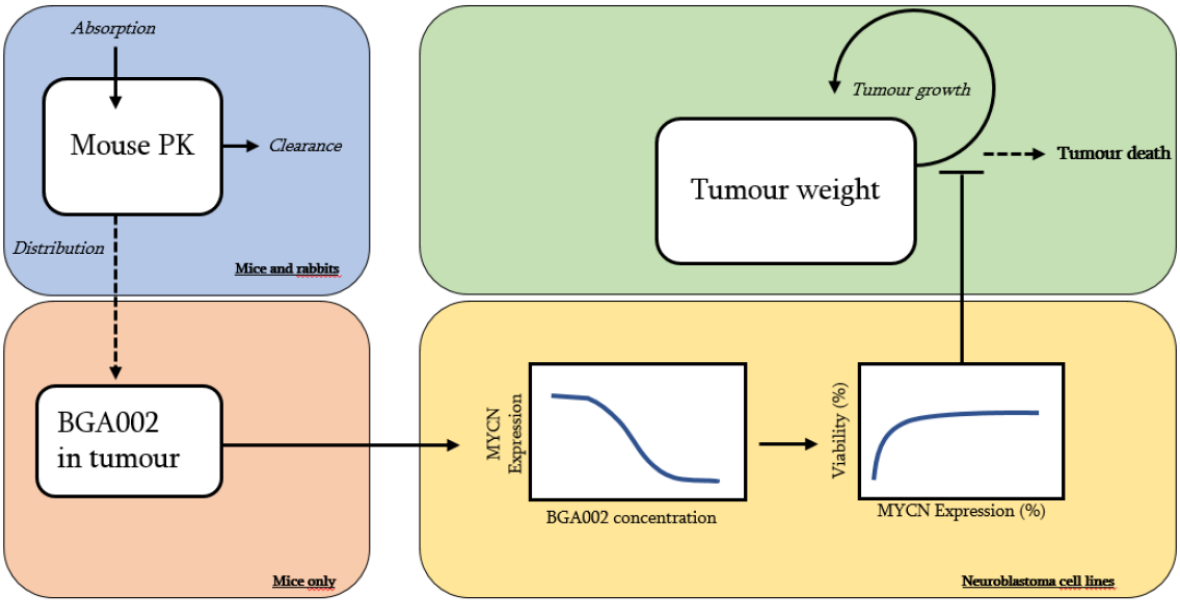

Figure 11 - PK/PD model representation, displaying the mechanism of action of BGA002 on neuroblastoma; Blue: PK model in mice. Red: PK at tumour site in mice. Yellow: *In vitro MYCN* expression linked to cell viability. Green: Tumour weight model in mice. The combined model describes the effect of BGA002 all the way from plasma to tumour concentrations into *MYCN* expression and cell viability, and ultimately changes in tumour weight; Ruotolo L. A rational-based PK/PD model for *MYCN* anti-gene PNA as a novel therapeutic strategy for the treatment of neuroblastoma in children. 2019.

4.3 – Literature Review

A broad literature research was performed to retrieve relevant publications about neuroblastoma, mainly on the PubMed platform. The name of the disease was combined with relevant terms, such as "rare disease", "paediatric disease", "clinical trial", "BGA002", "allometric scaling", "M&S" and "biomarker". Altogether, 90 references were reviewed, including articles, book sections, reports, guidelines and web pages.

5 – RESULTS

5.1 – Dose Rationale

The following graphs and tables display the data obtained from the simulations. These simulations were performed with a preclinical model which integrated *in vitro* and *in vivo* data (Figure 11).

A - Dose range selection

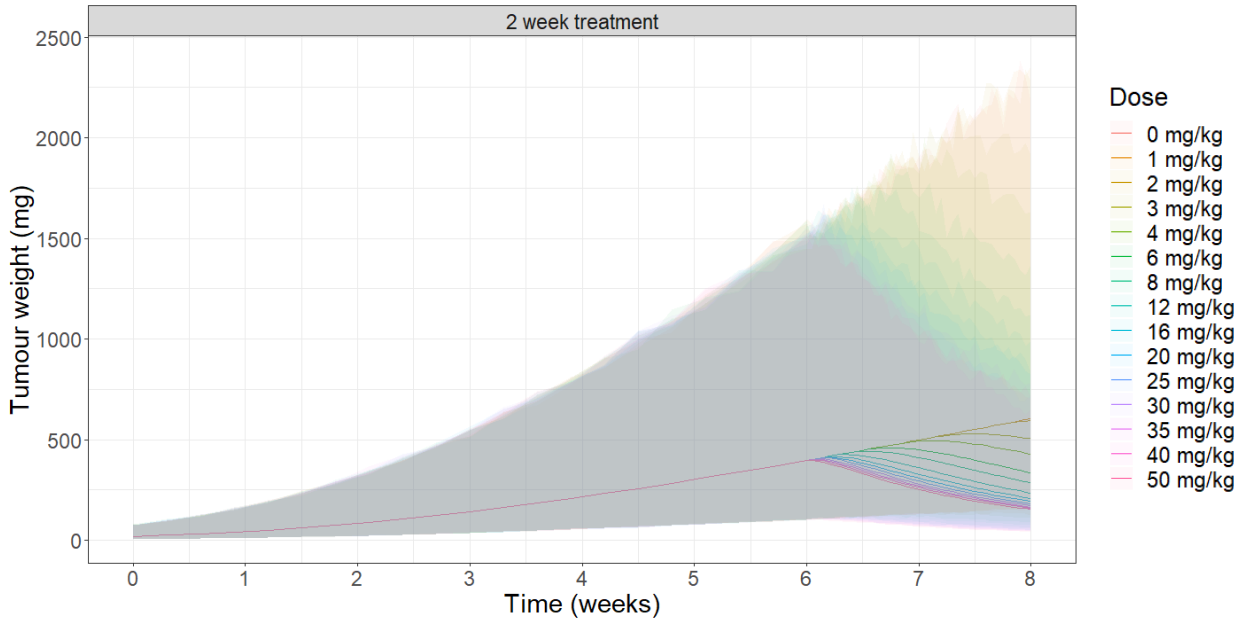

Figure 12 - Lineplot: Tumour weight (mg) vs Time (weeks); treatment with BGA002 starts at week 6 and it finishes at week 8; the shaded regions represent a y interval defined by a minimum and a maximum value which correspond, respectively, to the 5% and the 95% quantiles, for each dose

Figure 12 represents how the tumour weight changes over time, according to the model. Once again, these simulations are performed using a model that is based on preclinical studies. In this case, the trial duration was set to 8 weeks. and the treatment with BGA002 started at week 6. Before the beginning of treatment, at week 6, the tumour weight has a median of around 397 mg. From 0 mg/kg to 2 mg/kg, there does not seem to be a biological effect, while from 3 mg/kg to 50 mg/kg, there seems to be some biological effect. Since the supposed biological effect is not too clear, the data is now represented in a boxplot.

