FEBRUARY 1, 2021

GETTING TARGETED THERAPIES TO PEDIATRIC PATIENTS: THE ILTB AND THE PINOCCHIO STUDY

MAJOR INTERNSHIP REPORT

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Abstract

Molecular profiling is becoming standard of care in pediatric oncology, leading to an increase in knowledge about genetic aberrations at the basis of cancer development and subsequently to an increase of Targeted Therapies. Interesting novel therapies include the Tyrosine Kinase Inhibitors, Monoclonal antibodies, CAR-T cell therapies, and NK cell therapies. Approval for the usage of these novel targeted therapies in pediatric patients is lagging adult approval by a significant margin of 6 years. For this reason novel clinical trial designs in the form of master protocols are under development: to accelerate clinical trials in small subsets of pediatric patients. At the basis of a master protocol a profiling initiative molecularly profiles the patients and recommends enrolment into a specific clinical trial or a specific arm of a master protocol.

The International Leukemia Target Board (iLTB) is an European tumor board aiming to enroll all patients with a r/r hematological malignancy and no standard of care treatment option in Europe. After inclusion and discussion the iLTB recommends treating physicians the fitting clinical trials or therapies for their pediatric patient. For a molecular profiling initiative to work efficiently, a rigid framework to enroll the patient and store all their data is necessary. For this reason, iLTB database was designed and created. Several improvements on a standard clinical trial database design were thought up, considered, designed, and implemented to ensure a couple of core conditions: Easy enrolment of patients from anywhere in Europe, smooth data import and export, and, as a result, accelerating trial enrolment in small pediatric clinical trials.

Because of the absence of enough safety and efficacy data of targeted therapies in pediatric patients, targeted therapies are often not prescribed to them. In order to enlarge the current knowledge about the pharmacokinetics of targeted therapies in pediatric patients, the Pinocchio protocol was amended to also include Tyrosine Kinase Inhibitors. The study design, study outcomes, informed consent forms and database were designed and written, and the protocol was submitted to the Medical Ethics Committee receiving conditional approval. As soon as final approval is granted, patient enrolment into Pinocchio Stratum 2 can start.

Layman summary

Science has been able to cure most of the children that get cancer, since about 17 out of 20 children that get cancer will be alive 5 years after their diagnosis. For the 3 children that still pass away, current treatments are not effective and therefore new therapeutic options are necessary. Before a new therapy can be standardized in hospitals, the therapy must first be approved by the EMA (Europe) or FDA (US). For approval the therapy must be extensively tested on different patients in a controlled setting, named a clinical trial. Children for whom no further standard treatments are available can participate in these clinical trials and therefore get an innovative treatment early, while at the same time also helping in the process of getting the drug approved. The problem that the field of pediatric cancer research now faces is that new therapies are approved in adults much quicker than in children, because it is difficult to include enough patients into the pediatric clinical trial. For some rare forms of cancer there are not enough patients to perform the clinical trials necessary for the drug to be approved.

In this report, the process to setting up the database for the international Leukemia Target Board (iLTB) and the amendment of the Pinocchio study are described. The iLTB is a molecular profiling initiative aiming to include all European pediatric leukemia patients for whom there is no standard treatment option anymore. In setting up the iLTB database many challenges arose, since patients need to be enrolled locally from all over Europe. Challenges included the actual building and finetuning of the database, batch effect corrections, database hierarchy constructions, and simplifications for easy use. The amendment of the Pinocchio study aimed to include a new class of drugs (Tyrosine Kinase Inhibitors) to the existing protocol. For this, a whole new protocol has been written, Patient Informed Consent forms have been created, another database was made, and all documents were submitted to the METC. The Pinocchio study aims to create a framework to learn from every single patient treated with a TKI in the Princess Máxima Center, and is the first step in implementing Therapeutic Dose Monitoring.

While both these projects might seem very different, they come back to a central problem in pediatric oncology: data of drug behavior in pediatric patients is scarce. With these projects we wish to increase the knowledge on how pediatric patients respond to novel therapies. Both via enrolling them in as many clinical trials as possible, and by determining the behavior of a new class of drugs, the Tyrosine Kinase Inhibitors, in the body of children with cancer.

List of abbreviations

iLTB	International Leukemia Target Board
METC	Medical Ethics Committee
OS	Overall Survival
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
r/r	relapsed/refractory
FDA	Food & Drug Administration
PIP	Pediatric Investigation Plan
NSCLC	Non-Small Cell Lung Cancer
ALK	Anaplastic Lymphoma Kinase
TKI	Tyrosine Kinase Inhibitor
RTK	Receptor Tyrosine Kinase
CAR-T	Chimeric Antigen Receptor T-cells
EGF-R	Epidermal Growth Factor Receptor
VEGF-R	Vascular Endothelial Growth Factor Receptor
ALK	Anaplastic Lymphoma Kinase
FLT3	FMS Related Receptor Tyrosine Kinane 3
NTRK	Neurotrophic Tyrosine Receptor Kinase
MEK, MAP2K	Mitogen Activated Protein Kinase Kinase
MAPK, ERK	Mitogen Activated Protein Kinase), and the
BCR-ABL	Breakpoint Cluster Region-ABL proto-oncogene
TDM	Therapeutic Dose Monitoring
Pk	Pharmacokinetics
MTD	Maximum Tolerated Dose
OBD	Optimal Biologic Dose
PIF	Patient Informed Consent
eCRF	electronic Case Report Form
NCC	National Coordinating Center
DM	Data Manager
CPM	Count Per Milion

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Introduction

Over the last decades major improvements have been achieved in the treatment of pediatric patients with cancer. Overall survival rates for pediatric patients have been climbing since the 1970 at a steady pace. Consequently, at present time around 84% of pediatric oncology patients have an overall survival (OS) of over 5 years, compared to 48% in the mid-1970s (figure 1).[1–3] While this statistic is hopeful, for 15% of patients treatment is not successful. Children not responding to treatment will have refractory disease and therefore relapse.[4–7] In leukemic patients, 15-20% of pediatric Acute Lymphoblastic Leukemia (ALL) patients, 25% of pediatric Acute Myeloid Leukemia (AML) patients and 10% of pediatric lymphoma cases suffer a relapse.[4,8,9] After relapse, patients suffering from a hematological malignancy will see their chances of OS longer than 5 years decrease to about 40% in AML, between 20-50% in ALL (depending on cytogenetics and the timing of the relapse), and around 50% in lymphomas.[4,8,9] Furthermore, prognosis for brain tumors and neurological tumors is relatively low even without relapse.[2] Especially for the refractory/relapse group of pediatric patients novel therapeutic options are necessary. Therefore, innovative new treatments have been designed In the past years, but most of these groundbreaking new treatments start their development in adult-only clinical trials.[7,8]

