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# Studying Side Effects of Tyrosine Kinase Inhibitors in a Juvenile Rat Model with Focus on Skeletal Remodeling

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## Abstract

The tyrosine kinase (TK) inhibitor (TKI) imatinib provides a highly effective treatment for chronic myeloid leukemia (CML) targeting at the causative oncogenic TK BCR-ABL1. However, imatinib exerts off-target effects by inhibiting other TKs that are involved, e.g., in bone metabolism. Clinically, CML patients on imatinib exhibit altered bone metabolism as a side effect, which translates into linear growth failure in pediatric patients. As TKI treatment might be necessary for the whole life, long-term side effects exerted on bone and other developing organs in children are of major concern and not yet studied systematically. Here, we describe a new juvenile rat model to face this challenge. The established model mimics perfectly long-term side effects of TKI exposure on the growing bone in a developmental stage-dependent fashion. Thus, longitudinal growth impairment observed clinically in children could be unequivocally modeled and confirmed. In a “bench-to-bedside” manner, we also demonstrate that this juvenile animal model predicts side effects of newer treatment strategies by second generation TKIs or modified treatment schedules (continuous vs. intermittent treatment) to minimize side effects. We conclude that the results generated by this juvenile animal model can be directly used in the clinic to optimize treatment algorithms in pediatric patients.

**Keywords:** juvenile, growth, bone, tyrosine kinase inhibitor, side effects, CML

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## 1. Introduction

The introduction of tyrosine kinase inhibitors (TKIs) for targeted treatment of chronic myeloid leukemia (CML) marked a paradigm shift in the field of hemato-oncology [1, 2]. However, soon after CML became most successfully treated cancer—first in adults and thereafter in

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children—it was learned that chronic exposure to TKIs impaired modeling of the osseous skeleton as an off-target effect [3]. This skeletal side effect resulted in impaired longitudinal growth in not outgrown minors [4, 5]. With regard to a potential lifelong necessity of TKI intake, children with CML differ from a typical patient with CML who is about 60 years old [6]. Thus, the rationale of the research of TKIs' off-target effects is to generate a clear picture of early and late sequelae of long-term drug intake.

On this background, the essential objective of this chapter is the description of a juvenile (still growing) rat model that allows a chronic administration of TKIs via the drinking water in order (i) to mimic osseous changes observed in humans, (ii) to further characterize and investigate the causative pathophysiological mechanisms resulting in impaired bone growth, (iii) to test approaches in growing animals for ameliorating the off-target effect resulting in growth impairment, and (iv) to check further organs beside bone for long-term TKI toxicity.

In this chapter, sections describe i) the highly effective role that TKIs play in standardized attempts to operationally cure CML in adults as well as in children, ii) elucidate the role of the established juvenile male Wistar rat model to investigate with ease the skeletal changes at all developmental stages, and iii) focus on the administration of TKI via the drinking water over many weeks as an adequate and convenient way resulting in the achievement of therapeutic drug blood levels. TKI-induced changes in long bones, as well as vertebrae, can be investigated with dedicated small imaging devices while blood levels of bone turnover markers, growth hormone, and vitamin D metabolites can be followed at different stages of development. The results of these investigations as well as the derived hypothesis on the pathophysiological cascade, specifically how TKIs impair longitudinal bone growth, are in excellent agreement with clinical observations. In addition, the juvenile animal model is of value to monitor other long-term TKI side effects on the heart and fertility to generate an overall picture on all possible side effects.

## 2. Role of tyrosine kinase inhibitors in chronic myeloid leukemia treatment

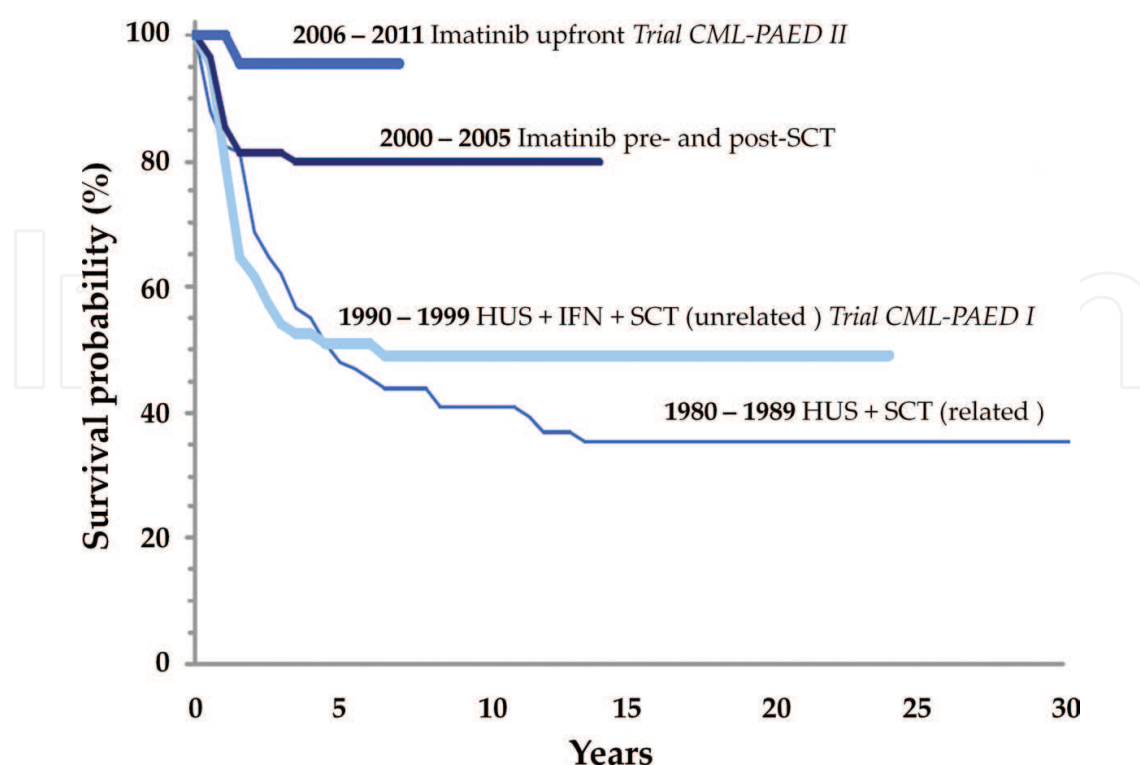
The principal function of tyrosine kinases (TKs) involves the regulation of multicellular aspects of the organism. By transferring a  $\gamma$ -phosphate group from adenosine triphosphate (ATP) to the hydroxyl group of tyrosine residues on signal transduction molecules, cell-to-cell signals, including growth, differentiation, adhesion, motility, and death, are transmitted [7]. Around 90 TK genes have been identified in the human genome [8]. Based on kinase domain structure, 58 are of transmembrane receptor type and can be grouped into 20 subfamilies and 32 are of cytoplasmic non-receptor type which falls into 10 subfamilies [8, 9]. TK receptors play a role in either transmembranous or intracellular signal transduction as they act as relay points controlling intracellular signaling pathways. Non-receptor TKs exhibit no transmembrane protein domain and are located in the cytoplasm. Generally, they are involved in signaling downstream of the receptor TKs.

In humans, TKs have been demonstrated to play significant roles in the development of many malignant diseases like chronic myeloid leukemia (CML) [8]. CML results from a reciprocal chromosomal translocation involving the c-abl proto-oncogene 1 (*ABL1*) on chromosome 9 and the breakpoint cluster region (BCR) on chromosome 22, thus forming the *BCR-ABL1*

oncogene [10, 11]. This t(9; 22) translocation or Philadelphia chromosome (Ph+) is a characteristic cytogenetic abnormality seen in 95% of patients with CML and in 15–30% of adult patients with acute lymphoblastic leukemia (ALL) [12, 13]. The *BCR-ABL1* oncogene codes for two forms of fusion transcripts: p190<sup>BCR-ABL1</sup> and p210<sup>BCR-ABL1</sup>, which are constitutively highly activated and subsequently dysregulate intracellular signaling by enhancing proliferative capability and resistance to apoptosis of hematopoietic stem or progenitor cells, leading to a massive increase in myeloid cell numbers.

About 1–1.5/100,000 residents are diagnosed with CML every year with an age peak between 50 and 60 years [14], representing around 20% of all cases of leukemia in adulthood [15]. Concerning pediatric patients, the frequency of diagnosis is about 0.05–0.40/100,000 residents per year within the age of 0–18 years [16]. Thus, CML represents one of the rarest leukemic disorders in childhood and adolescent age, accounting for only 2–3% of all children suffering from leukemia [16]. In terms of morphological characteristics, childhood CML is not different from adult CML. However, it is a matter of an ongoing debate whether and to what extend molecular differences exist between CML diagnosed at childhood or older age [6]. For example, pediatric CML shows a breakpoint distribution in the *BCR* gene more similar to adult Ph+ ALL [17].

Still, as the *BCR-ABL1* oncogene is the single molecular aberration causing the development of CML, specific TKIs like imatinib (Gleevec®, Novartis) have been developed to inhibit the BCR-ABL1 TK [19]. By achieving hematological and cytogenetic response in over 90% of the patients after a few months of imatinib treatment, imatinib has been very effective in inhibiting progression of CML (**Figure 1**) [1, 20–23].



**Figure 1.** Survival probabilities by year of diagnosis (1980–2013) of pediatric patients with CML in Germany [18].

However, some patients develop resistance to imatinib resulting in loss of treatment response or even leukemic relapse. Among other underlying mechanisms, BCR-ABL1 kinase domain mutations can cause varying degrees of drug insensitivity [24]. In order to counter these mechanisms, next generation TKIs have been developed like dasatinib (SPRYCEL®, Bristol-Myers-Squibb), nilotinib (Tasigna®, Novartis), bosutinib (BOSULIF®, Pfizer), and ponatinib (ICLUSIG®, Ariad Pharmaceuticals) with different affinities to the ATP-binding pocket of the BCR-ABL1 TK [25].

3. Imatinib as front-line treatment for pediatric CML

As imatinib has proven very effective in adult CML, its accelerated clinical approval was given in the year 2001 for adults with CML and without age restriction in 2003. In several studies, imatinib showed similar antileukemic efficacy in children compared to adults (Figure 1) [26, 27]. Typically, standard dose of imatinib (300 mg/m<sup>2</sup>) achieved a complete hematologic remission in 95% of the pediatric patients after 3 months, a complete cytogenetic remission in 80% after 12 months, and a major molecular remission (MR3.0 = 0.1 % ratio copy number of gene transcripts BCR-ABL1/control gene) in 60% after 18 months of treatment [16, 28].

Although imatinib acts relatively specifically against the dysregulated BCR-ABL1 TK, it is known that imatinib exerts off-target effects at therapeutic blood levels on other TKs (Table 1). The reason for this is the affinity of imatinib to the ATP-binding pocket of the kinase domain. This domain is a characteristic feature of many members of the kinome, including TKs (review of the human kinome: [29, 30], review structure of the TK: [8, 9]).

Nevertheless, imatinib treatment is generally well tolerated, showing mostly mild side effects. Neutropenia, thrombocytopenia, and anemia occur in up to 45, 20, and 10% of patients, respectively, who are in the chronic phase of CML and receive standard dose imatinib [2]. Nonhematologic adverse effects include nausea, skin rashes, peripheral edemas, muscle cramps, and elevated liver transaminase levels [2].

Studies with imatinib in adult patients also showed disturbed bone metabolism as a specific side effect [3] comprising altered calcium metabolism as well as increased trabecular mineralization and increased bone density in stamp biopsies [32]. In children, imatinib therapy has been associated with severe longitudinal growth retardation [4, 5, 33–41] (Figure 2), but the detailed mechanism how imatinib interferes with bone metabolism and the final consequences are not fully understood.