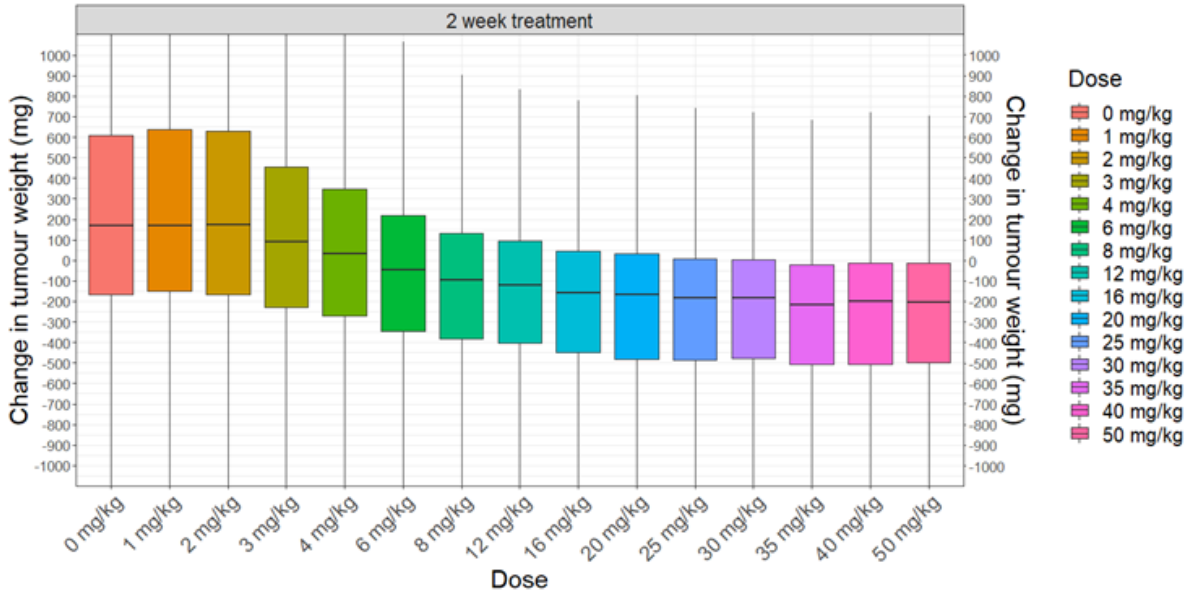

Figure 13 - Boxplot: Change in tumour weight (mg) (tumour weight at week 8 minus tumour weight at week 6), after 2 weeks of treatment with different doses of BGA002, in an 8-week trial; for presentation purposes the plot is zoomed in, so some upper and lower whiskers might be hidden

Figure 13 shows the difference in tumour weight after 2 weeks of treatment with a variety of different doses. The change in tumour weight is calculated by subtracting from the initial tumour weight (at week 6) the value of the tumour weight at the end of the treatment (week 8). In agreement with Figure 12, from 0 mg/kg to 2 mg/kg, there does not seem to be a biological effect. After 2 weeks of treatment, the change in tumour weight has a median of around 180 mg.

In addition, it is clearer that from 3 mg/kg to 50 mg/kg, there seems to be some biological effect. However, looking at doses 3 mg/kg and 4 mg/kg, we can see that the change in tumour weight still has a positive value for the median, so the tumour size did not decrease. This seems to suggest that the tumour is still growing, despite treatment.

Also, an interesting observation is that doses higher than 16 mg/kg may not offer any additional decrease in tumour size.

B – Treatment duration

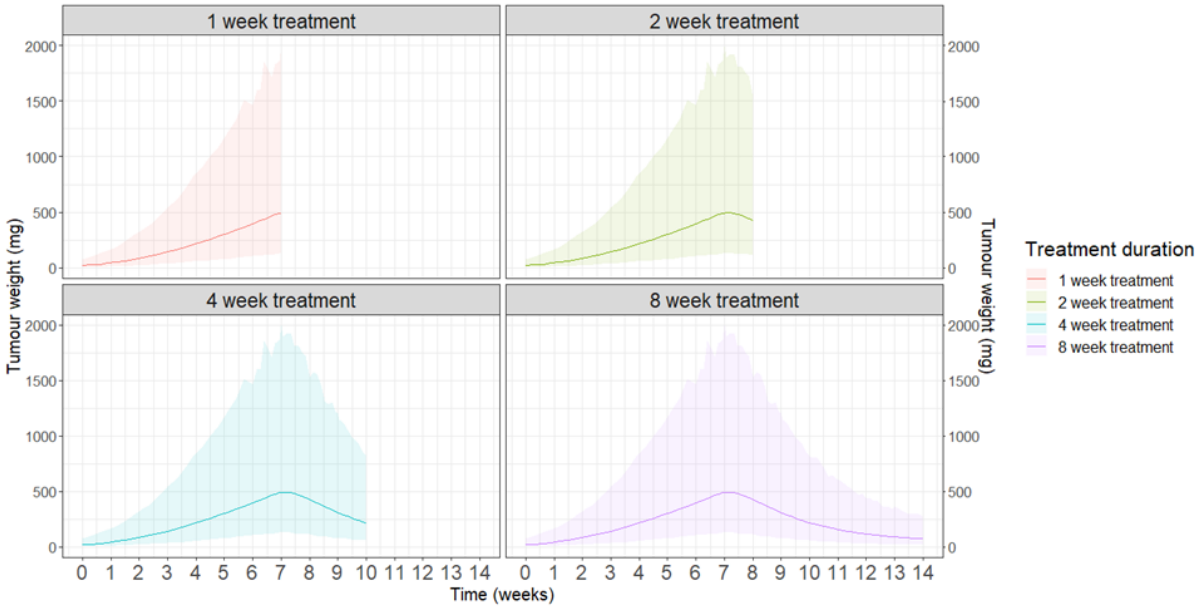

Figure 14 - Lineplots: Tumour weight (mg) vs Time (weeks), in a trial with four different treatment durations (1, 2, 4 or 8-week treatment); the treatment started at week 6; the shaded regions represent a y interval defined by a minimum and a maximum value which correspond, respectively, to the 5% and the 95% quantiles, for each dose

Figure 14 shows how the tumour weight varies over time, for four different treatment durations: either 1, 2, 4 or 8 weeks of treatment with the drug.