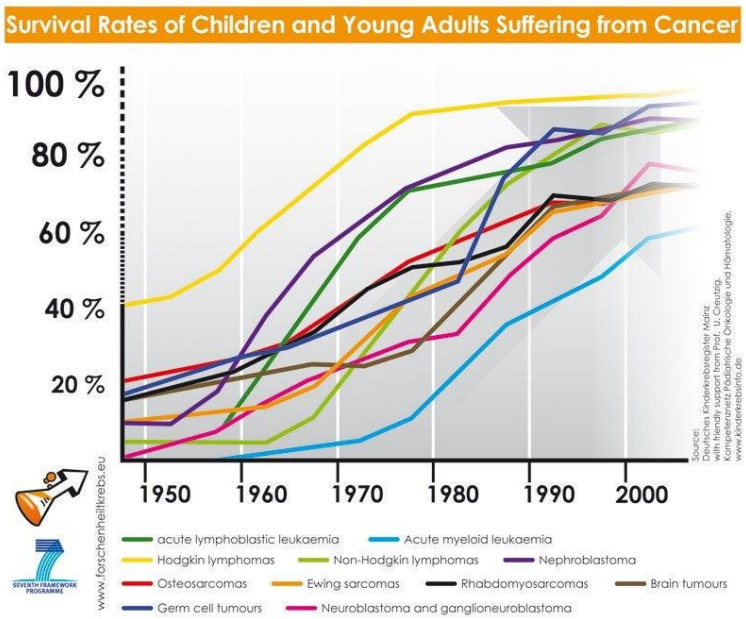

Figure 1: Percentage of pediatric and adolescent patients with an OS of 2 years per diagnosis. Especially patients suffering from Acute Myeloid Leukemia still have a bad prognosis, while patients suffering from Hodgkin lymphoma nearly always reach an OS of 2 years. Image taken from kinderkrebsinfo.de.[10]

To emphasize this, a study evaluated the median time between first-in-human and first-in-children clinical trials between 1997 and 2017. The average time between first-in-human enrollment and first-in-children enrollment was 6.5 years. Moreover, 6 out of 117 FDA-approved drugs with an oncologic indication included children in the initial indication.[11] Usually, pediatric clinical trials were starting inclusion at the time of first approval in adults. In 2007, the EMA introduced the 'Pediatric Investigation Plan' (PIP). Approval of novel therapies in children got an impulse, because PIP requires companies marketing a drug to include a market authorization plan for their drug in pediatric patients as a compulsory part of market authorization application. However, the PIP is often waived or deemed 'not necessary' in practice, as the specific diagnosis for which market approval is being asked rarely occurs in pediatric patients (e.g. non-small cell lung cancer, NSCLC). From within the research field a

switch from an indication-based PIP (e.g. NSCLC) to a target-based PIP (e.g. Anaplastic Lymphoma Kinase, ALK) is being proposed. The call for this shift from indication to target makes sense in oncology considering that over 50% of waivers of PIP obligations between 2012 and 2015 were drugs with a mechanism of action or target also relevant for pediatric patients.[11,12] The target-Based PIP would be a logical step considering that targeted therapies often have multiple target-based indications, compared to chemotherapeutic agents that have a broad diagnosis-wide indication. Having one target-based PIP for a targeted therapy would be more rational than having multiple diagnosis-based PIPs.

Increasingly small subsets of patients - Targeted therapy for children

Molecular profiling of pediatric patients with malignancies is becoming standard of care in specialized hospitals. Consequently, general diagnoses like 'leukemia' or 'lymphoma' are expanded to include the genetic aberrations present in the tumor (figure 2).[9,13] As a result, increasingly small subsets of patients are clustered together, and specific drugs, targeting exactly the genetic aberrations the tumor harbors have been developed. These targeted therapies are different to conventional chemotherapies, that target general cell division mechanisms.[12,14] Due to the increasingly small number of patients per diagnosis, a problem emerges: Enrolling enough patients into clinical trial that investigate targeted therapies for specific aberrations is becoming increasingly challenging.[15]

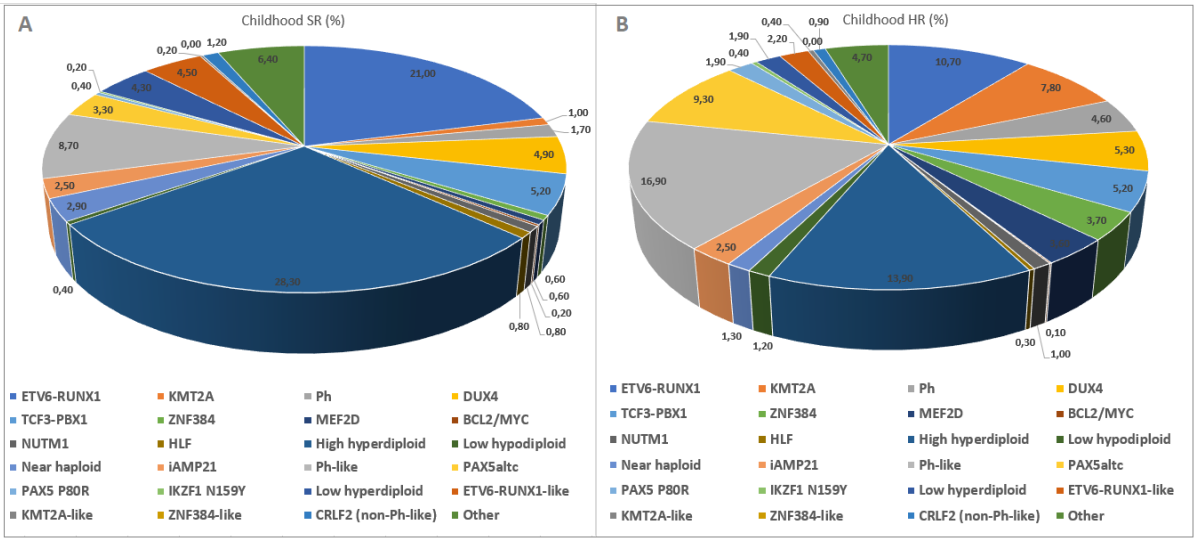

Figure 2: subdivision of B-cell Acute Lymphoblastic Leukemia. The first division is between standard risk (left) and high risk (right). Many different factors are at play, at different levels of cell organization. Chromosomal (hyperdiploid), genetic (KMT2A) or other. The fact that there is still a share of 'other' or 'unknown' origin means that there are still subtypes of B-ALL undiscovered. Figures were created using Microsoft Excel and data from Zhaohui Gu et al.[13]

How to allocate patients correctly – Tumor boards and master protocols

To ensure that all pediatric patients receive the best possible therapy, clinical research is adapting. As described above, targeted therapies and the cancer genome are being intensively investigated and knowledge of different genomic mutations at the basis of tumor development has increased. Consequently, number of patients that can be enrolled into specific trails becomes increasingly smaller, because the number of patients with the eligible genetic aberration is low. The targeted therapy is designed specifically for that small subset of patients, and consequently, to enroll enough patients, specific and adaptable 'master protocols' are under development.[16] Master protocols translate into 3 different types of clinical trials: Umbrella trials, basket trials, and platform trials. Umbrella trials study multiple targeted therapies for a single diagnosis.[17] Basket trials study one targeted therapy for multiple diagnoses or subtypes of diagnoses, and platform trials study multiple

targeted therapies for one disease, but in an ongoing manner. Therapies can be added or removed via a decision algorithm.[17,18]