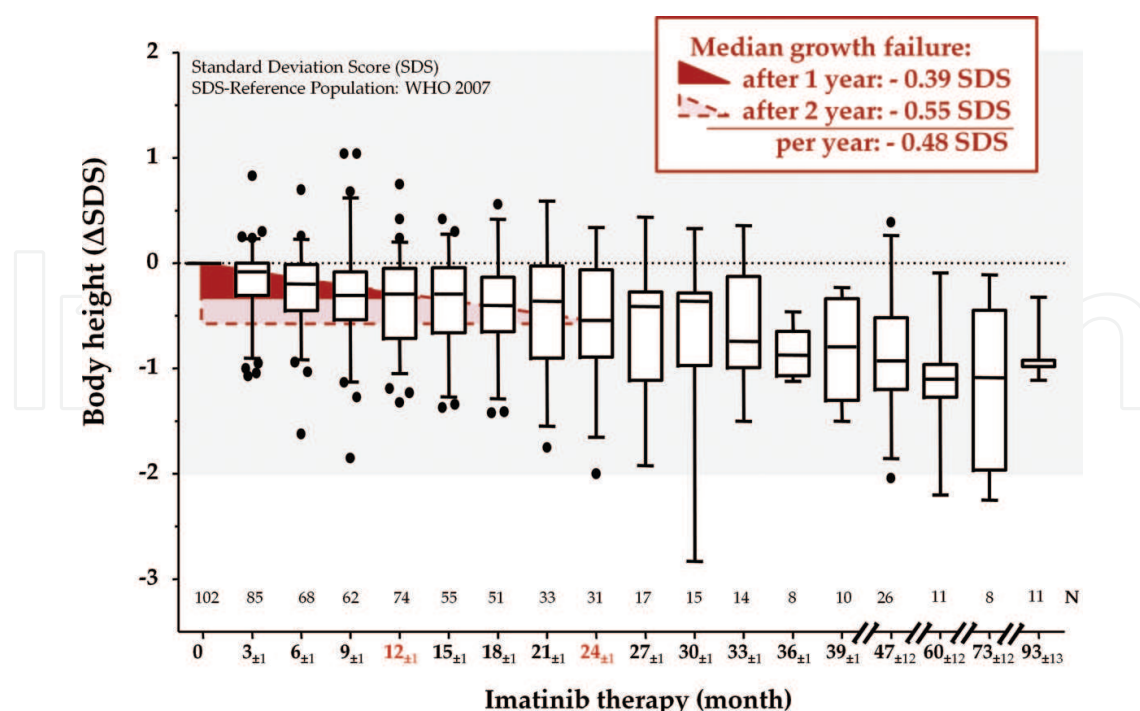
Regardless the type of kinase, imatinib binds to all structurally accessible ATP-binding pockets. Accordingly, other membrane-bound and cytosolic TKs, e.g., c-abl, PDGF-R α/β, c-KIT, and c-FMS [31, 42–44], are inhibited which play a major role in bone remodeling.

Tyrosine kinase	BCR-ABL1	c-abl	c-Kit	PDGF-Rα	PDGF-Rβ	c-FMS
IC <sub>50</sub> (μM)	0.25	0.19	0.15	0.10	0.39	1.42

IC, inhibitory concentration.

Table 1. Inhibitory effect of imatinib on selected TKs [31].





**Figure 2.** Growth failure in pediatric CML patients during imatinib treatment [4]. SDS: Standard deviation score. One hundred and two patients (54 male/48 female; median age 12 years, range: 1–18 years) at diagnosis of CML receiving imatinib as upfront treatment were enrolled retrospectively in the trial CML-PAED II during the period 02/2006 to 06/2014. Height standard deviation scores (SDS) were derived from WHO-AnthroPlus, version 1.04 software, a global growth-monitoring tool providing normal range values for the age cohorts from birth till 19 years. Eighty-one out of 102 patients fulfilled the criteria for continuous assessment of growth scheduled at 3- months intervals during imatinib exposure. Twenty-one patients were analyzed at intervals  $\neq$  3 months. Calculation:  $\Delta \text{SDS} = \text{SDS}_{\text{TKI therapy}} - \text{SDS}_{\text{Diagnosis}}$ ; data are shown as Whiskers box plot (median  $\pm$  5<sup>th</sup>/95<sup>th</sup> percentile).

Underlying dynamic processes of the growing skeleton are subject to strict regulation/communication of bone formation and resorption and can be easily influenced by interfering factors. At present, it is not possible to study simultaneously these complex bone remodeling processes such as the interaction of bone forming osteoblasts and bone resorbing osteoclasts by culturing systems *in vitro*. Therefore, it is only possible to study bone breakdown, bone structure, changes in the mineral content, and the overall structure of the bone *in vivo* in appropriate juvenile animal models.

In addition, TKI treatment for CML is not curative in most patients. Although first results from stopping TKI trials in adult patients after achieving sustainable deep molecular remission look promising most patients probably require a lifelong TKI treatment. This poses an increased risk to pediatric CML patients exposed to TKI treatment for decades as the long-term side effects on bone or other organs in a still growing organism presently are totally unknown.

#### 4. Juvenile animal model for chronic TKI exposure

All regulatory authorities (Food and Drug Administration (FDA), Health Canada, European Medical Agency (EMA)) require animal tests to be conducted before humans are exposed to a new molecular entity. In drug developmental process, every potential new therapeutic agent

has to pass clinical phase I-III studies in humans to verify safety, dosage, efficacy, side effects, and monitoring adverse reactions. All these studies are done in adult volunteers or adult patients if the disease under study occurs not exclusively at pediatric age [45]. Thus, in order to gain insight into side effects occurring specifically in the still growing organism during the preclinical research phase, *in vivo* studies in young growing animals are of main importance.

However, almost in all instances, primarily adult animal models are used in preclinical research (for reviews about the ongoing debate about animal models in clinical research see Refs. [46–48]). Adult animals were also used to study the influence of imatinib on the skeletal system [49, 50]. But as the growth process of the juvenile bone differs significantly from a mature bone, results described so far in adult patients/animals cannot readily be transferred to pediatric cohorts. Furthermore, Juvenile animal models cannot easily be selected as they are not established to match every single “research question” or disease on a routine basis.

Therefore, we describe here our established juvenile animal model to study side effects of a **chronic exposure** of imatinib primarily on the **growing bone** and to a lesser extent on other organs.

When establishing a juvenile animal model, several issues should be considered like the rodent species itself (mouse vs. rat), the strain (inbred vs. outbred), the overall speed of development (age when puberty starts), and convenient ways of drug administration in the situation of long-term exposure (intraperitoneal vs. subcutaneous vs. oral gavages vs. micro-osmotic pumps). Overall, the developmental stages must be comparable to human life.

#### 4.1. Mice versus rat

Most of the animals used in biomedical research are mice and rats because of their availability, ease of handling, and fast reproduction rate. Mice are an excellent model for human diseases because genetically they share 98% homology with human genome as well as a similar organization of their DNA and gene expression. However, the genome of a rat is smaller than its human equivalent but larger than that of a mouse.

Compared to mice, rats offer many advantages as, for example, their physiology is easier to monitor and is more like the corresponding human condition. But the most important advantage of the rat is its bigger size, not just because of the added ease to perform surgical procedures, but because of larger substructures (e.g., bone growth line, metaphysis) in organs thus influencing (i) which ratio of the organ is prone to an experimental lesion and (ii) the distance effects drugs exert to a specific anatomical area [51].

As we questioned about side effects of a chronic imatinib exposure on the growing organism, we were interested in the side effects on the long bones, which are much bigger in rats as compared to mice. As an additional benefit, we could also monitor side effects on other growing and developing organs like heart and testis as rats are a preferred model in cardiac and reproduction questions [52].

#### 4.2. Inbred versus outbred strain

In general, the difference between outbred and inbred strains lies in their genetic background. Inbred strains are characterized by almost 99% homogeneity of the genome resulting from a

long inbreeding of this strain, whereas outbred strains have a diverse genetic background. Due to this genetic characteristic, animals of inbred strains react nearly identical to a specific intervention, like medical treatment, surgery, etc., wherefore the influence of this intervention on a particular parameter can be identified more precisely. However, outbred strains reflect the natural situation more accurately as every individual is genetically different from the other. Every animal of an outbred strain will react slightly different to a specific intervention, which discloses all possible effects of this intervention on the metabolism and mimics more the situation in the clinic. Therefore, depending on the experimental question and if you need a genetic diversity in your test population, inbred or outbred strains are used. Our study focused on side effects of long-term TKI treatment on bone remodeling and to mimic the human situation, we choose juvenile rats of the outbred strain "Wistar."

#### **4.3. Male versus female**

We exclusively studied Wistar rats of male gender, as males tend to be more sensitive to bone influencing agents than female animals due to more rapid weight development and gender-specific hormones.

Prepubertal young Wistar rats triple their body weight, regardless of sex, from about 60 to 180 g in 14 days from the 3<sup>rd</sup> to 5<sup>th</sup> week of life due to the increasing growth hormone (GH) pulse amplitudes. The duration of GH pulses is significantly longer in males versus females, a pattern that continues throughout adulthood. Between 5<sup>th</sup> and 7<sup>th</sup> week of life, GH pulse amplitudes are similarly increased in both sexes [53, 54]. The rapid skeletal growth associated with this is particularly strongly influenced by interfering factors. In postpuberty, the growth slows down, especially in female rats, who weigh 200 g in the 8<sup>th</sup> week of life and 220 g in the 10<sup>th</sup> week of life. Contrary, male animals reach a body weight of 300 g postpubertally in the 8<sup>th</sup> week of life and 390 g in the 10<sup>th</sup> week of life. These differences in growth dynamics should also make postpubertal bone alterations due to TKI exposure more prominent in male animals.

Nevertheless, additional factors especially endocrine changes in hormones, such as testosterone, 17 $\beta$ -estradiol, and corticosterone, inducing and associated with the onset of puberty and puberty itself may be more important than GH to decide about the sex when setting up an animal model. It is commonly considered that puberty lasts until the 8<sup>th</sup> week of age [54]. However, onset of puberty in the rat (as measured by the age at vaginal opening and the onset of estrous cyclicity) occurs between 4<sup>th</sup> and 5<sup>th</sup> week in females, whereas in males (as measured by preputial separation which is an androgen-dependent event) occurs around 7<sup>th</sup> week of life depending on the strain used [55]. The onset of puberty in male Wistar rats based on the increase in plasma testosterone levels starts at 46–50 days of age and progressively increases until 76 days of age [56–58]. However, related to the increased production of estrogen and its positive influence on bone formation, trabecular bone density increases significantly both in women and in female rats with the onset of puberty [59, 60]. Because of this hormonal influence, effects on the bones, which are only mild, would be more difficult to detect in the female organism.

#### **4.4. Drug administration**

For chronic drug exposure, we choose administration via the drinking water. Drug application via subcutaneous (s.c.) or intraperitoneal injection (i.p.) or oral gavage is the most



accurate type of body weight-related exposure. However, young animals are prone to risks of injury and subsequent infection in the pharynx and/or esophagus [61, 62]. Micro-osmotic pumps could also be considered for s.c. administration but repeated implantation and removal of the pumps combined with the increased risk of infection should be taken into account [63]. For a detailed review of routes for chronic drug administration, see ref. [64]. However, due to the pharmacodynamics of the TKI, single shot by s.c. or i.p. administration would need at least two TKI applications daily over 10 weeks. Considering animal ethics as well as personal resources over several weeks including shifts on weekends, these numerous manipulations are hardly tolerable and affordable. For these reasons, the chosen intake of the drug via the drinking water was the most adequate and convenient form of chronic TKI exposure. Also, the stability of the TKIs in aqueous solution at room temperature facilitated this approach. Hence, the drug intake is dependent on the daily drinking volume considering age and associated body weight. Other possible interfering factors are loss of liquid when changing the water bottles or leaking water bottles and changes in the drinking behavior due to changes in the environment like fluctuation in the room temperature or humidity, or social conflicts between the animals. To counteract this, the care of the experimental animals, the measuring of the drinking volume, and the determination of weight gain were always carried out on a fixed schedule and by identical staff members including weekends.

Age-dependent drinking behavior of mammals varies. According to body weight, higher volumes are ingested by younger animals [65, 66]. Furthermore, rodents show a circadian rhythm of their food and drinking water intake. About 80% of the maximum daily intake of liquids occur at night [64]. This allows the conclusion that by administration via the drinking water, a peak level was achieved during the night, comparable to the single administration in human patients during the daytime.

4.5. Developmental stages

Due to the well-documented developmental stages of the rat, it is possible to carry out a comparison with human developmental stages in order to interpret the generated data in an orientated manner (Table 2).

Developmental stages	Rat	Human
Weaning	3 weeks	6 months
Puberty	7 weeks	12–14 years
Adolescent	8–11 weeks	15–20 years
Adult	>12 weeks	>20 years
Death	2–3 years	70–80 years

Table 2. Developmental stages of rat and human [67].

Considering the rapid maturation of the rats and the objective of examining the development (infancy, puberty, and young adulthood), we selected an exposure period of 10 weeks starting at 4 weeks of age.

Summing key issues in the juvenile animal model described, we chronically exposed healthy 4-week-old male Wistar rats to varying concentrations (low dose vs. high dose) of imatinib via drinking water over a period of 10 weeks while growing. We applied different treatment schedules to mimic possible new treatment strategies (continuous vs. intermittent). During the entire exposure time, the developmental stages from the end of weaning until young adolescence were covered (**Figure 3**). During ongoing imatinib exposure, a defined number of animals from each cohort were humanely sacrificed at prepubertal stage (age 6 weeks; after 2 weeks of exposure), at pubertal stage (age 8 weeks; after 4 weeks of exposure), and at postpubertal stage (age 14 weeks; after 10 weeks of exposure) [68].