After 1 week of treatment with the drug, there does not seem to be a decrease in the tumour weight. As for the other plots, there seems to be an observed effect.

In order to better evaluate the change in tumour weight, the data referring to 2-week and 4-week treatment will be represented in a boxplot.

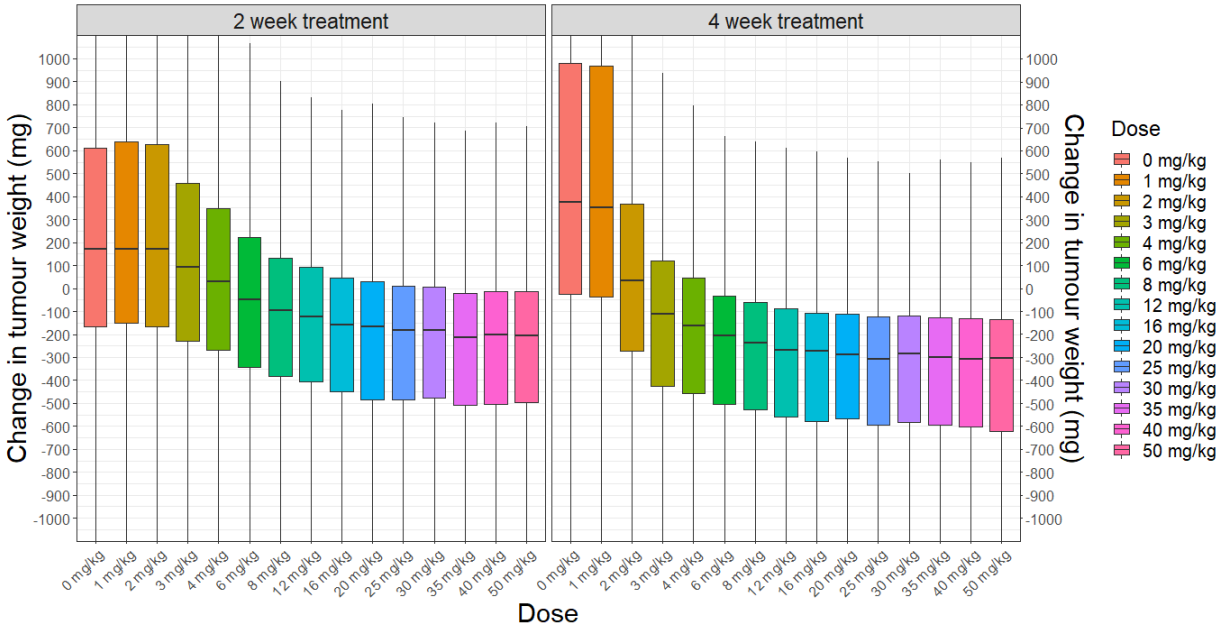

Figure 15 - Boxplot: Change in tumour weight (mg), after 2 weeks (*left*) or 4 weeks (*right*) of treatment with different doses of the drug

Figure 15 shows the difference in tumour weight after 2 weeks or 4 weeks of treatment with a variety of different doses. The change in tumour weight is calculated by subtracting from the

initial tumour weight (at week 6) the value of the tumour weight at the end of treatment (either week 8 or week 10). In agreement with Figure 13, from 0 mg/kg to 2 mg/kg, there does not seem to be a biological effect. In fact, in spite of treatment, the tumour weight rises, in more than 50% of the cases (the median value is above 0 mg).

Similarly, from 3 mg/kg to 50 mg/kg, there seems to be some biological effect.

In the same way as Figure 13, above 16 mg/kg there does not seem to be an additional decrease in tumour weight.

Table 6 shows the change in tumour weight after 2 weeks or 4 weeks of treatment with three different doses of the drug.

Dose	Treatment duration	Tumour weight (mg)				% Decrease	% Decrease with 2 extra weeks
		Week 6	Week 8	Week 10	Difference		
4 mg/kg	2 weeks	397	428	-	31	-8	**53**
	4 weeks		-	219	-178	45	
8 mg/kg	2 weeks		286	-	-111	28	**39**
	4 weeks		-	133	-264	66	
12 mg/kg	2 weeks		209	-	-188	47	**28**
	4 weeks		-	97	-300	76	

Table 6 – Comparison of the change in tumour weight after 2 weeks or 4 weeks of treatment with 4 mg/kg, 8 mg/kg and 12 mg/kg

If we look carefully at Table 6, it is possible to acknowledge that with 2 extra weeks of treatment, there is an additional decrease in tumour weight. This effect becomes less pronounced with higher doses of the drug. For instance, after 4 weeks of treatment with 4 mg/kg, there is a 53% extra decrease in tumour weight, compared with 2 weeks of treatment. However, after 4 weeks of treatment with 12 mg/kg, the percentage of extra decrease is about 28%.

Moreover, in the column "% Decrease" the negative sign means that the tumour weight increased, rather than decreased.

Study Design – Translating from mouse to human

In the previous section two different methods were presented, in order to calculate the human dose: Human Equivalent Dose (HED) Method, which is the one recommended by the FDA, and Allometric Scaling, which is the method that is going to be applied in the study design calculations. The key difference between these two methods is that the former is based on body surface area, and the latter is based on body size and change in clearance. Therefore, Allometric Scaling was the chosen method because it makes it possible to scale PK parameters directly and to simulate concentration-time profiles.