Examples of master protocols are the ESMART, and the HEM-I-SMART (currently under development) (figure 3).[18]

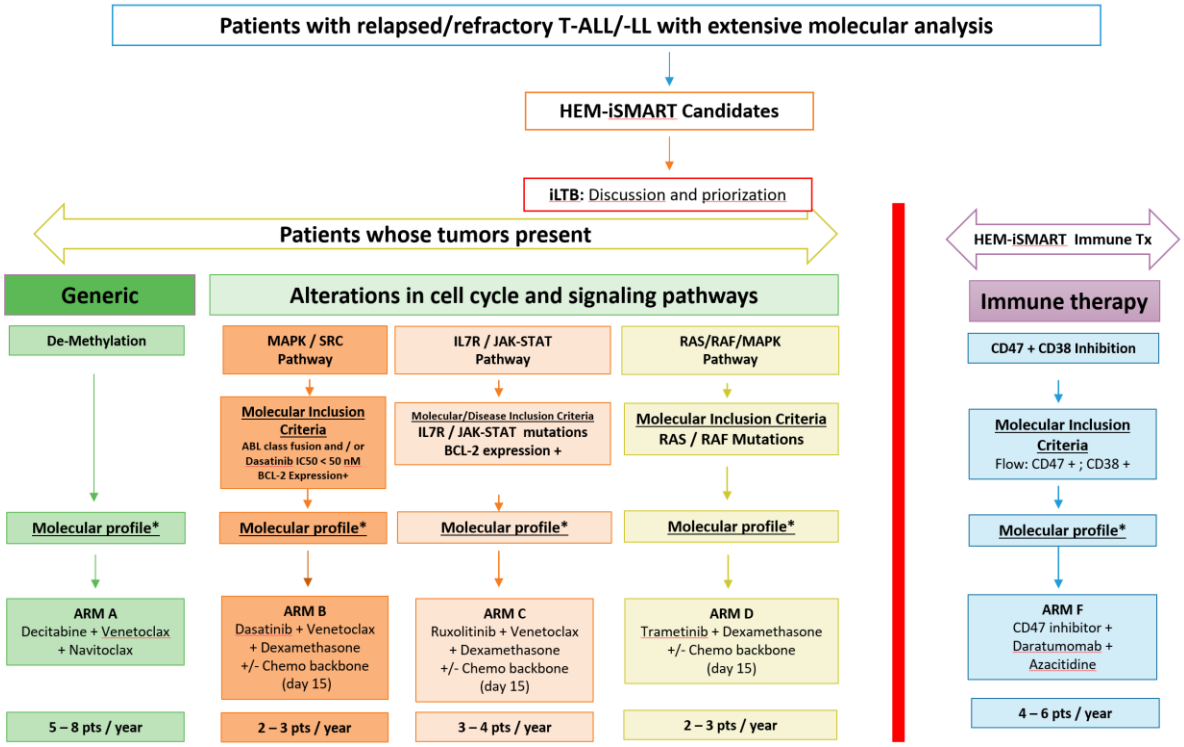

Figure 3: lay-out of the HEM-iSMART clinical trial. an example of a master protocol and specifically an umbrella trial. Patients with r/r T-ALL/LL are molecularly profiled and discussed in a molecular tumor board (the iLTB, see below). After profiling, a subdivision within the patient groups is made based on the results from molecular profiling. Based on the mutations found in the profiling, a patient is enrolled in a specific arm of the study. Expected enrollment per year is low, showing why an umbrella trial design was chosen.

At the basis of basket trials stands a profiling initiative or entity that divides the patients within the clinical trial on the basis of their molecular profile (e.g. on the basis of the results of DNA/RNA sequencing). Several methods of incorporating molecular profiling into standard care for patients with pediatric malignancies have been implemented. In these profiling initiatives patients are enrolled and extensively molecularly profiled. After enrolment a discussion follows. The result is a prioritized advice of treatment options for the patient.

Molecular profiling initiatives have are beneficial in multiple ways. First, because of the prioritized advice, the treating physician has the opinion of experts in the field of pediatric oncology and is therefore able to make a well-informed treatment decision for his/her patient. second, by consulting the experts and documenting treatment advice per diagnosis, treatment advice is automatically harmonized. Specific genetic events or signatures will therefore result in specific treatment suggestions demonstrated to be the most efficient. Third, by integrating innovative diagnostic tools (e.g. centralized drug response profiling and surface marker analysis with flow) into the discussion, the experts will always have the most data available to make an informed decision. Last, the prioritized advice is especially beneficial if the specific diagnosis of the patient is rare, and the treating physician has no prior experience with how to treat the patient or knowledge on which trials are currently open. If the advice leads to the inclusion of the patient into a small clinical trial, the trial will be accelerated, completed faster, and investigated drugs can be approved in a faster manner. One example of a

molecular profiling study is the American 'LEAP consortium'. Since opening, a total of 153 patients with a r/r hematological malignancy were enrolled. All of them were extensively molecularly profiled and discussed in a panel of experts. As a result, 17 patients eventually received a targeted therapy recommended by the panel of experts. In 11 patients actionable mutations were found that would not have been discovered using standard of care diagnostic protocols. For these patients the findings in the profiling study could literally be life-saving.[5] Other examples of molecular profiling studies include the PIPseq, INFORM, and ZERO. In PIPseq 56 patients with hematological malignancy were profiled. 40 had a targetable mutation, and 7 patients received targeted therapy. In INFORM 446 patients with potentially actionable target were identified, and consequently 147 received targeted therapy. In ZERO 247 patients with a high-risk pediatric malignancy were profiled, of which had 175 a targetable mutation. As a result, 43 patients received targeted therapy.[19–21] The two most important reasons for not receiving a targeted therapy recommended were the presence of standard-of-care protocols on which the patient started (LEAP) and no available safety and efficacy data of the targeted therapy in pediatric patients (PIPseq).[5,19]