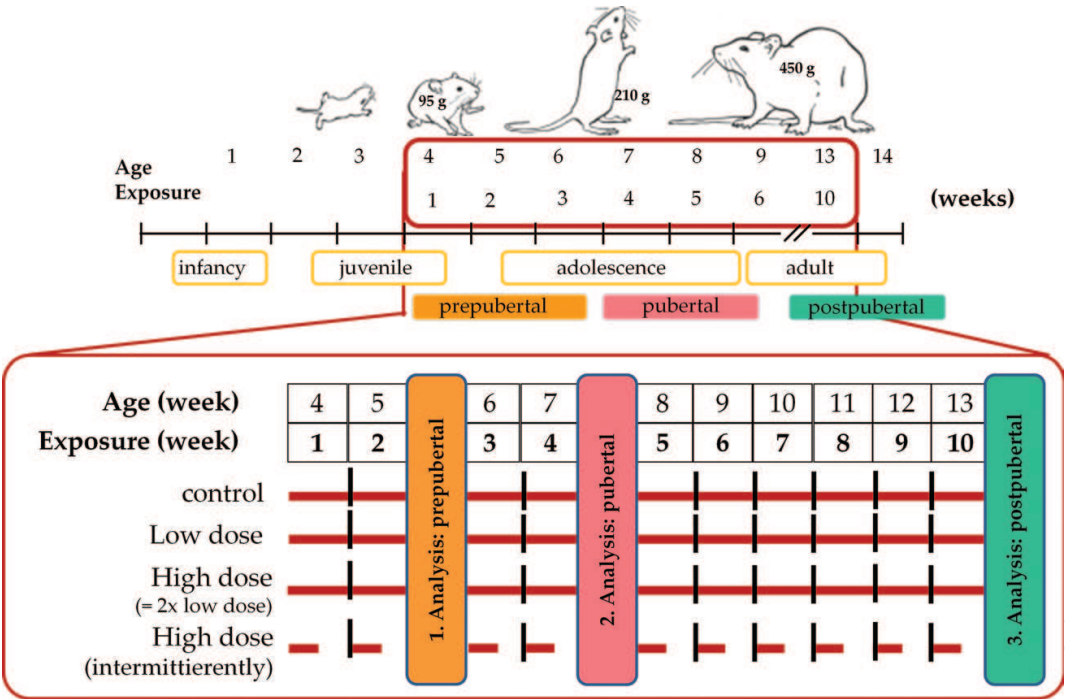


Figure 3. Experimental design of the juvenile animal model.

5. Side effects of chronic imatinib treatment on growing bone

At defined time points of analysis (**Figure 3**), blood serum was collected to measure TKI concentration by high-performance liquid chromatography (HPLC), biochemical markers of bone turnover, and hormone levels by ELISA technique. Long bones (tibia and femur) and lumbar vertebrae L1–L4 were isolated to determine bone length, vertebral height, bone mass,

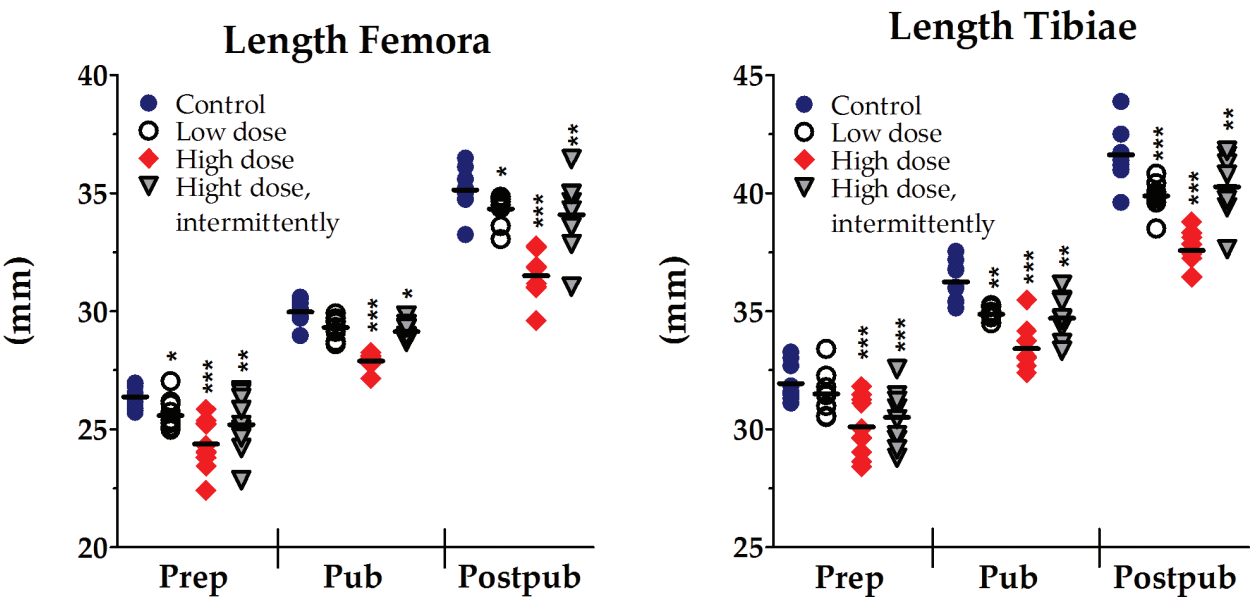
and strength by using quantitative computed tomography (pQCT), micro-computed tomography ( $\mu$ CT), and biomechanical testing [68].

5.1. TKI serum concentration

Imatinib mean serum levels of 1600 and 5600 ng/mL were achieved by continuous drug exposure via the drinking water to either low or high dose, respectively [68]. These serum concentrations match well with therapeutic imatinib levels of pediatric patients ranging from 2000 to 8000 ng/mL on imatinib administered at doses of 260–570 mg/m<sup>2</sup> daily [26], whereas in adult patients, serum levels in the range of ~1000–3400 ng/mL on imatinib doses of 400–600 mg daily were measured [69, 70]. Reflecting the half-life of imatinib in rats reported to be 12.3 h [71], serum levels of animals receiving high dose imatinib intermittently were below the detection limit of the assay (10.0 ng/mL) when serum was collected at the end of a 4-day period without drug exposure.

5.2. Long bone length and bone quality

During growth, a 10-week exposure to imatinib caused a significant reduction of the long bone length dose-dependently (Figure 4) [68]. These findings match with clinical data in children indicating that continuous administration of imatinib—even in high doses—does not result in a complete stop of growth, rather in a decelerated growth rate of the long bones [5, 36, 38–41, 72]. During growth, pQCT analysis of the bones revealed significantly reduced



**Figure 4.** Growth impairment of long bones by imatinib is dependent on the cumulative dose [68]. Prep: Prepubertal; Pub: Pubertal; Postpub: Postpubertal. Compared to controls, high dose imatinib (1000 mg/L daily) causes stronger longitudinal growth impairment than low dose exposure (500 mg/L daily). “On/off” exposure (3 days “on”, 4 days “off”) to high dose imatinib mitigates this effect. Of note, the cumulative dose resulting from 1000 mg/L administered “on/off” is approximately identical to 500 mg/L daily administered continuously. The resulting reduction in length reflects the cumulative dose administered.

trabecular bone mineral density (BMD) by imatinib exposure. Analysis of the 3D trabecular structure by  $\mu$ CT emphasizes these findings by demonstration of reduced bone volume density in combination with reduced trabecular number and connectivity [68]. Furthermore, our findings also indicated unchanged cortical BMD and cortical thickness during growth dose- and time-independently, whereas the bone strength of the femora was decreased after long-term exposure to high dose imatinib. This could be explained by decreased cross-sectional area, periosteal, and endosteal circumference of the femora, suggesting a blunted radial appositional bone growth [68]. With regard to pediatric patients, BMD measurements or increased fracture rates under long-term imatinib treatment are not published yet. However, intermittent treatment of the high dose mitigated all bony side effects of the long bones, which might offer a new perspective for pediatric patients.

### 5.3. Vertebrae height and quality

Concerning lumbar vertebra, 10-week imatinib exposure significantly reduced vertebral height combined with reduced trabecular BMD dose-dependently, whereas total BMD, cortical BMD, cross-sectional area, and cortical thickness were not affected [68, 73]. At the moment, only limited data are available on the effect of imatinib on vertebrae. In adult patients with CML, O'Sullivan et al. observed significantly increased lumbar spine BMD after 24 months of imatinib treatment as assessed by dual energy x-ray absorptiometry (DXA) [74], whereas Vandyke et al. observed unchanged BMD [75]. We predict from our animal model that imatinib also alters vertebral properties, but not to the same extent as in long bones [68].

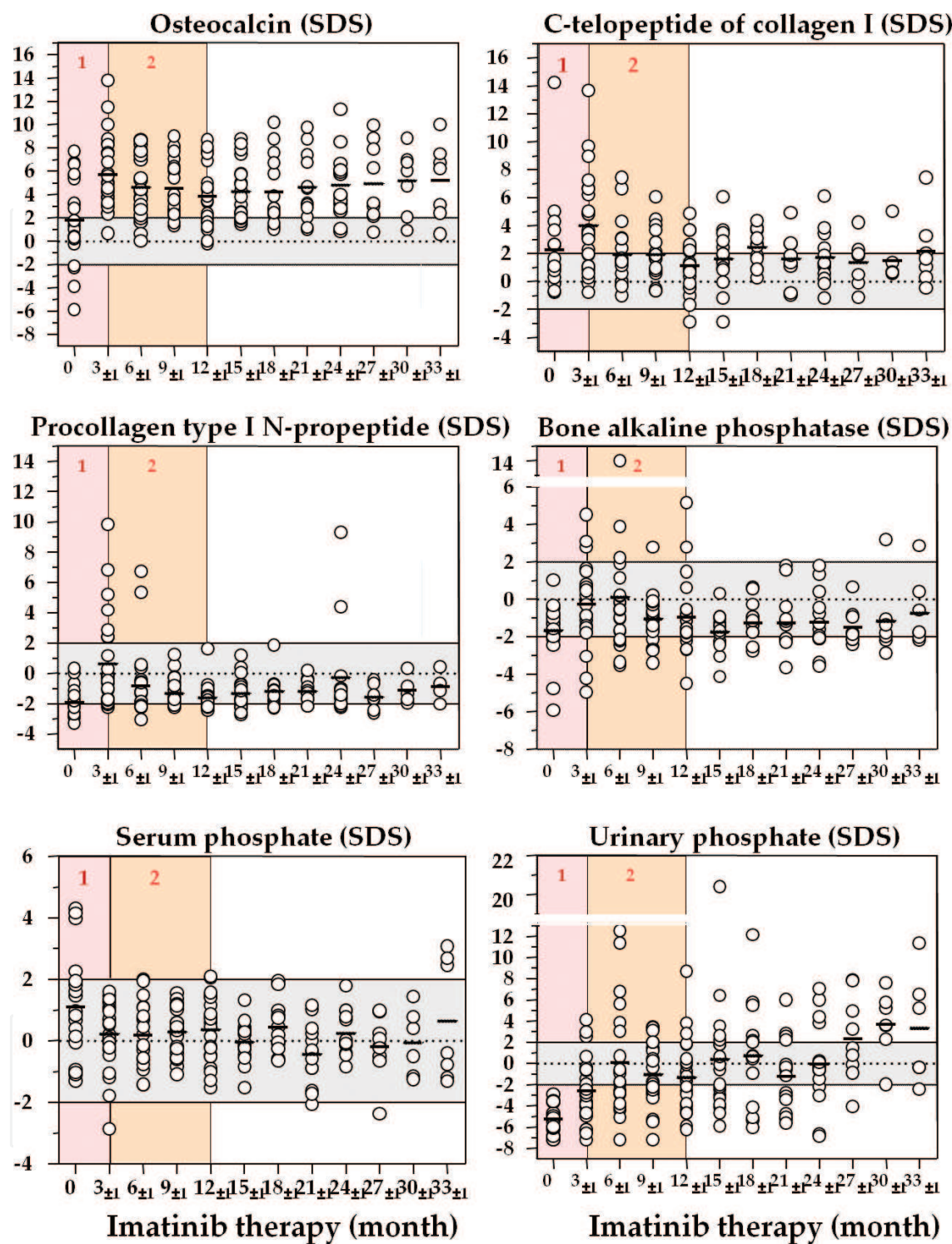
### 5.4. Bone turnover markers

The bone resorption marker tartrate-resistant acidic phosphatase (TRAP) revealed significantly decreased serum levels under continuous imatinib exposure indicating reduced osteoclast activity at all developmental stages [68]. This is confirmed by *in vitro* studies showing that imatinib impairs osteoclastogenesis leading to diminished numbers of TRAP-positive osteoclasts [49, 76]. However, bone resorption marker C-terminal collagen cross-links (CTX-I) revealed by trend elevated serum levels prepubertally, but normal levels during the ongoing exposure time, indicating nearly unchanged osteoclast activity during growth [68]. This is consistent with data from pediatric patients with CML describing by trend elevated CTX-I levels prepubertally while on imatinib [77].