The following formulas, described in the "Methods" section, are going to be used in the calculations, more specifically to convert an animal dose (mg/kg) into a human dose (mg):

$$HED\ (mg) = Animal\ dose\ (mg/kg) \times \left(\frac{W_{animal}\ (kg)}{W_{human}\ (kg)}\right)^{0.33} \times W_{human}\ (kg) \quad (5)$$

for the HED calculations, and:

$$Human\ dose\ (mg) = Animal\ dose\ (mg/kg) \times W_{animal}\ (kg) \times \left(\frac{W_{human}\ (kg)}{W_{animal}\ (kg)}\right)^{0.75} \quad (6)$$

for the allometric scaling calculations.

In both methods, the average mouse weight is considered to be 0.020 kg (50).

Before moving on to the study design calculations, Table 7 shows an example of the difference between human doses calculated using both methods, for different human weights.

Human weight (kg)	Human Dose (translated from a 10 mg/kg animal dose)	
	HED (mg)	Allometry (mg)
10	13	21
20	20	36
30	27	48
40	33	60
70	47	91
100	60	119
Ratio between the doses for the 100-kg person and 10-kg person	$\frac{60}{13} = 4.6$	$\frac{119}{21} = 5.7$

Table 7 - Conversion from a Mouse Dose (mg/kg) to a Human Dose (mg), obtained by two different methods: Human Equivalent Dose (HED) and Allometric Scaling; comparision of the doses for a 10-kg child and a 100-kg person

Also, Table 7 shows that in allometric scaling, the ratio between the doses for a 10-kg child and a 100-kg person is slightly higher than with the HED method.

As for the trial design calculations, it will be used an allometric scaling approach in order to translate the doses from mouse to human, using the following formula, explained before:

$$Human\ dose\ (mg) = Animal\ dose\ (mg/kg) \times W_{animal}\ (kg) \times \left(\frac{W_{human}\ (kg)}{W_{animal}\ (kg)}\right)^{0.75} \quad (6)$$

Table 7 shows an example of which dose would be administered according to different human weights, from 10 to 100 kg. For instance, for a group of children that weigh 30 kg, the increasing doses in the clinical trial would be 14 mg (dose 1), 19 mg (dose 2), 29 mg (dose 3), 39 mg (dose 4), 58 mg (dose 5) and 77 mg (dose 6).

Mouse dose (mg/kg)	Human dose (mg), for different human weights					
	10 kg	20 kg	30 kg	40 kg	70 kg	100 kg
3	6	11	14	18	27	36
4	8	14	19	24	36	48
6	13	21	29	36	55	71
8	17	28	39	48	73	95
12	25	43	58	72	109	143
16	34	57	77	96	146	190

Table 8 - Conversion from a Mouse Dose (mg/kg) to a Human Dose (mg), obtained by an Allometric Scaling approach; the highlighted column represents the values that are going to be used afterwards in the study design example

Figure 16 shows an example of the study design. In total, 16 subjects are planned to be enrolled and receive active investigational medicinal product. Before the beginning of the trial, there will be an initial screening that will last at least 1 week. After that, the 16 subjects will be divided into 4 cohorts of 4 patients each. In each cohort there will be 3 increasing doses. Each cohort of patients will receive the drug for 2 weeks, through a parenteral route.

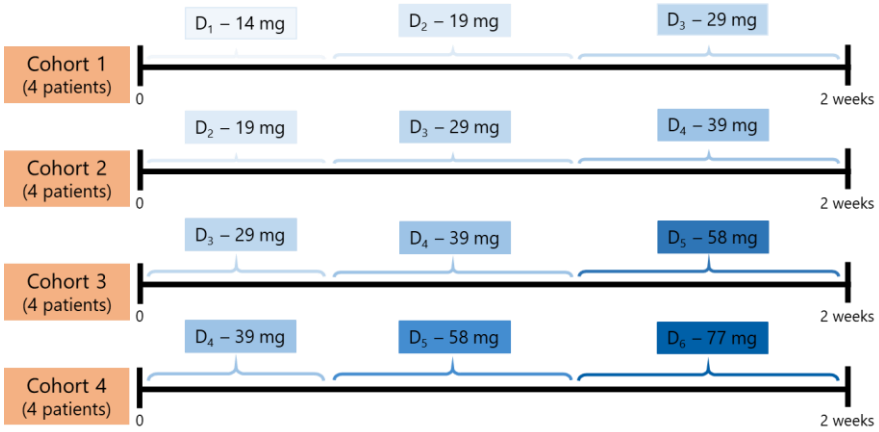

Figure 16 - Example of a study design, in a group of children with a body weight of 30 kg; there will be 4 cohorts of 4 patients each; in each cohort, 3 increasing doses of BGA002 will be administered; the trial duration will be 2 weeks

The dose that is going to be administered will vary according to the subject's weight (Table 8). For instance, for a subject that weighs 30 kg and belongs to cohort 1, the first dose of BGA002 that is going to be administered at day 1 is 14 mg. After the first dose, PK sampling will be collected and analysed to allow for dose adjustments depending on individual patient clearance values. Intra-patient titration will be used to ensure characterisation of the pharmacological effects (inhibition of *MYCN* expression) within each patient. PK and PD assessments will take place at days 1, 3, 5, 8 and 14 (Figure 17).

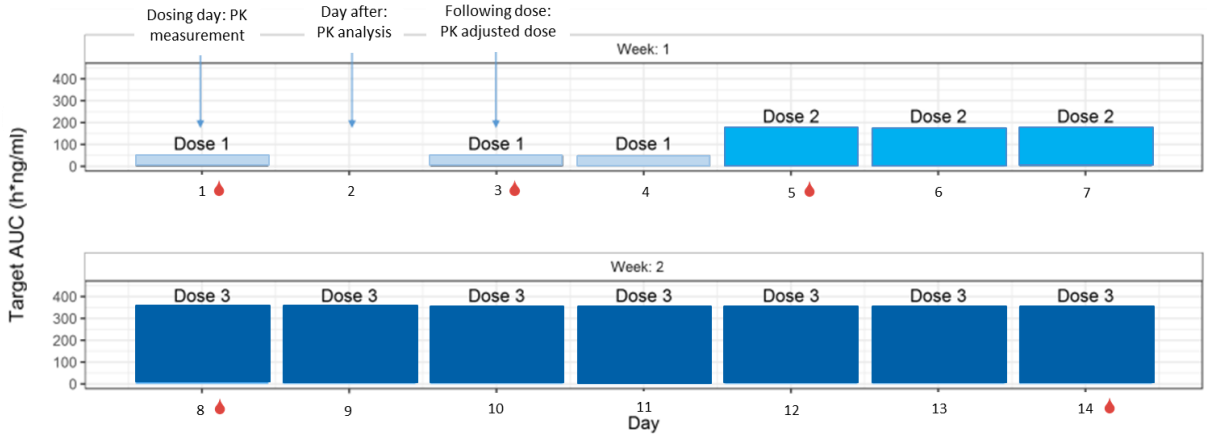

Figure 17 - Example of an adaptive trial design in a cohort of 4 patients; on day 1, these 4 patients will receive dose 1, according to their weight; treatment duration will be 2 weeks per cohort; PK and PD assessments are represented by a blood drop, in days 1, 3, 5, 8 and 14. Each bar represents the target AUC to be reached at each dose level during intra-patient titration