iLTB

The iLTB is a new molecular tumor board initiative currently being set up in Europe. The purpose is to enroll patients suffering from a r/r hematological malignancy with no standard of care treatment option. Because patients from all over Europe can be included, the iLTB is (one of the) first international tumor board. Patients are molecularly profiled by the local site or centrally, and not by the sponsor site of the study. Molecular profiling consists of DNA-, RNA-, and CD/Flow marker analysis. Drug response profiling is done centrally in Zurich. After profiling, patients are either discussed in a local molecular tumor board before being enrolled into the iLTB or directly enrolled into the iLTB. In the iLTB patients are discussed in a panel of experts based on the molecular profiling performed. Panel meetings are held weekly to ensure a quick turnover time between profiling and discussion in the iLTB. After the meeting, the treating physician will receive a prioritized advice based on actionable events identified by profiling. Actionable events are defined in the iLTB protocol as '*a tumor characteristic for which targeted therapy is approved or investigated in a clinical trial for any cancer indication, including but not limited to small molecules and immunotherapy*'.[22] The final treatment decision is made by the treating physician. The patient can be enrolled into a clinical trial (the HEM-iSMART, SeLuDex, etc.) or off label/compassionate use of a drug might be recommended. After discussion the patient is followed up every 3 months for 2 years to determine which treatment the treating physician prescribed, whether treatment was in line with the advice provided by the iLTB, and if no recommended treatment was started, the reason for not choosing a recommendation provided. An overview of the study design is provided in figure 4**.** the iLTB is an example of how to bring personalized medicine, a method of treating patients based on their personal genomic or cellular aberrations and drug sensitivities, to the patient. Recommendations done by the iLTB will include targeted therapies (tyrosine kinase inhibitors) for patients with specific mutations (BCR-ABL fusion proteins), CAR-T therapy against specific CD markers expressed on malignant cells, or combination therapies like inotuzumab ozogamicin (antibody-chemotherapy conjugate).[23] The iLTB aims to identify the percentage of patients that are treated according to their actionable events after iLTB discussion and harmonizing treatment prioritization in Europe.

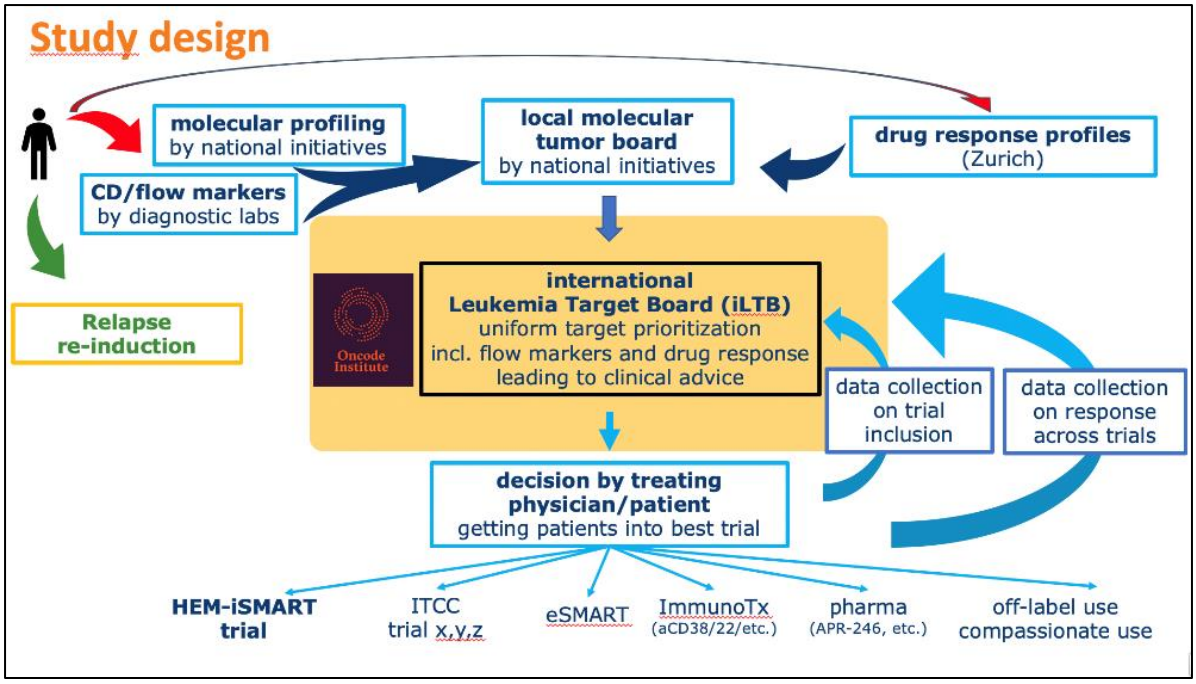

Figure 4: the study design of the international Leukemia Target Board study. Patients are diagnosed and molecularly profiled by the local site (treating hospital) before either being enrolled in a local tumor board, or directly into the iLTB. In the iLTB, patients are discussed, and targets are prioritized. A clinical advice is given. The treating physician finally decides how to treat the patient, either by enrolling them in a clinical trial, or by off-label compassionate use. Data on the treatment finally received is collected in the iLTB database.

Tyrosine Kinase Inhibitors

the targeted therapies recommended in the iLTB & LEAP study are a relatively new generation of anti-cancer drugs. With molecular profiling of tumors becoming standard of care, targeted therapies specifically designed for a genetic aberration are under development. One large group of interest is the Tyrosine Kinase Inhibitors (TKIs).[24–28] TKIs are a class of drugs inhibiting tyrosine kinases, that play a key role in cellular processes and their role in disease is well documented. Tyrosine kinases consist of two main groups, the transmembrane receptor kinases and the cytoplasmic kinases.[24]

The Receptor Tyrosine Kinase (RTK) group consists of an extracellular/ligand binding region, a single transmembrane helix, the juxta-membrane regulatory regions, and the protein tyrosine kinase domain (figure 5). Upon activation, the receptor dimers, and is phosphorylated three times per receptor unit (six times in total) leading to switching to the active conformation and subsequent downstream signaling.[24] Downstream signaling pathways include PI3K pathways, and RAS pathways, crucial in cell cycle regulation and proliferation. Consequently, constitutive activation of RTKs can lead to uncontrolled cell proliferation. The pathogenic activation of RTKs is mediated in 4 ways: an abundance in ligand (autocrine activation) A gain of function mutation (genomic mutation), genomic rearrangement (genomic fusions), or overexpression (often genomic amplification).[29] Generally, an activating mutation (gain of function mutation) in an RTK leads to consistent activation. Somewhere in the structure a mutation has occurred rendering ligand binding and consequent dimerization or phosphorylation unnecessary for 'on' signaling. Overexpression is mediated by an amplification of the gene of the RTK. Overexpression entails more protein is synthesized, and therefore more receptors are present on the cell membrane, leading to more downstream 'on' signaling.[29]