Under imatinib exposure, bone formation marker osteocalcin was decreased but procollagen type I (PINP) levels were by trend elevated, pointing to improved bone formation and mineralization [68]. *In vitro* assays using human isolated mesenchymal stem cells, primary rat osteoblasts, and mouse osteoblast-like cell line MC3T3-E1 revealed all increased mineralization combined with reduced proliferation under therapeutic imatinib concentration [50].

However, bone turnover markers of pediatric patients with CML exhibited a biphasic response during imatinib therapy with increasing levels within the first 3 months of treatment and a significant decline during long-term treatment (**Figure 5**) [77, 78].





**Figure 5.** Biphasic response (time period 1 [pink background], period 2 [brown background]) of bone remodeling to imatinib treatment in pediatric patients with CML. Data depicted from CML-PAED II study [78]. One hundred and nineteen patients (70 male/49 female, median age 12 years, range 1–18 years) received 260–340 mg imatinib/m<sup>2</sup> daily within 1 week after diagnosis of CML (0). Up to 30 patients (range 20–30) out of this cohort could successfully be monitored repeatedly over a median period of 3 years for all parameters planned to be analyzed by collecting blood and urine for 3- months under appropriate circumstances. Assays were performed in a central laboratory as described previously [77]. Age normalized reference values were used as standard deviation scores (SDS).



## 6. Non bone-related side effects of imatinib treatment

### 6.1. Growth hormone

Main length growth regulating factors at childhood and adolescence are GH and “insulin-like growth factor 1” (IGF-1), thyroid hormone (T3, T4), glucocorticoids, and sex hormones during puberty [79].

GH is secreted by pituitary somatotrophins in a pulsatile manner and acts on peripheral tissues, either directly or indirectly, through the stimulation of IGF-1 synthesis and secretion [80–82]. As reported, the increase in body height during childhood is initiated by promoting chondrocyte proliferation and endochondral ossification in the growth plate or induction of osteoblastogenesis, leading to linear bone growth [80].

Owing to growth, children and/or adolescents going through puberty are particularly vulnerable to a possible GH deficiency (GHD) under long-term imatinib treatment [5, 41]. Mimicking those findings in children on imatinib treatment, the juvenile animal model disclosed significantly lowered serum levels of IGF-1 binding protein 3 (IGF-BP3)—a stable and more accurately measurable degradation product of IGF-1—at all concentrations applied and at all ages investigated [37, 83, 84]. Data of clinical studies in pediatric CML patients under TKI therapy revealed IGF-1 and IGFBP-3 levels almost exclusively in the very low or deep pathological range when compared to age-matched controls, independent of treatment duration [83, 84].

### 6.2. Vitamin D and bone

Within the bone remodeling cycle, vitamin D plays a crucial role by influencing the overall mineralization and bone turnover of the skeleton. The main effects of the active vitamin D metabolite  $1.25(\text{OH})_2\text{D}_3$  comprises of stimulating the absorption of calcium/phosphorus from the gut to create optimal circumstances for bone mineralization, as well as stimulation of the osteoblast-mediated mineralization and osteoclast differentiation [85]. The consequences of vitamin D deficiency are secondary hyperparathyroidism and bone loss, leading to osteoporosis and fractures, mineralization defects, which may lead to osteomalacia in the long-term, and muscle weakness, causing falls and fractures [86].

Hypophosphatemia, associated with low serum levels of  $25(\text{OH})\text{D}_3$ ,  $1.25(\text{OH})_2\text{D}_3$ , calcium, and secondary hyperparathyroidism are known side effects in adult patients with CML under imatinib treatment [3]. An explanation for these findings is that imatinib directly stimulates bone formation while restraining resorption, resulting in a net flux of calcium from extracellular fluid into bone, a decreased serum calcium level, and a compensatory rise in the level of parathyroid hormone, which causes phosphaturia and modest hypophosphatemia [50]. Pediatric patients with CML also exhibit moderate secondary hyperparathyroidism in conjunction with pathologically low  $25(\text{OH})\text{D}_3$  and  $1.25(\text{OH})_2\text{D}_3$  levels but normal serum calcium and phosphate levels under imatinib therapy [77, 78]. Thereby these effects were independent of the duration of imatinib therapy, which underlined once again that

regulation and compensatory mechanisms on the growing skeleton are different from those in the adult skeleton.

How imatinib interferes with vitamin D synthesis and metabolism is poorly understood yet. So far only one study investigated *in vitro* the effect of imatinib on keratinocytes yet and revealed a competitive inhibition of CYP27B1, a vitamin D hydroxylating enzyme, by imatinib [87].

### 6.3. Fertility

TKs like c-kit and PDGF-R, which are inhibited “off-target” by imatinib, are involved not only in the bone remodeling process but also in the regulation of spermatogenesis [88], raising the question of testicular toxicities by imatinib treatment. Up to now, the influence of TKIs on the male reproductive endocrine system in pediatric patients with CML is still controversially discussed [89].

The first study in neonatal rats revealed that imatinib interferes with postnatal testicular development [90]. Investigations in the juvenile animal model starting at an older age (4 weeks) depicted unchanged testis weight but reduced testosterone levels under long-term imatinib exposure until young adulthood. Inhibin B, a protein that is predominantly produced in the testis controlling follicle stimulating hormone (FSH) [91], did not significantly differ from controls, at all doses, and by all application schemes tested [92]. A clinical study conducted in a small cohort of boys (age: 7.8–18.9 years) with CML receiving TKI treatment revealed testosterone and inhibin B levels within normal age-related reference ranges [83, 84, 92]. Therefore, severe testicular toxicity by imatinib seems to be unlikely.

However, a closer look on spermatogenesis in the juvenile animal model revealed that the spermatogenic cell counts were significantly decreased by high dose imatinib exposure (**Figure 6**). Additionally, during spermatogenesis cell cycle, the stage of the dominant cell proportion was shifted to more immature stages. Low dose and intermittent imatinib exposure attenuated these findings. Interestingly, spermatogenic cell proliferation was significantly lowered at all imatinib doses applied [93]. Thus, a delayed negative effect of long-term imatinib exposure on spermatogenesis cannot be excluded.

### 6.4. Cardiac side effects of TKI treatment

In the literature as well as indicated by the manufacturers in the specialist information, cardiotoxic and vascular side effects of imatinib and the next-generation TKIs are of special concern [94–99]. However, this primarily may play a role in older adult patients with CML (age > 65 years) under TKI treatment.

The juvenile animal model under discussion disclosed an increase in the relative heart weight ratio (= ratio of the heart weight to total body weight at sacrifice) under imatinib exposure. Another study found that imatinib treatment led to mitochondrial-dependent myocyte loss and cardiac dysfunction, occurring more severely in older mice, in part due to

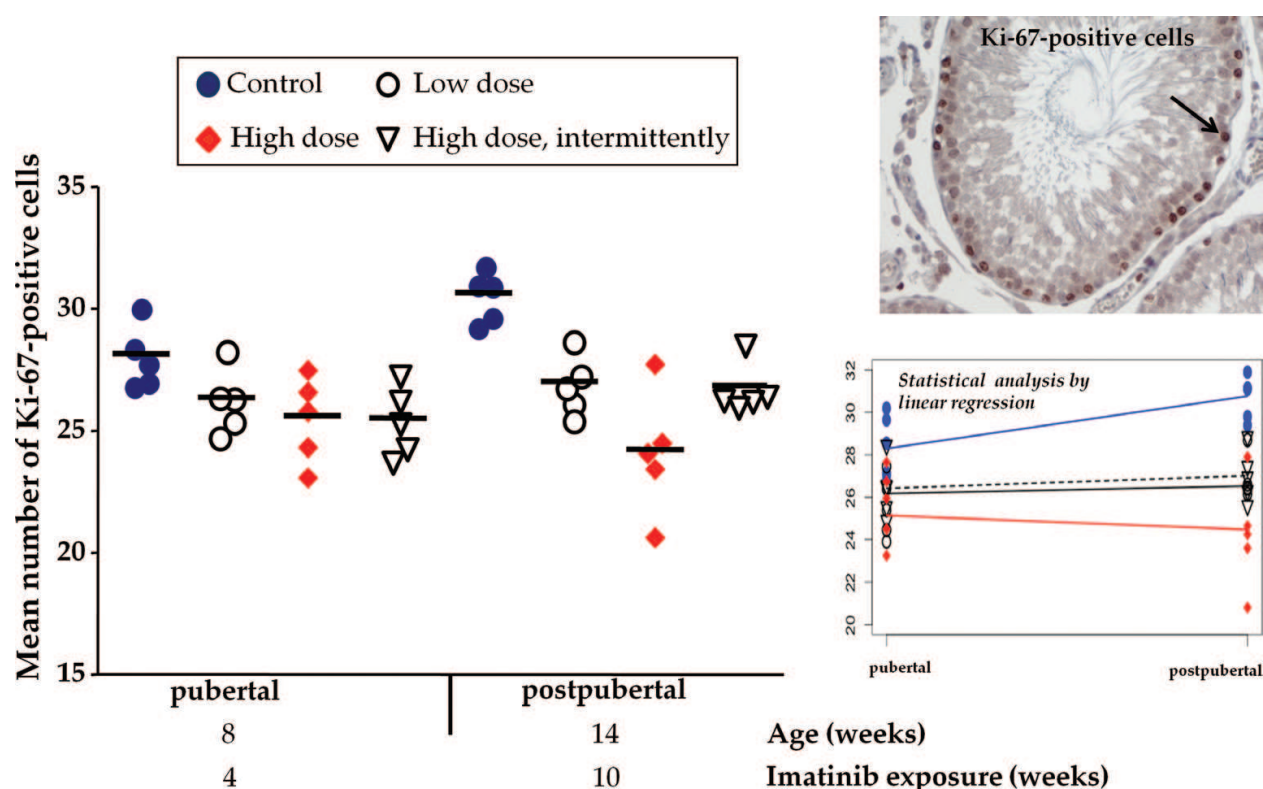
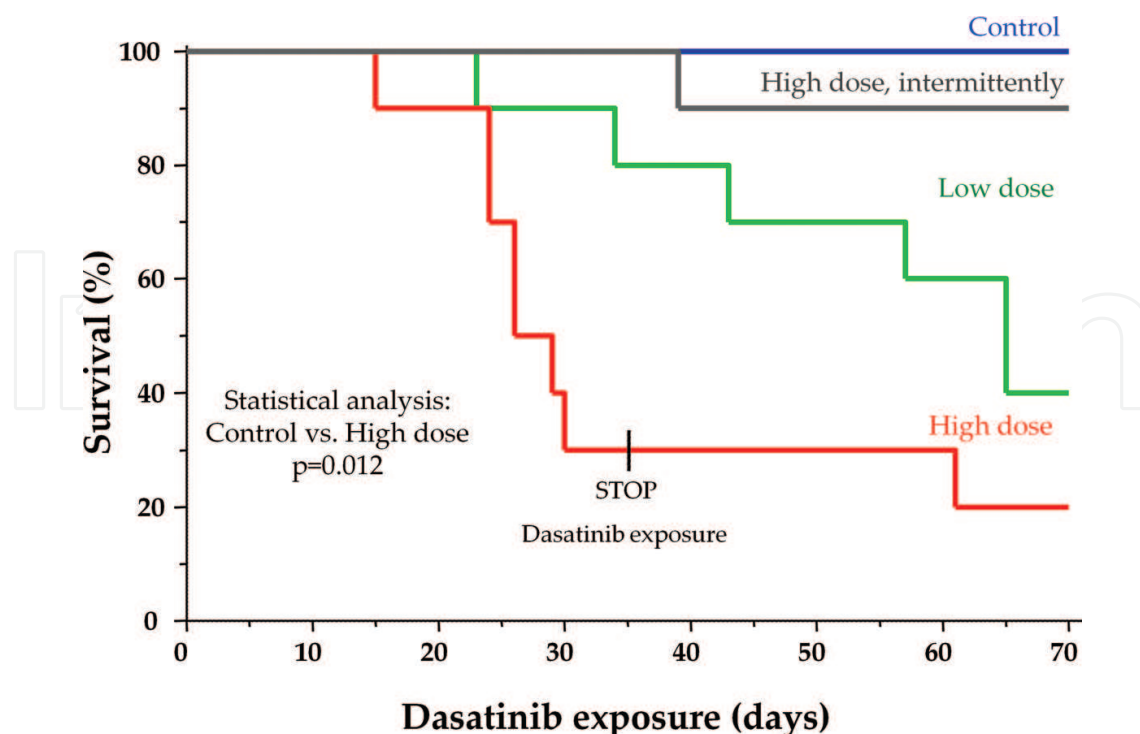


Figure 6. Number of proliferating testicular epithelium cells (Marker Ki67) in Wistar rats under imatinib exposure [93].

an age-dependent increase in oxidative stress [100]. This suggests that cardiac monitoring of older patients receiving imatinib therapy may be especially warranted.