For instance, that 30-kg subject will receive those 14 mg of BGA002 at day 1; PK measurements will also occur at day 1. In the following day, there will be a PK analysis to evaluate and possibly adjust the previous administered dose. Then, at days 3 and 4, there is another administration of dose 1 (14 mg) or a similar dose adjusted according to PK analysis.

Afterwards, at day 5, the dose is increased: in this subject, dose 2 would be 19 mg. This process would be followed through the rest of the 2-week trial.

5.2 – Clinical Study Protocol

Additionally, a clinical study protocol was elaborated: "A Double-Blind Phase I Study To Evaluate The Safety And Efficacy of BGA002 for the Treatment of Paediatric High-Risk Neuroblastoma".

Study Objectives and Purpose

Primary objectives

The primary objectives of this study are:

- To investigate the safety and tolerability of BGA002 in paediatric patients with high-risk neuroblastoma;
- To define the recommended phase II dose of BGA002.

Secondary objectives

The secondary objectives of this study are:

- To investigate the blood pharmacokinetics of BGA002 in paediatric patients with high-risk neuroblastoma;
- To estimate the acute and long-term toxicities of BGA002 for neuroblastoma;
- To investigate differences in *MYCN* expression reflective of efficacy during treatment with BGA002 in paediatric patients with high-risk neuroblastoma.

Selection and Withdrawal of Subjects

Patient population

Patients aged ≥ 3 years with high-risk neuroblastoma refractory to previous treatment, with *MYCN* amplification

Sample size

16 patients (number of subjects considered to be adequate, by BIOGENERA SpA)

Eligibility criteria

The Inclusion Criteria are the following:

- Subjects under study must be paediatric patients with a diagnosis of high-risk neuroblastoma. For the purposes of this study, the following definitions apply:
    - High-risk: any patient with *MYCN*-amplified neuroblastoma
- Diagnosis must be genetically confirmed
- Subjects must be male or female aged 3 years to 17 years

- Subjects must have a WHO performance status score of 0,1 or 2, or Lansky-Play Scale of 50% or greater at screening (45)
- Subject's legally authorised representative (LAR) must provide written informed consent and there must be written consent or assent (as age applicable and developmentally appropriate) by the subject before any study-related procedures are conducted
- Adequate pulmonary and cardiac function
- Adequate haematological, kidney and liver function:
  - ✓ Serum alanine transaminase (ALT) ≤ 2.5 x upper limit of normal (ULN)
  - ✓ Total serum bilirubin ≤ 1.5 x ULN
  - ✓ Absolute neutrophil count (ANC) ≥ 1500/μL
  - ✓ Platelets ≥ 100,000/μL
  - ✓ Haemoglobin ≥ 9.0 g/dL
  - ✓ Creatinine ≤ 1.5 x ULN
- Female patients of childbearing potential: documented negative pregnancy test and agreement to use effective contraception during treatment period
- National and, when needed, local ethical approval

The Exclusion Criteria are the following:

- Receiving other medications/therapies not stable (changed) within 4 weeks prior to the study
- Medical illness or other concern which would cause investigator to conclude subjects will not be able to perform the study procedures or assessments or would confound interpretation of data obtained during assessment
- Current enrolment in a clinical trial of an investigational drug or enrolment in a clinical trial of an investigational drug in the last 6 months
- Gastrointestinal disease which may interfere with the absorption, distribution, metabolism or excretion of the study medication and impact the interpretability of the study results
- BMI of less than 14.0 kg/m^2 or greater than 40.0 kg/m^2 at screening
- Patient has an acute or known chronic liver disease (e.g., chronic active hepatitis, cirrhosis)
- Patient has a known diagnosis of human immunodeficiency virus (HIV) infection
- Female patients who are pregnant or breast-feeding

Trial Design

This trial is a double-blind phase I study that aims to evaluate the safety and efficacy of BGA002 for the treatment of paediatric high-risk neuroblastoma.

Phase I trial designs are grouped into rule-based and model-based designs.

Traditionally, phase I trial designs are rule-based, such as the 3 + 3 design, where the dose-escalation or de-escalation depend on the toxicity of the current dose, through assigning groups of 3 subjects to a dose level (52).

In contrast, model-based designs estimate the dose-toxicity relationship with a statistical model. The model is used to assign patients to dose levels and to guide dose escalation; data from all enrolled patients are used. Even though the traditional 3 + 3 design is simpler and it is usually preferred by many investigators, model-based designs are more accurate, faster, cheaper and generally safer to establish the recommended phase II dose than rule-based designs. Therefore, a model-based design is going to be used (53,54).

To sum up, the selected dose range is 3-16 mg/kg and the treatment with the drug will last for 2 weeks.