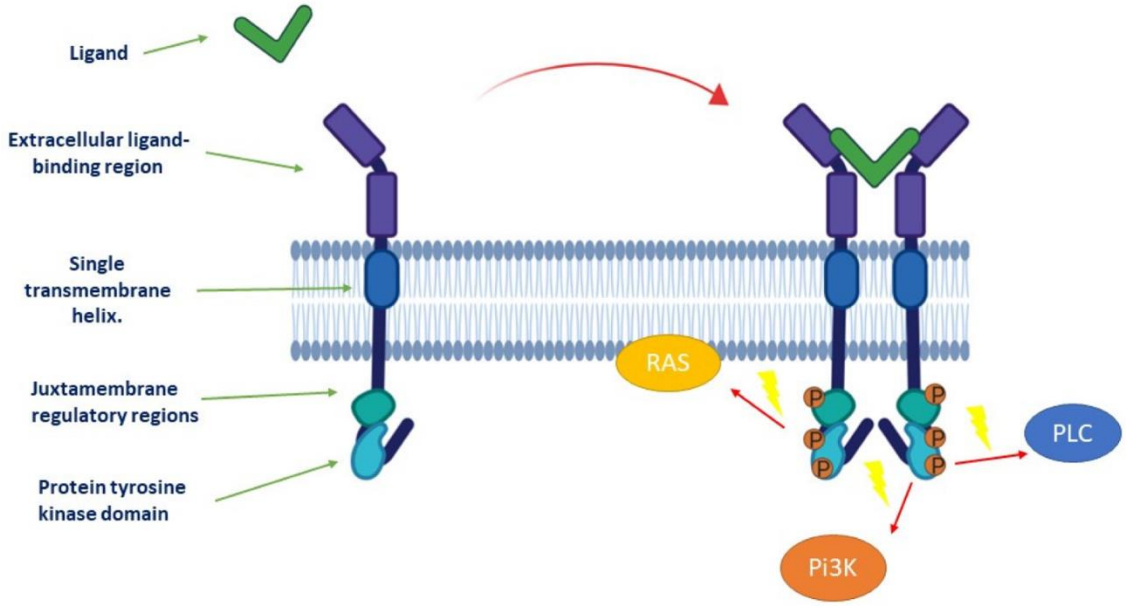

Figure 5: a Receptor Tyrosine Kinase. When binding ligand, the RTK typically dimers and auto phosphorylates. On the juxta membrane regulatory position regulatory proteins can bind to either alter the effect of the RTK or the downstream signaling. Important cellular pathways regulated via RTKs are the RAS pathway and Pi3K pathway. Figure by pottier et al.[24]

Examples of RTK inhibitors are the Epidermal Growth Factor Receptor (EGF-R) inhibitors, the Vascular Endothelial Growth Factor Receptor (VEGF-R) inhibitors, the Anaplastic Lymphoma Kinase (ALK) inhibitors, the FMS Related Receptor Tyrosine Kinane 3 (FLT3) inhibitors, and the Neurotrophic Tyrosine Receptor Kinase (NTRK) inhibitors. A list of developed RTKs can be found in supplementary table 1.

Apart from RTKs there is also a class of tyrosine kinases that reside in the cytoplasm, Cytoplasmic Tyrosine Kinases (CTKs). These proteins are present in distinct pathways required for cell proliferation and survival (MAP3K pathway, AKT/mTOR pathway). CTKs include the Mitogen Activated Protein Kinase Kinase (MEK, MAP2K), Mitogen Activated Protein Kinase (MAPK, ERK), and the onco-fusion protein Breakpoint Cluster Region-ABL proto-oncogene (BCR-ABL). Specific inhibitors to target CTKs have been developed. A full list of TKIs, their target, and indications can be found in supplementary table 1.

The first TKI receiving approval was imatinib, a BCR-ABL fusion protein inhibitor. Approval was granted in 2001 for adult patients and in 2003 for pediatric patients. Quickly thereafter ALK inhibitors, MEK inhibitors, EGF-R inhibitors, VEGF-R inhibitors, BCR-ABL inhibitors, FLT-3 inhibitors, and NTRK inhibitors also gained approval in adults. Disappointingly, only a handful of TKIs are currently approved for usage in the pediatric population. BCR-ABL inhibitors are approved (imatinib, nilotinib, dasatinib) for myeloid leukemias, the ALK-inhibitor crizotinib for anaplastic large cell lymphoma, and the VEGF-R inhibitor vandetanib for testicular cancer in children older than 5 (supplementary table 1).

Differences between adults and pediatric patients

A new way of dosing therapeutics in pediatric patients is via Therapeutic Dose Monitoring (TDM). Which is being intensively investigated in cytoplasmic agents.[30] In TDM, dosing regimens are adaptable, based on Pk properties that are investigated during administration of a drug. In TKIs TDM is less advanced because of a couple of challenges: first, all TKIs are administered orally. Therefore, the pharmacokinetic profile is generally more complex than chemotherapies, conventionally administered via intravenous central line. This leads to more complex blood sampling because no central line is present to acquire samples, like in chemotherapeutics. Secondly, large inter-patient

differences in pharmacokinetics (Pk) of TKIs are observed, because food, the formulation in which the drug is given, genetic factors, and concomitant medication influence the TKI's disposition.[31] Apart from the external factors mentioned, the pediatric population is heterogeneous in terms of body composition and organ maturation (liver and kidney), creating even larger differences in pharmacokinetics. Third, the majority of TKIs investigated to be used in pediatric malignancies are still in early stages of development and exposure-efficacy and exposure-toxicity relationships are not yet established. Therefore, target exposure is often based on adults. [32] fourth, dose finding trials are often still designed based on the concept of a maximum tolerated dose (MTD, increasing dosing until toxicities), while for TKIs the optimal biologic dose (OBD) might be lower.[33] Last, there is an increasing number of available TKIs seeking market approval and as mentioned above, the indication-based PIP is often waived. This leads to clinical trials generally opening for adults but not for pediatric patients. Nonetheless, an exposure-response- and exposure-toxicity relationship have been characterized for some TKIs (imatinib, dasatinib, bosutinib, nilotinib, pazopanib, sunitinib, vemurafenib and everolimus) in adults. This would suggest a possible advantage of individualized exposure strategies for both adults and children.[14,32] However, the lack of Pk data from pediatric patients generates friction when trying to achieve optimal dosing strategies, and actively discourages physicians from prescribing TKIs to pediatric patients.[19]

in this internship the framework for the international leukemia target board (iLTB), an European profiling initiative, is drawn up. This framework consists of a database in which local sites will enroll patients themselves. Additionally, a treatment protocol to gather specific Pk data in children receiving TKI therapies outside of clinical trials measuring Pk data was written. This report will describe the process of creating a database for the innovative iLTB study, as well as the submission of a new stratum to the Pinocchio-Study, assessing Pk data in children receiving TKIs.

Material & Methods

iLTB

Database

For the creation of the iLTB framework database, electronic Case Report Forms (eCRFs) were compiled based on the lay-out of various studies. The eCRFs of different already running clinical trials were used as a basis. The eCRFs used were from:

- SeLuDex
- IntReALL
- INFORM
- iTHER
- iTHER2

studies were analyzed and compared. The ITHER eCRFs (the study with the end points most resembling the iLTB) were taken to function as a baseline. In excel, the SeLuDex, IntReAll, and INFORM eCRFs were compared field for field to add, remove, and rephrase the iLTB eCRFs. after comparison of other eCRFs, the first draft version was created in Excel, and sent to- and discussed with the central data managers, principal investigators, and physicians in the Princess Máxima Center.