As cardiac side effects were also observed with the use of dasatinib, in the experiment conducted with the juvenile animal model, this 2<sup>nd</sup>-generation TKI was tested for safety, efficacy, and dose response. Surprisingly, animals died spontaneously in a dose- and exposure time-dependent manner (Figure 7). Data of the surviving animals that were sacrificed according to the experimental set-up schedule (Figure 3) disclosed—dependent on the cumulative dose administered—increased relative heart weights, impaired heart ejection fraction as assessed by echocardiography, and elevated brain natriuretic peptide (BNP) serum levels, an indicator of cardiac dysfunction [101]. Data of this unexpected high toxicity can be explained by the serum elimination half-life time of dasatinib which is rather short and in the range of 2–3 h in rodents [102]. As known from clinical data on treatment of CML by dasatinib, it is not mandatory to achieve steady state drug blood levels as the intracellular concentration of dasatinib is responsible for efficacy, which is sufficiently achieved by once daily drug administration. Initial trials in humans based on drug administration twice daily were characterized by high toxicity requiring treatment interruption or reduction to once daily dosing [103]. Thus, the juvenile rat model also mimics this situation as a continuous intake of small doses of dasatinib via the drinking water evidently is associated with higher toxicity.

Initially, inhibition of the c-abl kinase was assumed to be the reason for cardiac toxicity by TKI [94]. But an extensive *in vitro* study of 18 TKIs on myocytes showed that their relative ability to inhibit ABL1 or ABL2 did not correlate with myocyte damage, revealing that inhibition



**Figure 7.** Survival rate of juvenile Wistar rats under chronic dasatinib exposure [101].

of other kinases like MEK1 and MEK2 could be responsible for the cardiotoxicity. However, it was reported that all TKIs induce myocyte damage correlating with their kinase inhibitor selectivity [97]. So, we conclude that it might be prudent to carefully monitor cardiac function in still growing individuals with CML if treated with TKI continuously over long periods.

## 7. Hypothesized model of osseous damage and clinical relevance

### 7.1. Model of action of imatinib on bone remodeling

Despite the knowledge accumulated so far, the detailed mechanism how imatinib impairs bone remodeling and growth remains yet speculative. In *in vitro* studies, it was shown that imatinib impairs osteoblastogenesis as well as osteoclastogenesis revealing its effect on bone remodeling [49, 50, 104, 105]. However, long bone growth is not only based on the balanced action of bone formation and bone resorption but also depends on the endochondral bone formation at the epiphyseal line of the long bones. Here, the column structure of the epiphyseal line, achieved and maintained by chondrocytes, is of main importance. In general, the epiphyseal line or growth plate is divided into different zones: reserve zone (RZ), proliferative zone (PZ), and the hypertrophic zone (HZ) followed by the primary spongiosa (PS)—the initial trabecular bone. The transition zone between HZ and PS is the osteochondral junction (OJ) (**Figure 8A**).

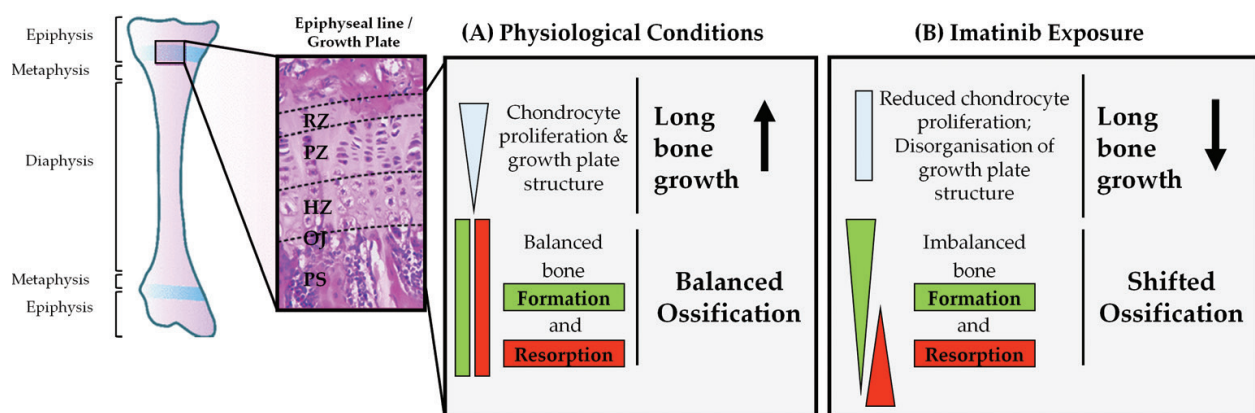
During growth, new cartilage is formed at one side of the epiphyseal growth plate and is gradually replaced by bone. The work by Nurmio et al. disclosed a disorganization of the epiphyseal line by imatinib treatment of neonatal rats (1–15 days old) (**Figure 9**) [106].



Instead of the typical long, smooth proliferating chondrocyte columns at the epiphyseal line, a thin, disorganized layer of proliferative cells was detected after imatinib treatment resulting in a decreased thickness of PZ and increased the thickness of the HZ. This is in line with an *in vitro* study revealing an inhibitory effect of imatinib on chondrocyte proliferation [107]. Nurmio et al. also observed that imatinib treatment led to a bone resorption arrest and increased bone formation at the OJ [106].

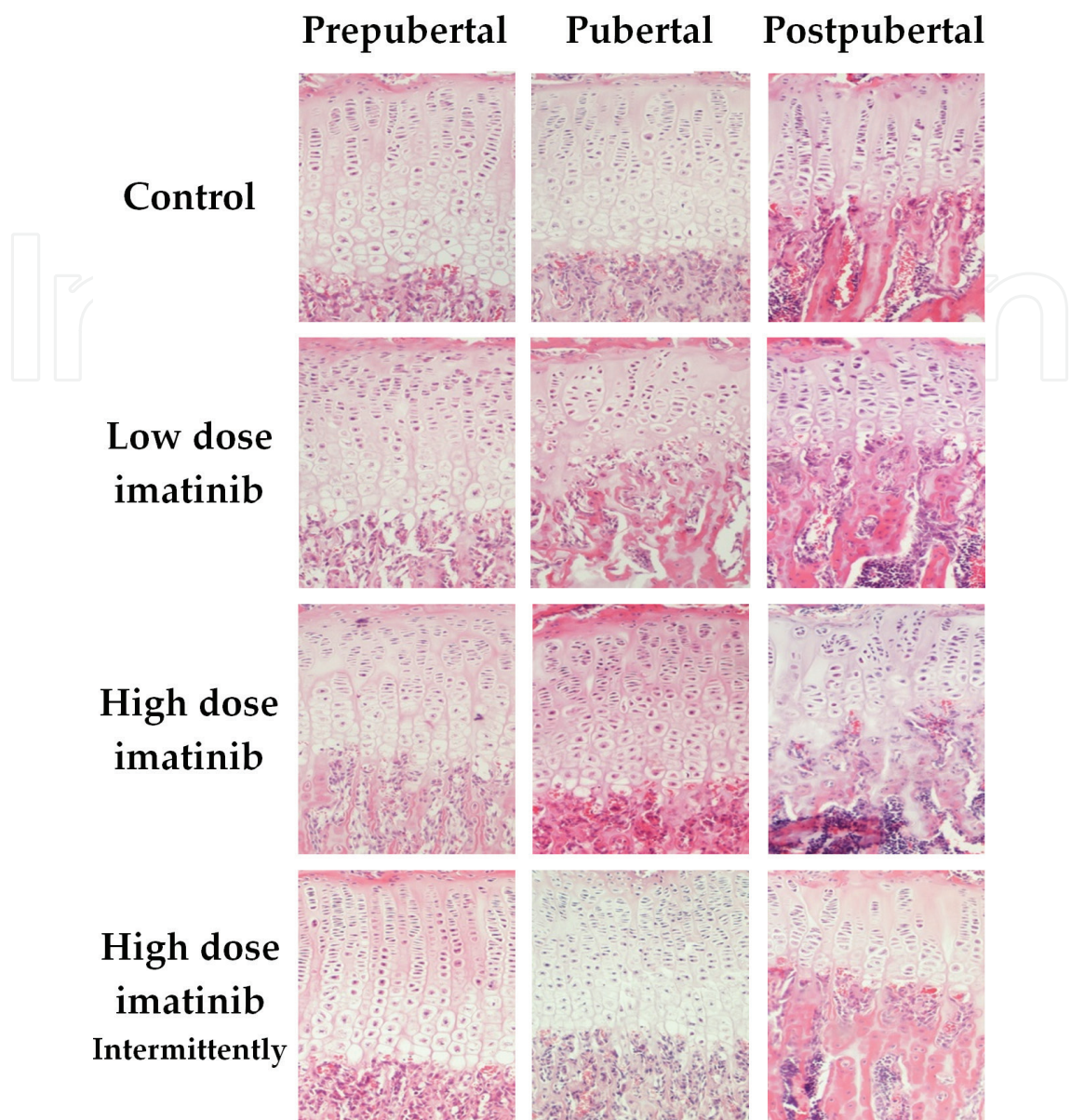
However, combining our data [68] with data from Nurmio et al. [106] and Vandyke et al. [107], it can be hypothesized that imatinib exposure alters metabolism and remodeling of the growing bone in a temporal-spatial stepwise fashion (**Figure 8B**). In the first instance, migration, proliferation, and activity of chondrocytes will be impaired by imatinib leading to a disturbed organization of the growth plate impairing longitudinal bone growth [106]. Altered growth hormone secretion under imatinib treatment as shown before in the growing organism [39, 41, 83] may aggravate this growth impairment. Thereafter, ongoing drug exposure causes a spatial activity shifting of bone remodeling: initially, the formation will be elevated and shifted to the area of the osteochondral junction, whereas the activity of bone resorption remains unchanged but will be spatially shifted to the distal area of the trabecular bone [106]. Finally, under long-term imatinib treatment, osteoblastogenesis and osteoclastogenesis will be impaired [104, 108], hampering bone remodeling during growth.

As an interesting approach, our juvenile animal model demonstrated that intermittent imatinib exposure will ameliorate growth impairment in rats. The inhibitory effect is not irreversible and we assume that during the days “OFF” imatinib exposure catch-up growth occurred. Therefore, drug administration following a schedule with “days on drug” and “days off drug” might reduce some skeletal side effects in pediatric patients. A single trial in older adults has already proven that intermittent TKI treatment is sufficient to control CML once remission



**Figure 8.** Schematic overview of physiologic bone growth (A) and under imatinib exposure (B). On the left side, longitudinal section of the epiphyseal line of a rodent proximal tibial metaphysis is depicted [109]. The epiphyseal plate separates the epiphysis from the metaphysis and is important for endochondral bone formation. The growth plate is divided into reserve zone (RZ), proliferative zone (PZ), and the hypertrophic zone (HZ). The transition of HZ to the primary spongiosa (PS)—the initial trabecular network formed after the vascular invasion and matrix calcification—is the osteochondral junction (OJ). Under physiological conditions, longitudinal growth occurs by endochondral ossification. In this process, new cartilage is formed at one side of the epiphyseal growth plate and is gradually replaced by bone. Chondrocytes of the growth plate are initially in a resting state in the RZ. They differentiate through proliferative and hypertrophic stages (PZ, HZ) as the growth plate moves past. This programmed differentiation pathway ends in cell death in the HZ and the replacement of cartilage by bone by osteoblasts in the OJ resulting in the PS. (For detailed review, see Ref. [110]).





**Figure 9.** Disorganization of the femoral epiphyseal line by long-term imatinib exposure. 2  $\mu$ m sections of decalcified femora were stained with hematoxylin-eosin (magnification 100  $\times$ ). Controls show the typical “column” structure of the epiphyseal line and its physiological narrowing with increasing age. However, under imatinib exposure, the cellular architecture is more disorganized in a dose- and time-dependent manner.

has been achieved [111, 112]. However, the length and frequency of intervals to allow catch-up growth in children on TKI treatment still have to be defined and at least in our rat model, this approach did not recover the biomechanical strength of the long bones.

7.2. Clinical relevance

The established juvenile rat model mimics to a gross extent side effects of long-term TKI exposure on the growing bone in a developmental stage-dependent fashion. Impairment of longitudinal growth, as observed in children under imatinib treatment, could be unequivocally modeled and confirmed.

Our hypothesis of spatiotemporal shifting of skeletal formation and resorption under imatinib is supported by clinical observations of a biphasic reaction of corresponding osseous metabolism serum markers in adult and pediatric patients with CML. In adult patients, an increase in bone formation occurred accompanied by elevated bone formation markers in the serum within the first months of therapy [74]. Pediatric CML patients display a biphasic response of bone formation and bone resorption by increasing levels within the first 3 months of imatinib treatment followed by a significant decline until 12 months of treatment (**Figure 5**) [77, 78].