Primary Outcome Measures

- Tumour response, based on changes in tumour size and the number of *MYCN* copy number

Secondary Outcome Measures

- Progression-free survival
- Overall Survival
- Safety and tolerability
- Pharmacokinetic profile
- Pharmacodynamics of BGA002
- Pharmacogenetics

Study design

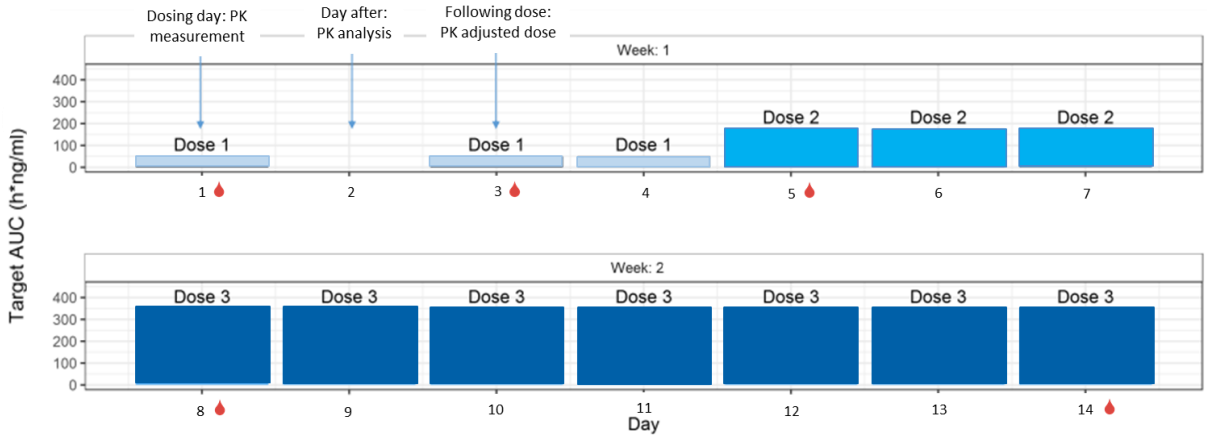

Figure 18 - Example of an adaptive trial design in a cohort of 4 patients; on day 1, these 4 patients will receive dose 1, according to their weight; treatment duration will be 2 weeks per cohort; PK and PD assessments are represented by a blood drop, in days 1, 3, 5, 8 and 14. Each bar represents the target AUC to be reached at each dose level during intra-patient titration

6 – DISCUSSION

In the previous section, modelling and simulations (M&S) proved to be an extremely useful tool in clinical drug development. A model-based approach allows clinical drug developers to explore different doses, treatment durations, trial designs, using only a software and a model that was created based on preclinical data. Without M&S, we would not be able to simulate and predict exposure-response relationships. In the previous graphs and tables, only some doses were tested. However, with the model that was built by Dr. Luca (40), it is possible to interpolate and extrapolate to other doses, to determine which dose is the safest and most effective. In fact, the model demonstrated that BGA002 influences tumour growth leading to a decrease in tumour weight, depending on drug concentration. Drug concentration affects *MYCN* expression and, consequently, tumour cells viability (40).

Based on the results from FiguresFigure 12 and Figure 13, the selected dose range is 3-16 mg/kg. An evaluation of the data suggests that until 2 mg/kg there does not seem to be an observed effect. Moreover, from 16 mg/kg until 50 mg/kg there does not seem to be an added efficacy, which could be explained by some biological processes. One reason for this could be the limited death rate of tumour cells. This means that from 16 mg/kg, the death rate of tumour cells reaches a limit that does not go any higher, regardless of increasing doses of BGA002. In addition, higher doses with no apparent additional efficacy could mean an unnecessary increase of toxicity and consequent adverse events.

In what regards Figures Figure 14 and Figure 15, the aim was to explore different treatment durations, taking into account efficacy, safety and trial costs. Based on the previous data, the 1-week treatment was excluded, since there is no observed effect on the tumour weight. Regarding the 8-week treatment, even though there is a clear decline of the tumour weight, factors such as uncertain safety issues and high costs probably would not make a 14-week trial the best option.

As for the 2-week and 4-week treatment, Figure 15 suggests that in both cases there is a decrease in the tumour weight. Now, does the change in tumour weight become much greater, with two extra weeks of treatment? Surely, if we look at Figure 14 and Table 6, we notice that the 4-week treatment seems to have a greater impact in the tumour weight and, consequently, higher efficacy. However, how much higher is the efficacy? Is it worth it, does it justify a longer duration and higher costs of the trial? Or is the 2-week treatment enough to observe an effect in the tumour weight?

Table 6 provides a clearer view on this question, since it compares the difference in tumour weight after 2 weeks and 4 weeks of treatment. Indeed, 2 extra weeks of treatment do cause a higher percentage of decrease in tumour weight. For instance, after 4 weeks of treatment with 8 mg/kg, there is a 66% decrease in tumour weight, compared with a 28% decrease in tumour weight after 2 weeks of treatment with the same dose. Therefore, 2 additional weeks of treatment provide an extra 39% decrease in tumour weight. It is tempting to say that the 4 weeks of treatment is the best option for this trial. Nevertheless, in my opinion, this does not necessarily mean that there is an improvement on the benefit-risk ratio. Firstly, it is important to remind that this is not only a first-in-human trial, so there is little information about safety in humans, but also that the trial subjects are children. According to the EMA Guidelines, clinical trials in children should be designed in order to be as safe as possible. Hence, 2 additional weeks of treatment could mean a higher risk for the trial subjects. Secondly, the model was created based on preclinical studies that went on for 2 weeks. Thus, extrapolating to 4 weeks would mean an increased uncertainty. Finally, extending the treatment duration for 2 additional weeks would have an impact on the trial costs and duration.

Overall, in my view, the 2-week treatment is the best choice for the treatment duration, since it showed an efficacy in decreasing the tumour size, it is safer and it takes almost half of the time and it costs about half as much, comparing to the 4-week treatment. To sum up, the selected dose range is 3-16 mg/kg and the treatment with the drug will last for 2 weeks. If a 2-week treatment proves to be safe enough, there is the possibility of exploring longer treatment durations such as a 4-week treatment, in a future trial.

Furthermore, in the clinical trial, an intra-patient dose escalation will be performed. According to the EMA, an intra-patient dose escalation is recommended when the adult MTD is unknown. That is the case with neuroblastoma, since it almost exclusively affects children. An intra-patient dose escalation increases the chance of the patient receiving an effective dose. One drawback of this strategy is that it may mask possible cumulative toxic effects of the drug, or make them harder to differentiate from chronic or delayed toxic effects.

However, using M&S also has some limitations. For instance, the validation of the work is a difficult task, since there is no real data we can use to validate and confirm our results. Moreover, the model was built based on preclinical studies such as *in vitro* experiments on neuroblastoma cell lines and *in vivo* experiments in mice. Even though human cell lines were studied, the preclinical data obtained do not reflect the actual response that children with neuroblastoma would develop. One reason for this is that the animal models did not have any immune response, so the results do not entirely correspond to the real response that would be obtained in humans.