The program 'Alea Data Management' is used for the building of the database. The excel, extensively reviewed and modified, was used as a basis. Separate forms were created:

- Registration

- baseline
    - disease history
    - treatment history
    - biopsy
- profiling
    - analyses
    - events
    - documents
- meeting
- advice
- follow-up

After the first draft was created in Alea, various other molecular profiling cases (iTHER, iTHER2) were entered, and the database was changed if needed. After careful internal review, access to the Alea database was granted to representatives from Charité (Berlin), Heidelberg university hospital, and Newcastle Hospital for reviewing.

a gene list of 240 known oncogenes was compiled from data from ITHER, ITHER 2, and from data from a local bioinformatics exper. This gene list was discussed extensively, and some genes were added in retrospect. The gene list was pasted into an alea dropdown, to make selection easier. In this dropdown extra room for future genes of interest is left, and options to make suggestions for missing genes is added to Alea as well. The full list can be found in supplementary data table 2.

A reference cohort of 30 cases per diagnosis (AML, ALL, Lymphoma) will be registered in the iLTB database. By creating a separate eCRF 'reference cohort data'. This eCRF will be hidden for all participants. A separate table was created in which housekeeping gene CPM values can be stored. Data for the reference cohort was requested via an internal BioBank request.

Clinical trial list

to generate a list of open clinical trials for hematological malignancies, a search was conducted on clinicaltrials.gov. the advanced search option was used. 4 different searches were conducted:

1. Leukemia
    a. Search term: 'leukemia'
    b. Study type: 'interventional study'
    c. Study recruitment: 'recruiting'
    d. Age range 'child' (birth-17)
2. Leukemia
    a. Search term: 'leukemia'
    b. Study type: 'interventional study'
    c. Study recruitment: 'recruiting'
    d. Age range 'custom' (≤21)
3. Lymphoma
    a. Search term 'lymphoma'
    b. Study type: 'interventional study'
    c. Study recruitment: 'recruiting'
    d. Age range 'child' (birth-17)
4. Lymphoma
    a. Search term 'lymphoma'

b. Study type: ‘interventional study’
c. Study recruitment: ‘recruiting’
d. Age range ‘custom’ (≤21)

Results were exported to Excel and separated by column. Irrelevant columns were removed. Data was cleaned up to fit properly the columns. Studies conducted outside of Europe were systematically removed. Only relevant interventional studies were kept. A summary of ITCC studies was created based on presentations given at the ITCC meeting (Paris, 2021) and formatted to the same columns as the results from clinicaltrials.gov. all studies were pasted under one another, and duplicates were removed to create a shortlist of open pediatric clinical trials in Europe.

Pinocchio protocol

The Pinocchio protocol amendment started with a wide literature search of TKIs. Articles describing either pediatric or adult clinical trials as well as all SmPCs of approved drugs were read and summarized in the introduction of the protocol. Blood sampling schedules conform visiting schedules and with room for patient preferences were drawn up. Informed consent forms were written, checked by research nurses, and approved. A data management plan was drawn up and the database and eCRFs were built in castor. The eCRFs were taken from stratum 1 and modified for stratum 2. A lab manual was written for handling of samples after sampling and before analysis. Approval for this study was granted by the METC of the Erasmus medical center in Rotterdam.

Results

iLTB database

the finalized database in Alea contains a total of 10 different eCRFs. At the start of enrollment, the registration tab is filled in. In this tab the exclusion- and inclusion criteria for enrollment are assessed and contact details of the treating physician are collected. The ITCC site the physician is connected to is specified, and a preference for the week number in which discussion takes place is selected. After the registration tab is completed, the patient will be assigned a study number, and the rest of the available data can be entered. The treatment history, date of relapse, number of relapses, specific cytogenetics and other relevant medical history can be noted down here. The diagnosis of the relapse is automatically copied to the follow-up. The treatment history and biopsy details can be written down in separate CRFs as well. An overview of CRFs and their connection to one another can be found in figure 6.

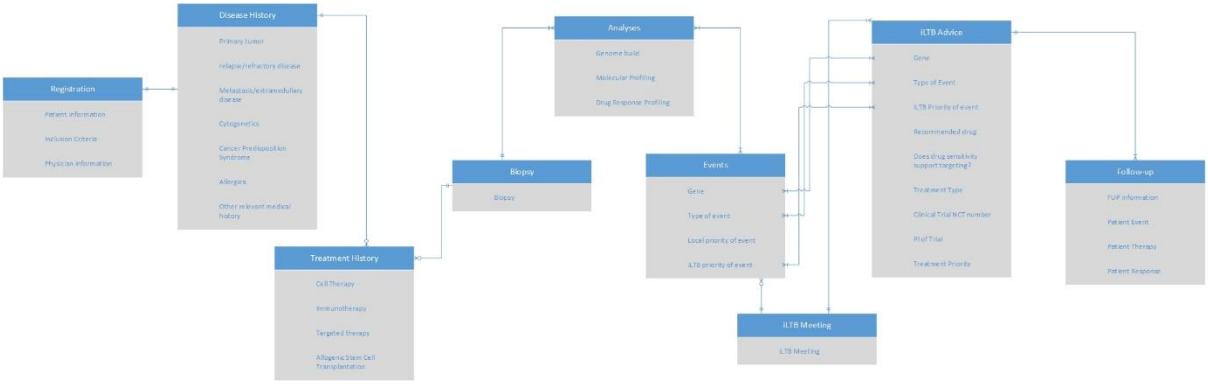

Figure 6: simplified database diagram for the iLTB database. the start and endpoints of lines indicate the relationship between two forms. || means one and only one. 0< means 0 or more, and |< means 1 or more. The figure is enlarged in ***supplementary data figure 1***

Registration of actionable events

After registration is completed, data about profiling analyses can be submitted, and actionable events can be registered. Especially the registration of actionable events is important for the discussion, and therefore an extensive eCRF is build. A list of 240 known oncogenes is added to the 'gene' dropdown, with the option to 'specify other' if the gene of interest is missing (supplementary table 2). after specifying the gene of interest, a total of 14 different types of events can be registered. Small Nuclear Variants (SNVs), deletions, bi-allelic deletions, insertions, fusion genes, gene breaks, (over- and under) expression, and surface marker (with and without RNA) expression. Dependent on the type of event selected, type-specific field will appear. The fields of interest per event type are provided in table 1.

Table 1: overview of possible actionable events and thereafter requested information for the iLTB Events page

Event type	Event-specific fields appearing
SNV	Chromosome number, genomic position, name of reference transcript, amino acid change relative to the reference transcript, Variant allele frequency DNA
Small insertion/deletion	Chromosome number, genomic position, start genomic position, name of reference transcript, amino acid change relative to the reference transcript, variant allele frequency DNA, Variant allele frequency RNA
Deletion	Chromosome number, number of copies
Bi-allelic deletion	Chromosome number, number of copies
Amplification	Chromosome number, number of copies
Fusion gene	Chromosome number, fusion gene partner, number of discordant reads
Gene break	Chromosome number genomic position
Expression	Local Z Score, CPM value, reference cohort, Z score iLTB reference cohort
Over-expression	Local Z Score, CPM value, reference cohort, Z score iLTB reference cohort
Under-expression	Local Z Score, CPM value, reference cohort, Z score iLTB reference cohort
Surface marker protein expression	Flow result, percentage of positive cells, RNA expression available, Local Z Score, CPM value, reference cohort, Z score iLTB reference cohort
Surface marker RNA expression	Local Z Score, CPM value, reference cohort, Z score iLTB reference cohort
Signature	Free text
Extreme drug sensitivity	Free text
Other event	Free text

all documentation the local center might have, including an overview of actionable events identified by the local center and the eventual presentation within the iLTB discussion, can be uploaded into the database and can be easily found in the patient dossier.