Furthermore, we could show that long-term imatinib exposure may result in reduced bone strength possibly posing an elevated fracture risk in pediatric patients. Since 2001, adult CML patients are treated with imatinib but until now, no elevated fracture rates have been described in these patients [113]. As pediatric CML patients are treated with imatinib only since the beginning of this millennium, there is still no long-term experience. Our animal model also revealed that intermittent imatinib treatment mitigated skeletal effects on the growing bone, thus pointing toward a possibility to improve the risk-benefit ratio of long-term TKI exposure in pediatric patients. First clinical data in adults look promising but further studies must be carried out to determine whether the intermittent exposure is also sufficiently effective for the control of CML [111, 112]. Regarding pediatric patients, the results from the juvenile animal model and the clinical experience from adult patients with CML should be combined. This approach can be expected to harbor great potential in translational research.

## 8. Other animal models

The aim of the animal model described in this chapter was to evaluate side effects on bone remodeling rather than gaining further insight into the biology of CML (e.g., to study elementary mechanisms of CML disease progression) or on a more efficient antileukemic treatment exerted by new drugs (e.g., exploring why resistance develops under TKI therapy) [114, 115]. For these essential questions, the reader is kindly referred to the detailed body of literature on establishing and maintaining acute lymphatic or myeloid leukemic cells in xenograft models, transgenic models, and syngeneic models using a broad range of species [116–119], whereas mice are used mostly in orthotopic animal models [120–123].

Our research described, focused on the question how bone metabolism is affected by TKI treatment as an off-targeted side effect and therewith induced structural and mechanical osseous changes in healthy not-outgrown animals [124]. Bone remodeling has been studied in many species and resulted in the current available knowledge [125–131]. Evidently, the financial burden of animal maintenance and drug doses to be administered when sequelae of chronic exposure are investigated are much lower using small animals like mice and rats. Especially in these species, the time periods concerning defined stages of development are shorter, thus requiring drug exposure only for 2–3 months in order to mimic one to two decades in humans [132].

Most importantly, any intervention on the bone during chronic TKI exposure of the animals was minimized. Bone growth and repair is governed by regulatory mechanisms other than that of the outgrown organism. Therefore, the model described here differs principally from

experiments investigating bone healing and growth after surgical procedures performed on the skeleton (for a comprehensive review see Refs. [131, 133, 134]).

Ethical concerns in the last decades resulted in the establishment of studying bone growth and development preclinically in *ex vivo* cultures mostly making use of embryonic bone of mouse or rat strains [135, 136]. For an overview on conventional versus static versus 3D dynamic bioreactor models as well as a chorioallantoic membrane (CAM)-culture systems, the reader is kindly referred to a comprehensive review by Abubakar et al [137]. The composition of the nursing cell culture medium in these models is a crucial step. However, concerning TKIs whose metabolism in juvenile rodents is still poorly characterized and pleiotropically influences bone remodeling (e.g., impact on synthesis of growth hormone and insulin-like growth factor, liver metabolism, vitamin D metabolism, renal function, etc.) evidently not all components can be added to a cell culture medium mimicking correctly the *in vivo* situation. Therefore, our investigations had to be restricted to a genetically unchanged – “healthy” – animal model to study the side effects of long-term TKI exposure on bone remodeling during growth and in addition on other developing organs.

## 9. Conclusion

Long-term toxicity resulting from off-target effects of TKIs can be assessed conveniently by administering TKIs via the drinking water to juvenile male Wistar rats over a prolonged period. During all developmental phases (prepubertal, puberty, postpubertal, and adult), drug blood levels are obtained corresponding to data in humans. The juvenile animal model disclosed reduced long bone length and diminished vertebral height combined with reduced bone mass density and reduced breaking strength dose-dependently after chronic exposure to imatinib. Thus, the juvenile animal model depicted here mimics perfectly clinical observations on osseous changes observed in pediatric patients with CML. Furthermore, intermittent exposure of the high TKI dose mitigated the skeletal side effect and therefore represented a possible treatment option for pediatric patients suffering from longitudinal growth retardation under imatinib therapy. The juvenile animal model might also be of value to predict sequelae of TKI treatment in other human organs following exposure over decades.

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## References

- [1] Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: Results of a phase II study. *Blood*. 2002;**99**:3530-3539. Available from: <http://www.bloodjournal.org/content/bloodjournal/99/10/3530.full.pdf>
- [2] Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *The New England Journal of Medicine*. 2002;**346**:645-652. DOI: 10.1056/NEJMoa011573
- [3] Berman E, Nicolaides M, Maki RG, et al. Altered bone and mineral metabolism in patients receiving imatinib mesylate. *The New England journal of Medicine*. 2006;**354**:2006-2013. DOI: 10.1056/NEJMoa051140
- [4] Tauer JT, Nowasz C, Sedlacek P, et al. Impairment of longitudinal growth by tyrosine kinase inhibitor (TKI) treatment—Data from a large pediatric cohort with chronic myeloid leukemia (CML). *Blood*. 2014;**124**(21):522. Available from: <http://www.bloodjournal.org/content/124/21/522?sso-checked=true>.
- [5] Millot F, Guilhot J, Baruchel A, et al. Growth deceleration in children treated with imatinib for chronic myeloid leukaemia. *European Journal of Cancer*. 2014;**50**:3206-3211. DOI: 10.1016/j.ejca.2014.10.007
- [6] Hijiya N, Schultz KR, Metzler M, et al. Pediatric chronic myeloid leukemia is a unique disease that requires a different approach. *Blood*. 2016;**127**:392-399. DOI: 10.1182/blood-2015-06-648667
- [7] Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000;**103**:211-225. Available from: [http://www.cell.com/cell/pdf/S0092-8674\(00\)00114-8.pdf](http://www.cell.com/cell/pdf/S0092-8674(00)00114-8.pdf)

- [8] Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. *Oncogene*. 2000;**19**:5548-5557. DOI: 10.1038/sj.onc.1203957
- [9] Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001;**411**:355-365. DOI: 10.1038/35077225
- [10] de Klein A, van Kessel AG, Grosveld G, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1982;**300**:765-767. DOI: 10.1038/300765a0
- [11] Bartram CR, de Klein A, Hagemeijer A, et al. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1983;**306**:277-280. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/6580527>
- [12] Faderl S, Talpaz M, Estrov Z, et al. The biology of chronic myeloid leukemia. *The New England Journal of Medicine*. 1999;**341**:164-172. DOI: 10.1056/nejm199907153410306
- [13] Shawver LK, Slamon D, Ullrich A. Smart drugs: Tyrosine kinase inhibitors in cancer therapy. *Cancer Cell*. 2002;**1**:117-123. DOI: 10.1016/S1535-6108(02)00039-9
- [14] Quintas-Cardama A, Cortes JE. Chronic myeloid leukemia: Diagnosis and treatment. *Mayo Clinic Proceedings*. 2006;**81**:973-988. DOI: 10.4065/81.7.973
- [15] An X, Tiwari AK, Sun Y, et al. BCR-ABL tyrosine kinase inhibitors in the treatment of Philadelphia chromosome positive chronic myeloid leukemia: A review. *Leukemia Research*. 2010;**34**:1255-1268. DOI: 10.1016/j.leukres.2010.04.016
- [16] Suttorp M, Eckardt L, Tauer JT, et al. Management of chronic myeloid leukemia in childhood. *Current Hematologic Malignancy Reports*. 2012;**7**:116-124. DOI: 10.1007/s11899-012-0113-6
- [17] Krumbholz M, Karl M, Tauer JT, et al. Genomic BCR-ABL1 breakpoints in pediatric chronic myeloid leukemia. *Genes, Chromosomes & Cancer*. 2012;**51**:1045-1053. DOI: 10.1002/gcc.21989. <http://www.kinderkrebsregister.de/dkkr-gb/latest-publications/annual-reports.html?L=1>
- [18] Kaatsch P, Spix C. German Childhood Cancer Registry—Annual Report 2015 (1980-2014). Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI) at the University Medical Center of the Johannes Gutenberg University, Mainz, Germany; 2015
- [19] Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nature Medicine*. 1996;**2**:561-566. DOI: 10.1038/nm0596-561
- [20] Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *The New England Journal of Medicine*. 2001;**344**:1031-1037. DOI: 10.1056/nejm200104053441401
- [21] Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *The New England Journal of Medicine*. 2001;**344**:1038-1042. DOI: 10.1056/nejm200104053441402



- [22] Soverini S, Martinelli G, Iacobucci I, et al. Imatinib mesylate for the treatment of chronic myeloid leukemia. *Expert Review of Anticancer Therapy*. 2008;**8**:853-864. DOI: 10.1586/14737140.8.6.853
- [23] Deininger MW, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with imatinib. *Pharmacological Reviews*. 2003;**55**:401-423. DOI: 10.1124/pr.55.3.4
- [24] O'Hare T, Eide CA, Deininger MW. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood*. 2007;**110**:2242-2249. DOI: 10.1182/blood-2007-03-066936
- [25] Rix U, Hantschel O, Durnberger G, et al. Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. *Blood*. 2007;**110**:4055-4063. DOI: 10.1182/blood-2007-07-102061
- [26] Champagne MA, Capdeville R, Krailo M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: Results from a children's oncology group phase 1 study. *Blood*. 2004;**104**:2655-2660. DOI: 10.1182/blood-2003-09-3032
- [27] Millot F, Guilhot J, Nelken B, et al. Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. *Leukemia*. 2006;**20**:187-192. DOI: 10.1038/sj.leu.2404051
- [28] de la Fuente J, Baruchel A, Biondi A, et al. Managing children with chronic myeloid leukaemia (CML): recommendations for the management of CML in children and young people up to the age of 18 years. *British Journal of Haematology*. 2014;**167**:33-47. DOI: 10.1111/bjh.12977
- [29] Manning G, Whyte DB, Martinez R, et al. The protein kinase complement of the human genome. *Science (New York, NY)*. 2002;**298**:1912-1934. DOI: 10.1126/science.1075762
- [30] Taylor SS, Kornev AP. Protein kinases: Evolution of dynamic regulatory proteins. *Trends in Biochemical Sciences*. 2011;**36**:65-77. DOI: 10.1016/j.tibs.2010.09.006
- [31] Fabian MA, Biggs 3rd WH, Treiber DK, et al. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotechnology*. 2005;**23**:329-336. DOI: 10.1038/nbt1068
- [32] Fitter S, Dewar AL, Kostakis P, et al. Long-term imatinib therapy promotes bone formation in CML patients. *Blood*. 2008;**111**:2538-2547. DOI: 10.1182/blood-2007-07-104281
- [33] Mariani S, Giona F, Basciani S, et al. Low bone density and decreased inhibin-B/FSH ratio in a boy treated with imatinib during puberty. *Lancet*. 2008;**372**:111-112. DOI: 10.1016/s0140-6736(08)61023-5
- [34] Schmid H, Jaeger BA, Lohse J, et al. Longitudinal growth retardation in a prepuberal girl with chronic myeloid leukemia on long-term treatment with imatinib. *Haematologica*. 2009;**94**:1177-1179. DOI: 10.3324/haematol.2009.008359
- [35] Kimoto T, Inoue M, Kawa K. Growth deceleration in a girl treated with imatinib. *International Journal of Hematology*. 2009;**89**:251-252. DOI: 10.1007/s12185-008-0251-8