Despite these limitations, the results obtained through M&S are still a good starting point to further plan and conduct a phase I clinical trial in children with high-risk neuroblastoma. Without M&S, it would be a much more difficult challenge to answer some questions related, for example, to which initial dose should be administered, how long should the treatment last, and which trial design should be conducted.

Taking into account the preclinical data and the simulations performed, BGA002 seems to be a promising compound for neuroblastoma therapy, due to its effect on *MYCN* inhibition and on the reduction of the tumour weight. However, there is still a lack of information as to which other cellular pathways might be involved in neuroblastoma development. For that reason, besides the *MYCN* gene, other biomarkers involved in tumour growth and tumour viability should be explored.

Having in mind that the current standard of care for children with high-risk neuroblastoma is based on intensive chemotherapy, the development of a molecularly targeted agent such as BGA002 would be an enormous advantage for these patients. Ideally, administering these patients with a specific agent, would induce the death of neuroblastoma cells, while posing minimal harm to healthy cells. Moreover, the possibility of combining BGA002 with traditional cytotoxic drugs would represent a new option for the management of neuroblastoma patients. This combinational strategy would not only provide a synergistic effect, but also reduce the dose of the cytotoxic drug, which would result in a better safety profile and consequently, fewer adverse events, which is particularly important in the paediatric population.

7 – CONCLUSION

The clinical development of drugs in rare diseases and in the paediatric population poses many challenges, mainly related to a lack of information about the disease, drug safety concerns and a limited number of patients. One way to circumvent some of these difficulties is through the implementation of modelling and simulations (M&S). M&S may prove to be an extremely helpful tool to predict human PK/PD data, extrapolate data from adults to children, interpolate data between different paediatric age groups, and explore different doses, treatment durations and trial designs. Additional benefits of M&S include substantial savings in animal’s lives, time and costs of the trials, while still yielding good-quality results that meet the legal requirements.

The implementation of M&S is encouraged by the European Medicine’s Agency, to provide complementary PK/PD information and guide the process of dose selection and dose escalation. The EMA also supports the use of an allometric scaling approach to predict human PK, including the PK of different paediatric age subsets.

To sum up, the use of a model-based approach, compared to a traditional approach, is essential in the planning and conduct of first-in-human clinical trials. In my opinion, a wider implementation of M&S in future clinical trials could be driven by an increased flexibility of drug developers and further discussion by the regulatory authorities.

REFERENCES

1. European Parliament and the Council of the EU. Regulation (EC) Nº 141/2000 on orphan medicinal products. Off J Eur Union. 2000;
2. European Medicines Agency. Orphan designation: Overview. [cited 2020 Feb 19]; Available from: https://www.ema.europa.eu/en/human-regulatory/overview/orphan-designation-overview
3. EURORDIS-Rare Diseases Europe [Internet]. Available from: https://www.eurordis.org/about-rare-diseases
4. Kaufmann P, Pariser AR, Austin C. From scientific discovery to treatments for rare diseases - the view from the National Center for Advancing Translational Sciences - Office of Rare Diseases Research. Orphanet J Rare Dis. 2018;13(1):1–8.
5. NORD - National Organization for Rare Disorders. Available from: https://rarediseases.org/
6. Food and Drug Administration Safety and Innovation Act (FDASIA). Food and Drug Administration; 2012 p. 1–140.
7. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE. Overview and recent advances in the treatment of neuroblastoma. Expert Rev Anticancer Ther [Internet]. 2017;17(4):369–86. Available from: http://dx.doi.org/10.1080/14737140.2017.1285230
8. European Medicines Agency. Public summary of opinion on orphan designation: Temozolomide for the treatment of neuroblastoma. 2019;(December). Available from: https://www.ema.europa.eu/en/documents/orphan-designation/eu/3/19/2188-public-summary-positive-opinion-orphan-designation-temozolomide-treatment-neuroblastoma_en.pdf
9. Logviss K, Krievins D, Purvina S. Characteristics of clinical trials in rare vs. common diseases: A register-based Latvian study. PLoS One [Internet]. 2018;13(4):1–14. Available from: http://dx.doi.org/10.1371/journal.pone.0194494
10. Kempf L, Goldsmith JC, Temple R. Challenges of developing and conducting clinical trials in rare disorders. Am J Med Genet. 2018;176(4):773–83.
11. Brasil S, Pascoal C, Francisco R, Ferreira VDR, Videira PA, Valadão G. Artificial intelligence (AI) in rare diseases: Is the future brighter? Genes (Basel). 2019;10(12).
12. Carlier A, Vasilevich A, Marechal M, De Boer J, Geris L. In silico Clinical Trials for Pediatric Orphan Diseases. Sci Rep [Internet]. 2018;8(1):1–9. Available from: http://dx.doi.org/10.1038/s41598-018-20737-y
13. Bellanti F, Della Pasqua O. Modelling and simulation as research tools in paediatric drug development. Eur J Clin Pharmacol. 2011;67(SUPPL. 1).
14. ICH International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. E11 (R1) Addendum to ICH E11: Clinical Investigation of Medicinal Products in the Pediatric Population. ICH; 2017.
15. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. Nat Rev Dis Prim. 2016;2.