Overexpression analysis

To be able to assess (over)expression of oncogenes a calculation was drafted by bio-informaticians (figure 7). To be able to calculate the necessary Z-Score, a reference cohort tab was introduced into the database. The reference cohort tab can hold the values of the population (figure 7) necessary for Z-Score calculation of oncogenes. At the bottom of the 'events' page a location was created for the filling of the housekeeping gene count per million (CPM) values.

Z-score = (X – mean(population)) / sd(population)

- Population here means some reference cohort
- X = gene ratio's calculated by: CPM value gene / median(CPM values housekeeping genes)

Figure 7: calculation for Z-Scores. The X is calculated via the CPM value of the housekeeping genes in combination with the CPM value of the oncogene. After X is calculated, the mean CPM and SD of the CPM value of the oncogene from the reference cohort is taken to calculate the Z-Score of the oncogene. The reference cohort will be 30 cases per diagnosis (AML, ALL, Lymphoma).

iLTB advice

The end goal of the iLTB is to give patients a prioritized advice based on their genetic aberrations. To orchestrate this, a separate form is created in Alea, the iLTB advice. The events registered in the 'events' tab including the gene of interest, the type of event and the priority of the event are copied to the advice tab automatically when the events tab is submitted (figure 8). Extra advice might also be added, and a treatment advice is given. The possibilities of treatment might include enrollment into a clinical trial, off label/compassionate use of a therapy, or recommendation of an already approved therapy.

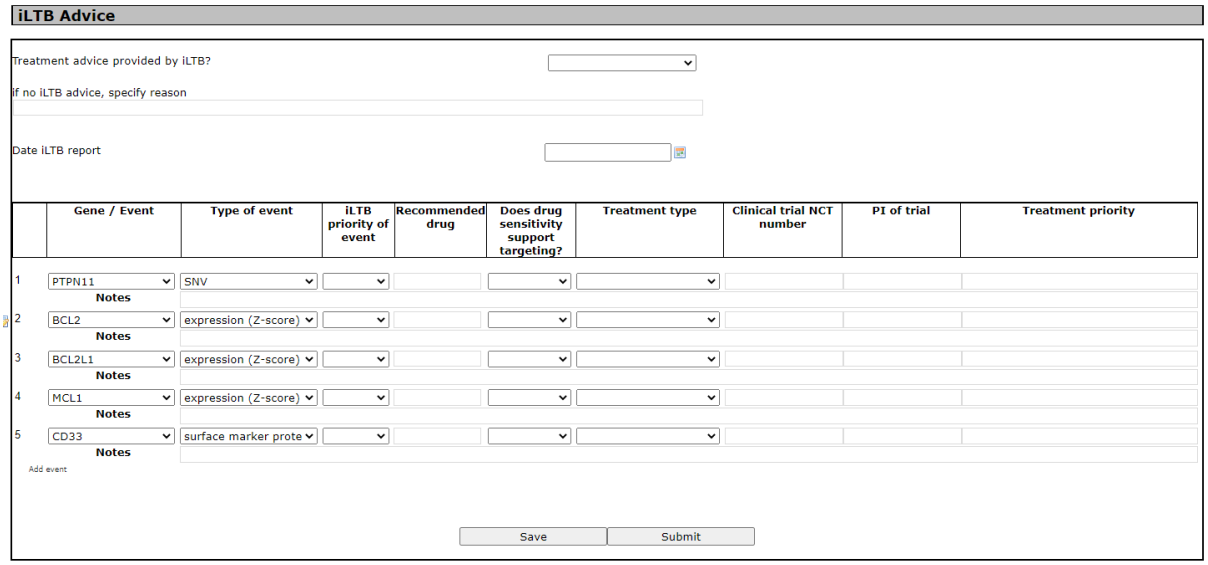

Figure 8: the CRF for patient advice in the iLTB. Gene, type of even and the iLTB priority given to the event are copied from the 'events' tab and therefore are prefilled automatically. The recommended drug can be specified, and if drug sensitivity supports targeting. If the recommended therapy is a clinical trial, a NCT number can be provided, as well as the PI of the trial to speed up enrollment. Treatment priority should be numbered in the final column.

Follow-up

The follow-up section is created with the outcome measures of the iLTB in mind. The events of the patient in the months between last follow-up and current follow-up can be recorded. Since the iLTB discusses patients with specifically aggressive diseases, the option to specify if patient status changed within the 3 months since last follow-up. If 'yes' is answered to the question a table opens to specify events within the 3 months of follow-up. If the patient received targeted therapy recommended by the iLTB, the number of the advice can be registered. The start day of treatment can be filled in and

the dosing regimen specified since the start date is of interest in determining the turnover time of the iLTB discussion. The time between diagnosis, profiling, iLTB meeting, and start of treatment needs to be documented for the endpoints of the iLTB. If no targeted treatment recommended by the iLTB was given to the patient, 17 different reasons on why not can be provided to ensure that all possibilities can be captured in the database. By elucidating every possible reason for not receiving treatment, insights will be gained on how to increase the enrollment into clinical trials or the usage of off-label drugs. The response of the patient after one cycle of treatment is another outcome measure of the iLTB and should therefore also be recorded in the database. Diagnosis specific outcome measures can be selected from the dropdown menu (figure 9). Follow-up will take place every 3 months for 2 years after enrollment.