- [36] Bansal D, Shava U, Varma N, et al. Imatinib has adverse effect on growth in children with chronic myeloid leukemia. *Pediatric Blood & Cancer*. 2012;**59**:481-484. DOI: 10.1002/pbc.23389
- [37] Narayanan KR, Bansal D, Walia R, et al. Growth failure in children with chronic myeloid leukemia receiving imatinib is due to disruption of GH/IGF-1 axis. *Pediatric Blood & Cancer*. 2013;**60**:1148-1153. DOI: 10.1002/pbc.24397
- [38] Giona F, Mariani S, Gnessi L, et al. Bone metabolism, growth rate and pubertal development in children with chronic myeloid leukemia treated with imatinib during puberty. *Haematologica*. 2013;**98**:e25–e27. DOI: 10.3324/haematol.2012.067447
- [39] Hoernicht SL, Schweiger B, Zeitler P, et al. Acquired growth hormone deficiency in a girl with chronic myelogenous leukemia treated with tyrosine kinase inhibitor therapy. *Pediatric Blood & Cancer*. 2011;**56**:671-673. DOI: 10.1002/pbc.22945
- [40] Rastogi MV, Stork L, Druker B, et al. Imatinib mesylate causes growth deceleration in pediatric patients with chronic myelogenous leukemia. *Pediatric Blood & Cancer*. 2012;**59**:840-845. DOI: 10.1002/pbc.24121
- [41] Shima H, Tokuyama M, Tanizawa A, et al. Distinct impact of imatinib on growth at pre-pubertal and pubertal ages of children with chronic myeloid leukemia. *The Journal of Pediatrics*. 2011;**159**:676-681. DOI: 10.1016/j.jpeds.2011.03.046
- [42] Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. *Nature Biotechnology*. 2011;**29**:1046-1051. DOI: 10.1038/nbt.1990
- [43] Dewar AL, Cambareri AC, Zannettino AC, et al. Macrophage colony-stimulating factor receptor c-fms is a novel target of imatinib. *Blood*. 2005;**105**:3127-3132. DOI: 10.1182/blood-2004-10-3967
- [44] Taylor JR, Brownlow N, Domin J, et al. FMS receptor for M-CSF (CSF-1) is sensitive to the kinase inhibitor imatinib and mutation of Asp-802 to Val confers resistance. *Oncogene*. 2006;**25**:147-151. DOI: 10.1038/sj.onc.1209007
- [45] de Melo-Martín I, Sondhi D, Crystal RG. Novel therapies, high-risk pediatric research, and the prospect of benefit: Learning from the ethical disagreements. *Molecular Therapy*. 2012;**20**:1095-1102. DOI: 10.1038/mt.2012.90
- [46] Mak IWY, Evaniew N, Ghert M. Lost in translation: Animal models and clinical trials in cancer treatment. *American Journal of Translational Research*. 2014;**6**:114-118. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3902221/pdf/ajtr0006-0114.pdf>
- [47] Denayer T, Stöhr T, Van Roy M. Animal models in translational medicine: Validation and prediction. *New Horizons in Translational Medicine*. 2014;**2**:5-11. DOI: 10.1016/j.nhtm.2014.08.001
- [48] Hepple B, Peckham C. *The Ethics of Research Involving Animals*. London, UK: Nuffield Council on Bioethics; 2005. ISBN: 1904384102. Available from: <http://nuffieldbioethics.org/project/animal-research/>

- [49] Ando W, Hashimoto J, Nampei A, et al. Imatinib mesylate inhibits osteoclastogenesis and joint destruction in rats with collagen-induced arthritis (CIA). *Journal of Bone and Mineral Metabolism*. 2006;**24**:274-282. DOI: 10.1007/s00774-006-0684-1
- [50] Grey A, O'Sullivan S, Reid IR, et al. Imatinib mesylate, increased bone formation, and secondary hyperparathyroidism. *The New England Journal of Medicine*. 2006;**355**:2494-2495. DOI: 10.1056/NEJMc062388
- [51] Cozzi J, Fraichard A, Thiam K. Use of genetically modified rat models for translational medicine. *Drug Discovery Today*. 2008;**13**:488-494. DOI: 10.1016/j.drudis.2008.03.021
- [52] Iannaccone PM, Jacob HJ. Rats! *Disease Models & Mechanisms*. 2009;**2**:206-210. DOI: 10.1242/dmm.002733
- [53] Krinke GJ. *The Handbook of Experimental Animals: The Laboratory Rat*. In: Bullock G, Bunton TE, editors. New York: Academic Press; 2000
- [54] Zemunik T, Peruzovic M, Capkun V, et al. Reproductive ability of pubertal male and female rats. *Brazilian Journal of Medical and Biological Research = Revista brasileira de pesquisas medicas e biologicas*. 2003;**36**:871-877. Available from: <http://www.scielo.br/pdf/bjmr/v36n7/4675.pdf>
- [55] Sengupta P. A scientific review of age determination for a laboratory rat: How old is it in comparison with human age? *Biomedicine International*. 2011;**2**:81-89. Available from: <http://www.bmijournal.org/index.php/bmi/article/view/80>
- [56] Zanato VF, Martins MP, Anselmo-Franci JA, et al. Sexual development of male Wistar rats. *Brazilian Journal of Medical and Biological Research = Revista brasileira de pesquisas medicas e biologicas*. 1994;**27**:1273-1280. Available from: <http://europepmc.org/abstract/med/8000350>
- [57] Freudenberger CB. A comparison of the Wistar albino and the Long-Evans hybrid strain of the Norway rat. *American Journal of Anatomy*. 1932;**50**:293-349. DOI: 10.1002/aja.1000500207
- [58] Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *Journal of Reproduction and Fertility*. 1978;**54**:103-107. Available from: <http://www.reproduction-online.org/content/54/1/103.full.pdf>
- [59] Boot AM, de Ridder MA, Pols HA, et al. Bone mineral density in children and adolescents: Relation to puberty, calcium intake, and physical activity. *The Journal of Clinical Endocrinology and Metabolism*. 1997;**82**:57-62. DOI: 10.1210/jcem.82.1.3665
- [60] Sirois I, Cheung AM, Ward WE. Biomechanical bone strength and bone mass in young male and female rats fed a fish oil diet. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*. 2003;**68**:415-421. Available from: [http://www.plefa.com/article/S0952-3278\(03\)00066-8/fulltext](http://www.plefa.com/article/S0952-3278(03)00066-8/fulltext)
- [61] Damsch S, Eichenbaum G, Tonelli A, et al. Gavage-related reflux in rats: Identification, pathogenesis, and toxicological implications (review). *Toxicologic Pathology*. 2011;**39**:348-360. DOI: 10.1177/0192623310388431

- [62] Brown AP, Dinger N, Levine BS. Stress produced by gavage administration in the rat. *Contemporary Topics in Laboratory Animal Science*. 2000;**39**:17-21. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/11178310>
- [63] Tauer JT, Hofbauer LC, Jung R, et al. Micro-osmotic pumps for continuous release of the tyrosine kinase inhibitor bosutinib in juvenile rats and its impact on bone growth. *Medical Science Monitor Basic Research*. 2013;**19**:274-278. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24185529>
- [64] Nebendahl K. Routes of administration. In: Bullock G, Bunton TE, editors. *The Handbook of Experimental Animals: The Laboratory Rat*. New York: Academic Press; 2000. pp. 463-482
- [65] Tober-Meyer BK, Bieniek HJ, Kupke IR. Studies on the hygiene of drinking water for laboratory animals. 2. Clinical and biochemical studies in rats and rabbits during long-term provision of acidified drinking water. *Laboratory Animals*. 1981;**15**:111-117. DOI: 10.1258/002367781780959071
- [66] Bachmanov AA, Reed DR, Beauchamp GK, et al. Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behavior Genetics*. 2002;**32**:435-443. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1397713/>
- [67] Pass D, Freeth G. The rat. *Anzccart News*. 1993;**6**:1-4. Available from: [https://www.adelaide.edu.au/ANZCCART/docs/fact-sheets/TheRat\\_3Arch.pdf](https://www.adelaide.edu.au/ANZCCART/docs/fact-sheets/TheRat_3Arch.pdf)
- [68] Tauer JT, Hofbauer LC, Jung R, et al. Impact of long-term exposure to the tyrosine kinase inhibitor imatinib on the skeleton of growing rats. *PLoS One*. 2015;**10**:e0131192. DOI: 10.1371/journal.pone.0131192
- [69] Picard S, Titier K, Etienne G, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood*. 2007;**109**:3496-3499. DOI: 10.1182/blood-2006-07-036012
- [70] Peng B, Hayes M, Resta D, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2004;**22**:935-942. DOI: 10.1200/jco.2004.03.050
- [71] Bende G, Kollipara S, Movva S, et al. Validation of an HPLC method for determination of imatinib mesylate in rat serum and its application in a pharmacokinetic study. *Journal of Chromatographic Science*. 2010;**48**:334-341. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20515524>
- [72] Vandyke K, Zannettino ACW. Effects of tyrosine kinase inhibitors on growth in paediatric patients. In: Akhtari M, Elhemaidi I, editors. *Imatinib/Chemical Structure, Pharmacology and Adverse Effects*. Vol. 1. Hauppauge, NY: NovaScience Publisher; 2013
- [73] Tauer JT, Hofbauer LC, Suttrop M. Impact of the tyrosine kinase inhibitors imatinib, dasatinib, and bosutinib in young rats on the vertebral body. *Blood*. 2013:1472. Available from: <http://www.bloodjournal.org/content/122/21/1472>.



- [74] O'Sullivan S, Horne A, Wattie D, et al. Decreased bone turnover despite persistent secondary hyperparathyroidism during prolonged treatment with imatinib. *The Journal of Clinical Endocrinology and Metabolism*. 2009;**94**:1131-1136. DOI: 10.1210/jc.2008-2324
- [75] Vandyke K, Fitter S, Drew J, et al. Prospective histomorphometric and DXA evaluation of bone remodeling in imatinib-treated CML patients: Evidence for site-specific skeletal effects. *The Journal of Clinical Endocrinology and Metabolism*. 2013;**98**:67-76. DOI: 10.1210/jc.2012-2426
- [76] El Hajj Dib I, Gallet M, Mentaverri R, et al. Imatinib mesylate (Gleevec) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. *European Journal of Pharmacology*. 2006;**551**:27-33. DOI: 10.1016/j.ejphar.2006.09.007
- [77] Jaeger BA, Tauer JT, Ulmer A, et al. Changes in bone metabolic parameters in children with chronic myeloid leukemia on imatinib treatment. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2012;**18**:CR721–CR728. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23197234>
- [78] Tauer JT, Glauche I, Suttorp M. Changes in bone metabolic parameters under imatinib treatment in children with chronic myeloid leukemia (CML). *Blood*. 2015;**126**(23):1574. Available from: <http://www.bloodjournal.org/content/126/23/1574>
- [79] van der Eerden BC, Karperien M, Wit JM. Systemic and local regulation of the growth plate. *Endocrine Reviews*. 2003;**24**:782-801. DOI: 10.1210/er.2002-0033
- [80] Tritos NA, Klibanski A. Chapter nine—Effects of growth hormone on bone. In: Felipe FC, editor. *Progress in Molecular Biology and Translational Science*. Vol. 138. Academic Press, London, UK; 2016. pp. 193-211
- [81] Kimura F, Tsai CW. Ultradian rhythm of growth hormone secretion and sleep in the adult male rat. *The Journal of Physiology*. 1984;**353**:305-315. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1193308/pdf/jphysiol00591-0314.pdf>
- [82] Gamble KL, Berry R, Frank SJ, et al. Circadian clock control of endocrine factors. *Nature Reviews Endocrinology*. 2014;**10**:466-475. DOI: 10.1038/nrendo.2014.78
- [83] Ulmer A, Tabea Tauer J, Glauche I, et al. TK inhibitor treatment disrupts growth hormone axis: Clinical observations in children with CML and experimental data from a juvenile animal model. *Klinische Padiatrie*. 2013;**225**:120-126. DOI: 10.1055/s-0033-1343483
- [84] Ulmer A, Tauer JT, Suttorp M. Impact of treatment with tyrosine kinase inhibitors (TKIs) on blood levels of growth hormone-related parameters, testosterone, and inhibin b in juvenile rats and pediatric patients with chronic myeloid leukemia (CML). *Blood*. 2012;**120**(21):3752. Available from: <http://www.bloodjournal.org/content/120/21/3752>
- [85] Yoshida T, Stern PH. How vitamin D works on bone. *Endocrinology and Metabolism Clinics of North America*. 2012;**41**:557-569. DOI: 10.1016/j.ecl.2012.04.003
- [86] Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2011;**25**:585-591. DOI: 10.1016/j.beem.2011.05.002