16. European Medicines Agency. Modelling and simulation: questions and answers [Internet]. 2018. Available from: https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/clinical-pharmacology-pharmacokinetics/modelling-simulation-questions-answers#paediatrics-section
17. Guideline on the Evaluation of Anticancer Medicinal Products in Man. Eur Med Agency [Internet]. 2017;(September). Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/10/WC500195215.pdf
18. Gilbert SF. Developmental Biology. 8th ed. Sinauer Associates Inc.; 2006.
19. Nakagawara A, Li Y, Izumi H, Muramori K, Inada H, Nishi M. Neuroblastoma. Jpn J Clin Oncol. 2018;48(3):214–41.
20. Marshall GM, Carter DR, Cheung BB, Liu T, Mateos MK, Meyerowitz JG, et al. The prenatal origins of cancer. Nat Rev Cancer. 2014;14(4):277–89.
21. Heck JE, Ritz B, Hung RJ, Hashibe M, Boffetta P. The epidemiology of neuroblastoma: A review. Paediatr Perinat Epidemiol. 2009;23(2):125–43.
22. Cheung NK V., Dyer MA. Neuroblastoma: Developmental biology, cancer genomics and immunotherapy. Nat Rev Cancer. 2013;13(6):397–411.
23. Zeid R, Lawlor MA, Poon E, Reyes JM, Fulciniti M, Lopez MA, et al. HHS Public Access. 2018;50(4):515–23.
24. Smith V, Foster J. High-Risk Neuroblastoma Treatment Review. Children. 2018;5(9):114.
25. Montemurro L, Raieli S, Angelucci S, Bartolucci D, Amadesi C, Lampis S, et al. A novel MYCN-specific antigene oligonucleotide deregulates mitochondria and inhibits tumor growth in MYCN-amplified neuroblastoma. Cancer Res. 2019;79(24):6166–77.
26. G. Gardner D, M. Shoback D. Greenspan's Basic and Clinical Endocrinology. In: 10th ed. McGraw-Hill Education; 2017. p. 924.
27. Maclagan K. The Association for Clinical Biochemistry and Laboratory Medicine [Internet]. [cited 2020 May 25]. Available from: http://www.acb.org.uk/Nat Lab Med Hbk/Dopamine.pdf
28. Abeling NGGM, Goorden SMI, Fiocco M, Tytgat GAM. Catecholamines profiles at diagnosis : Increased diagnostic sensitivity and correlation with biological and clinical features in neuroblastoma patients. Eur J Cancer. 2017;72:235–43.
29. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging system: An INRG Task Force report. J Clin Oncol. 2009;27(2):298–303.
30. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. Lancet. 2007;369(9579):2106–20.
31. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33(27):3008–17.
32. Herd F. Treatment of Patients with Low / Intermediate Risk Neuroblastoma. 2015;2(JANUARY).

33. High-Risk Neuroblastoma Study 1 of SIOP-Europe. 2009.

34. Elliott M, Gray J, Tweddle D, Gaze M, Ramanujachar R, Wheeler K, et al. CCLG Neuroblastoma SIG statement: Management of high-risk neuroblastoma. V1.0 Treatment and management of newly diagnosed patients with high-risk neuroblastoma (not patients currently registered on HR-NBL-1) Statement from CCLG Neuroblastoma SIG. 2017;(June):1–5. Available from: http://www.cclg.org.uk/write/MediaUploads/Member area/Treatment guidelines/CC

35. Peinemann F, Kahangire DA, Dalen EC Van, Berthold F. Rapid COJEC versus standard induction therapies for high-risk neuroblastoma. Cochrane Libr. 2015;1–85.

36. Elliott M, Gray J, Tweddle D, Gaze M, Ramanujachar R, Wheeler K, et al. Statement from CCLG Neuroblastoma SIG: Treatment and management of patients with high-risk neuroblastoma. 2019;(March). Available from: https://www.cclg.org.uk/write/MediaUploads/Member area/Treatment guidelines/Ne

37. Huang M, Weiss WA. Neuroblastoma and MYCN. Cold Spring Harb Perspect Med. 2013;3(10).

38. Pellestor F, Paulasova P. The peptide nucleic acids (PNAs), powerful tools for molecular genetics and cytogenetics. Eur J Hum Genet. 2004;694–700.

39. Gupta A, Mishra A, Puri N. Peptide nucleic acids: Advanced tools for biomedical applications. J Biotechnol. 2017;395(July):148–59.

40. Ruotolo L. A rational-based PK/PD model for MYCN anti-gene PNA as a novel therapeutic strategy for the treatment of neuroblastoma in children. 2019.

41. Braun K, Peschke P, Pipkorn R, Lampel S, Wachsmuth M, Waldeck W, et al. A Biological Transporter for the Delivery of Peptide Nucleic Acids (PNAs) to the Nuclear Compartment of Living Cells. J Mol Biol. 2002;318(2):237–43.

42. European Medicines Agency. Public summary of opinion on orphan designation 16-base single-stranded peptide nucleic acid oligonucleotide linked to 7- amino acid peptide for the treatment of neuroblastoma. 2014;(March). Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2009/10/WC500006083.pdf

43. European Parliament and the Council of the EU. Regulation (EC) Nº 536/2014 clinical trials on medicinal products for human use. Off J Eur Union. 2014;

44. ICH International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH E8 - General Considerations for Clinical Trials. Reproduction. 1998;(March).

45. Note for Guidance on Evaluation of Anticancer Medicinal Products in Man - Addendum on Paediatric Oncology. Eur Med Agency. 2004;(January).

46. Guideline on Clinical Trials in Small Populations [Internet]. European Medicines Agency 2006. Available from: https://www.ema.europa.eu/en/clinical-trials-small-populations

47. Mahmood I. Misconceptions and issues regarding allometric scaling during the drug development process. Expert Opin Drug Metab Toxicol [Internet]. 2018;14(8):843–54. Available from: https://doi.org/10.1080/17425255.2018.1499725

48. Huang S-M, Lertora J, Atkinson Jr. A, Markey S. Principles of Clinical Pharmacology.

In: 3rd ed. Academic Press; 2012. p. 652.

49. Mahmood I. Initiation of Pediatric Clinical Trials for Coagulation Factors: Application of Pharmacokinetics and Allometry to First-in-Pediatric Dose Selection. J Clin Pharmacol. 2019;59(6):829–34.

50. Center for Drug Evaluation and Research. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. US Dep Heal Hum Serv [Internet]. 2005;(July):1–27. Available from: http://www.fda.gov/downloads/Drugs/Guidance/UCM078932.pdf

51. Mahmood I. What is Allometric Scaling and How Does it Support Drug Development? [Internet]. 2019. Available from: https://www.nuventra.com/resources/blog/what-is-allometric-scaling-in-drug-development/

52. Wang Y, Wang J. Model Based vs. Rule Based Designs in Phase I Dose Finding Clinical Trials. Clin Trials Pract. 2016;1(1).

53. van Brummelen EMJ, Huitema ADR, van Werkhoven E, Beijnen JH, Schellens JHM. The performance of model-based versus rule-based phase I clinical trials in oncology. J Pharmacokinet Pharmacodyn. 2016;43(3):235–42.

54. Frangou E, Holmes J, Love S, McGregor N, Hawkins M. Challenges in implementing model-based phase I designs in a grant-funded clinical trials unit. Trials. 2017;18(1):1–7.