- [87] Mehlig LM, Garve C, Tauer JT, et al. Inhibitory effects of imatinib on vitamin D(3) synthesis in human keratinocytes. *Molecular Biology Reports*. 2015;**11**:3143-3147. DOI: 10.3892/mmr.2014.3074
- [88] Zhang M, Zhou H, Zheng C, et al. The roles of testicular c-kit positive cells in de novo morphogenesis of testis. *Scientific Reports*. 2014;**4**:5936. DOI: 10.1038/srep05936
- [89] Samis J, Lee P, Zimmerman D, et al. Recognizing endocrinopathies associated with tyrosine kinase inhibitor therapy in children with chronic myelogenous leukemia. *Pediatric Blood & Cancer*. 2016;**63**:1332-1338. DOI: 10.1002/pbc.26028
- [90] Nurmio M, Toppari J, Zaman F, et al. Inhibition of tyrosine kinases PDGFR and C-Kit by imatinib mesylate interferes with postnatal testicular development in the rat. *International Journal of Andrology*. 2007;**30**:366-376. discussion 376. DOI: 10.1111/j.1365-2605.2007.00755.x
- [91] Meachem SJ, Nieschlag E, Simoni M. Inhibin B in male reproduction: Pathophysiology and clinical relevance. *European Journal of Endocrinology*. 2001;**145**:561-571. Available from: <http://www.eje-online.org/content/145/5/561.full.pdf>
- [92] Tauer JT, Ulmer A, Glauche I, et al. Long-term imatinib treatment does not cause testicular toxicity in male adolescents with chronic myeloid leukemia and in a juvenile rat model. *Klinische Padiatrie*. 2014;**226**:169-174. DOI: 10.1055/s-0034-1372643
- [93] Girke V, Tauer JT, Glauche I, et al. Impact of long-term tyrosine kinase inhibitor exposure on spermatogenesis in juvenile rats. *Blood*. 2016;**128**(22): 1884. Available from: <https://ash.confex.com/ash/2016/webprogram/Paper90120.html>.
- [94] Kerkela R, Grazette L, Yacobi R, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nature Medicine*. 2006;**12**:908-916. DOI: 10.1038/nm1446
- [95] Bhawe M, Akhter N, Rosen ST. Cardiovascular toxicity of biologic agents for cancer therapy. *Oncology (Williston Park, NY)*. 2014;**28**:482-490. Available from: <http://www.cancer-network.com/oncology-journal/cardiovascular-toxicity-biologic-agents-cancer-therapy>
- [96] Haguet H, Droux J, Mullier F, et al. Risk of arterial and venous occlusive events in chronic myeloid leukemia patients treated with new generation BCR-ABL tyrosine kinase inhibitors: A systematic review and meta-analysis. *Expert Opinion on Drug Safety*. 2017;**16**:5-12. DOI: 10.1080/14740338.2017.1261824
- [97] Hasinoff BB, Patel D, Wu X. The myocyte-damaging effects of the BCR-ABL1-targeted tyrosine kinase inhibitors increase with potency and decrease with specificity. *Cardiovascular Toxicology*. 2016. DOI: 10.1007/s12012-016-9386-7
- [98] Galinsky I, Buchanan S. Practical management of dasatinib for maximum patient benefit. *Clinical Journal of Oncology Nursing*. 2009;**13**:329-335. DOI: 10.1188/09.cjon.329-335
- [99] Orphanos GS, Ioannidis GN, Ardavanis AG. Cardiotoxicity induced by tyrosine kinase inhibitors. *Acta Oncologica (Stockholm, Sweden)*. 2009;**48**:964-970. DOI: 10.1080/02841860903229124

- [100] Maharsy W, Aries A, Mansour O, et al. Ageing is a risk factor in imatinib mesylate cardiotoxicity. *European Journal of Heart Failure*. 2014;**16**:367-376. DOI: 10.1002/ejhf.58
- [101] Geidel P, Tauer JT, Steinbronn N, et al. Cardiac failure in juvenile rats caused by continuous long-term exposure to the tyrosine kinase inhibitor dasatinib can be circumvented by an intermittent application schedule. *Blood*. 2013;**122**(21):3984. Available from: <http://www.bloodjournal.org/content/122/21/3984>.
- [102] Kamath AV, Wang J, Lee FY, et al. Preclinical pharmacokinetics and in vitro metabolism of dasatinib (BMS-354825): A potent oral multi-targeted kinase inhibitor against SRC and BCR-ABL. *Cancer Chemotherapy and Pharmacology*. 2008;**61**:365-376. DOI: 10.1007/s00280-007-0478-8
- [103] McCormack PL, Keam SJ. Dasatinib: A review of its use in the treatment of chronic myeloid leukaemia and Philadelphia chromosome-positive acute lymphoblastic leukaemia. *Drugs*. 2011;**71**:1771-1795. DOI: 10.2165/11207580-000000000-00000
- [104] Jonsson S, Hjorth-Hansen H, Olsson B, et al. Imatinib inhibits proliferation of human mesenchymal stem cells and promotes early but not late osteoblast differentiation in vitro. *Journal of Bone and Mineral Metabolism*. 2012;**30**:119-123. DOI: 10.1007/s00774-011-0323-3
- [105] Vandyke K, Fitter S, Dewar AL, et al. Dysregulation of bone remodeling by imatinib mesylate. *Blood*. 2010;**115**:766-774. DOI: 10.1182/blood-2009-08-237404
- [106] Nurmio M, Joki H, Kallio J, et al. Receptor tyrosine kinase inhibition causes simultaneous bone loss and excess bone formation within growing bone in rats. *Toxicology and Applied Pharmacology*. 2011;**254**:267-279. DOI: 10.1016/j.taap.2011.04.019
- [107] Vandyke K, Dewar AL, Fitter S, et al. Imatinib mesylate causes growth plate closure in vivo. *Leukemia*. 2009;**23**:2155-2159. DOI: 10.1038/leu.2009.150
- [108] O'Sullivan S, Naot D, Callon K, et al. Imatinib promotes osteoblast differentiation by inhibiting PDGFR signaling and inhibits osteoclastogenesis by both direct and stromal cell-dependent mechanisms. *Journal of bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*. 2007;**22**:1679-1689. DOI: 10.1359/jbmr.070719
- [109] Wongdee K, Krishnamra N, Charoenphandhu N. Endochondral bone growth, bone calcium accretion, and bone mineral density: How are they related? *The Journal of Physiological Sciences*. 2012;**62**:299-307. DOI: 10.1007/s12576-012-0212-0
- [110] Gilbert SF. Osteogenesis: The development of bones. In: *Developmental Biology*. 6th ed. Sunderland (MA): Sinauer Associates; 2000. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK10056/>
- [111] Russo D, Malagola M, Skert C, et al. Managing chronic myeloid leukaemia in the elderly with intermittent imatinib treatment. *Blood Cancer Journal*. 2015;**5**:e347. DOI: 10.1038/bcj.2015.75

- [112] Russo D, Martinelli G, Malagola M, et al. Updating long-term outcome of intermittent imatinib (INTERIM) treatment in elderly patients with Ph<sup>+</sup>-CML. *Blood*. 2014;**124**(21):1794. Available from: <http://www.bloodjournal.org/content/124/21/1794?sso-checked=true>.
- [113] Farmer S, Horváth-Puhó E, Vestergaard H, et al. Chronic myeloproliferative neoplasms and risk of osteoporotic fractures; a nationwide population-based cohort study. *British Journal of Haematology*. 2013;**163**:603-610. DOI: 10.1111/bjh.12581
- [114] Fava C, Morotti A, Dogliotti I, et al. Update on emerging treatments for chronic myeloid leukemia. *Expert Opinion on Emerging Drugs*. 2015;**20**:183-196. DOI: 10.1517/14728214.2015.1031217
- [115] Kang Y, Hodges A, Ong E, et al. Identification of drug combinations containing imatinib for treatment of BCR-ABL<sup>+</sup> leukemias. *PLoS One*. 2014;**9**:e102221. DOI: 10.1371/journal.pone.0102221
- [116] Sontakke P, Jaques J, Vellenga E, et al. Modeling of chronic myeloid leukemia: An overview of in vivo murine and human xenograft models. *Stem Cells International*. 2016;**2016**:1625015. DOI: 10.1155/2016/1625015
- [117] Harrison NR, Laroche FJ, Gutierrez A, et al. Zebrafish models of human leukemia: Technological advances and mechanistic insights. *Advances in Experimental Medicine and Biology*. 2016;**916**:335-369. DOI: 10.1007/978-3-319-30654-4\_15
- [118] Duran-Struuck R, Matar AJ, Huang CA. Myeloid leukemias and virally induced lymphomas in miniature inbred swine: Development of a large animal tumor model. *Frontiers in Genetics*. 2015;**6**:332. DOI: 10.3389/fgene.2015.00332
- [119] Ma W, Ma N, Chen X, et al. An overview of chronic myeloid leukemia and its animal models. *Science China Life Sciences*. 2015;**58**:1202-1208. DOI: 10.1007/s11427-015-4965-6
- [120] Giotopoulos G, van der Weyden L, Osaki H, et al. A novel mouse model identifies cooperating mutations and therapeutic targets critical for chronic myeloid leukemia progression. *The Journal of Experimental Medicine*. 2015;**212**:1551-1569. DOI: 10.1084/jem.20141661
- [121] Schneckenleithner C, Hoelbl-Kovac A, Sexl V. Modeling BCR/ABL-driven malignancies in the mouse. *Methods in Molecular Biology (Clifton, NJ)*. 2015;**1267**:263-282. DOI: 10.1007/978-1-4939-2297-0\_12
- [122] Askmyr M, Agerstam H, Lilljebjorn H, et al. Modeling chronic myeloid leukemia in immunodeficient mice reveals expansion of aberrant mast cells and accumulation of pre-B cells. *Blood Cancer Journal*. 2014;**4**:e269. DOI: 10.1038/bcj.2014.89
- [123] Wicklein D, Schmidt A, Labitzky V, et al. E- and p-selectins are essential for repopulation of chronic myelogenous and chronic eosinophilic leukemias in a scid mouse xenograft model. *PLoS One*. 2013;**8**:e70139. DOI: 10.1371/journal.pone.0070139



- [124] Pogoda P, Priemel M, Schilling AF, et al. Mouse models in skeletal physiology and osteoporosis: Experiences and data on 14,839 cases from the Hamburg Mouse Archives. *Journal of Bone and Mineral Metabolism*. 2005;**23**(Suppl):97-102. Available from: <http://link.springer.com/article/10.1007/BF03026332>
- [125] Parra-Torres AY, Valdés-Flores M, Orozco L, et al. Molecular aspects of bone remodeling. In: Flores MV, editor. *Topics in Osteoporosis*. Rijeka: InTech; 2013. Ch. 01
- [126] Favus MJ. Primer on the metabolic bone diseases and disorders of mineral metabolism. In: Favus MJ, editor. 4th ed. Hagerstown, Maryland, USA: Lippincott Williams & Wilkins; 1999. p. 502. ISBN: 0-7817-2038-9
- [127] Wang Q, Seeman E. Skeletal Growth and Peak Bone Strength. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 8th ed. John Wiley & Sons, Inc.; 2013. pp. 127-134. ISBN: 978-1-118-45388-9. DOI: 10.1002/9781118453926.ch16
- [128] Yang T, Grover M, Joeng KS, et al. Human Fetal and Neonatal Bone Development. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 8th ed. John Wiley & Sons, Inc.; 2013. pp. 119-126. ISBN: 978-1-118-45388-9. DOI: 10.1002/9781118453926.ch15
- [129] Holm IA. Skeletal Complications of Childhood Cancer. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 8th ed. John Wiley & Sons, Inc.; 2013. pp. 734-740. ISBN: 978-1-118-45388-9. DOI: 10.1002/9781118453926.ch89
- [130] Inui A, Itamoto K, Takuma T, et al. Age-related changes of bone mineral density and micro-architecture in miniature pigs. *The Journal of Veterinary Medical Science*. 2004;**66**:599-609. Available from: [https://www.jstage.jst.go.jp/article/jvms/66/6/66\\_6\\_599/\\_pdf](https://www.jstage.jst.go.jp/article/jvms/66/6/66_6_599/_pdf)
- [131] Allori AC, Sailon AM, Pan JH, et al. Biological basis of bone formation, remodeling, and repair-part III: Biomechanical forces. *Tissue Engineering Part B, Reviews*. 2008;**14**:285-293. DOI: 10.1089/ten.teb.2008.0084
- [132] Kilborn SH, Trudel G, Uhthoff H. Review of growth plate closure compared with age at sexual maturity and lifespan in laboratory animals. *Contemporary Topics in Laboratory Animal Science*. 2002;**41**:21-26. Available from: <http://www.ingentaconnect.com/content/aalas/jaalas/2002/00000041/00000005/art00005?crawler=true>
- [133] Viateau V, Logeart-Avramoglou D, Guillemain G, et al. Animal models for bone tissue engineering purposes. In: Conn PM, editor. *Sourcebook of Models for Biomedical Research*. Totowa, NJ: Humana Press; 2008. pp. 725-736
- [134] Muschler GF, Raut VP, Patterson TE, et al. The design and use of animal models for translational research in bone tissue engineering and regenerative medicine. *Tissue Engineering Part B, Reviews*. 2010;**16**:123-145. DOI: 10.1089/ten.TEB.2009.0658
- [135] Wood MW, Hart LA. Selecting appropriate animal models and strains: Making the best use of research, information and outreach. 6th World Congress on Alternatives and Animal Use in the Life Sciences; August 21-25, 2007; Tokyo, Japan. AATEX; 2008. pp. 303-306

- [136] Kojima H. The use of 3-D models as alternatives to animal testing. *Alternatives to Laboratory Animals*. 2015;**43**:P40-P43. Available from: <http://pilas.org.uk/wp-content/uploads/2015/10/Opinion-Kojima-FINAL.pdf>
- [137] Abubakar AA, Noordin MM, Azmi TI, et al. The use of rats and mice as animal models in *ex vivo* bone growth and development studies. *Bone & Joint Research*. 2016;**5**:610-618. DOI: 10.1302/2046-3758.512.bjr-2016-0102.r2