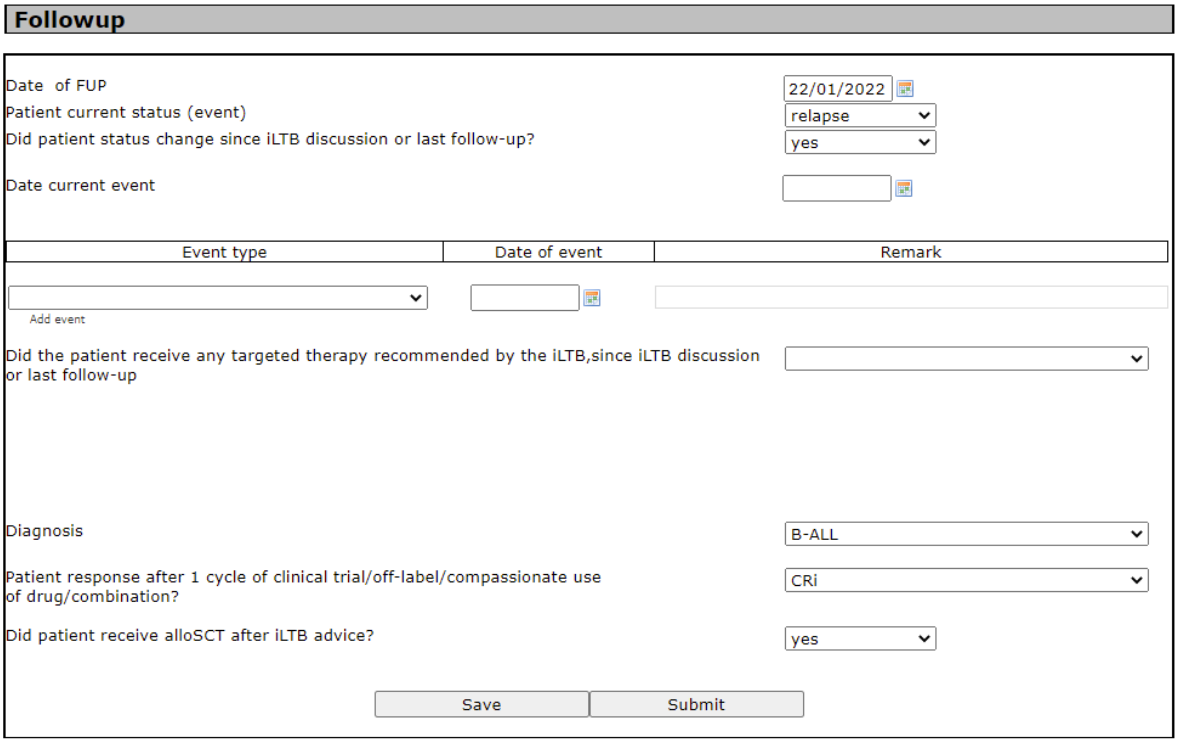

Figure 9: the follow-up section from the iLTB database. In the follow-up section the current status of the patient, received therapy and whether this therapy was recommended by the iLTB, and diagnosis specific clinical response after one cycle of treatment can be recorded.

Database hierarchy

To enroll patients from across Europe, the database was built with a clear hierarchy in mind. A specific center should be able to see and extract data from all patients enrolled by that center, but not by any other center. National Coordinating Centers (NCCs) should be able to see and extract data from all patients enrolled in their country. The PI, administrator, and Central Data Manager (CDM) of the study should be able to see and extract everything in the database. In (figure 10) the database hierarchy has been visualized.

Level	Role/Form	Registration	Disease History	Treatment History	Biopsy	Reference Cohort Data	Analyses	Events	Documents	iLTB Meeting	Advice	Followup
Local	Physician	E	E	E	E	H	E	E	E	R	R	E
	Data manager	E	E	E	E	H	E	E	E	R	R	E
National	PI	E	E	E	E	H	E	E	E	R	R	E
	Biologist	E	E	E	E	H	E	E	E	R	R	E
	National Coordinator	E	E	E	E	H	E	E	E	R	R	E
	Data Manager	E	E	E	E	H	E	E	E	R	R	E
Central	PI	E	E	E	E	R	E	E	E	E	E	E
	Coordinator	E	E	E	E	E	E	E	E	E	E	E
	Trial Manager	E	E	E	E	E	E	E	E	E	E	E
	Data Manager	E	E	E	E	E	E	E	E	E	E	E

Figure 10: overview of database hierarchy. Database roles have been split in three levels: Local, National, and Central. per CRF a different role is ascribed with E = Edit, R = Read, and H = Hidden. Another added dimension to the hierarchy is that local roles will only see patients enrolled by the local site. National roles will see all patients enrolled from that country, and the central roles will see all patients.

Pinocchio

A major amendment has been filed and reviewed by the METC of the Erasmus MC. After revision, minor changes have been implemented and the documents have been submitted again, awaiting final approval by the METC. The amendment aims to include 8 classes of TKIs into the Pinocchio protocol initially written to only include Pk Sampling of chemotherapeutic agents. Anaplastic Lymphoma Kinase (ALK) inhibitors, Mitogen-activated Protein Kinase (MEK) inhibitors, Break point Cluster Region – Abelson Oncoprotein (BCR-ABL) inhibitors (Vascular) Epidermal Growth Factor Receptor (VEGF-R and EGFR) inhibitors, multikinase inhibitors, FMS-Like Tyrosine Kinase 3 (FLT3) inhibitors, and Neurotrophic Tyrosine Receptor Kinase inhibitors were included. The protocol was deliberately written to specify classes of TKIs instead of specific TKIs to assure that new agents entering clinical trials could be added to the protocol without further amending. Three informed consent forms dependent on age of participant (<12 years, 12-15 years, >16 years) were created. Inclusion- and inclusion criteria were created.

Inclusion criteria are:

1. Planned to receive ALK inhibitors, MEK inhibitors, BCR-ABL inhibitors, VEGF-R inhibitors, EGF-R inhibitors, FLT3 inhibitors, NTRK inhibitors, or multi-kinase inhibitors
2. Age ≤21 years
3. Signed informed consent form (ICF)

Exclusion criteria are:

1. Already included in a clinical trial assessing PK parameters
2. Down syndrome
3. For adolescent girls of child-bearing age: pregnancy (orally inquired, a test is not necessary)
4. Any other disease/circumstance that may influence the participation of the subject in a negative way

The plan is to enroll around 20 patients per compound. With help from research nurses, physicians, trial managers, and the pharmacokinetics lab a dynamic sampling schedule was drawn up to minimize study participation burden on participants (table 2).

Table 2: Table of the sampling schedule of the Pinocchio study. Week numbers are given above, and in the week numbers of sampling days can be chosen. These days will be selected for days the patient needs to be in the Princess Máxima Center for standard of care appointments. The week number of treatment can be specified, and marked on which days Pk sampling should take place

	Week 3						
Study period (day)	1	2	3	4	5	6	7

PK sampling*		x			x		

The intra-day sampling schedule consists of three measurements. One before taking the daily dose of TKI, one around three hours after administration of the daily dose, and one six hours after taking the daily dose. All measurements can be taken from the same venipuncture, because contrary to stratum one, patients will seldom have a central line (Table 3).

Table 3: intraday sampling schedule. One sample will be done before dosing of the drug, and two samples will be taken after dosing. One after three hours and one after 6 hours.

Timing	PK sampling
Pre dose*	yes
3 hours post-dose (± 60 min) * *	yes
6 hours post-dose (± 60 min) * *	yes

After blood sampling is completed, the blood will be stored and analyzed. How blood should be stored is documented in the Pinocchio stratum 2 lab manual.

Database

The database for stratum 2 was created separately from stratum 1 in Castor. The patient is first registered. Patient details, diagnosis details, and in-and exclusion criteria are assessed. After registration is completed, a 'course' can be added for a specific patient. this 'course' is the same for the 2-5 measurements the patient will get. In the course the TKI measured can be selected, together with the dose, start date of current therapy, date of TKI dosing and time of TKI dosing. After specifying information about the TKI treatment, standard blood work is assessed, including creatinine, ALAT, and ASAT. When standard blood work is completed, all measurements of the specific research day can be added. Specified should be which sample number is taken (pre-dose, +3hrs, +6hrs), the actual sampling time, and if SAE did occur during sampling. After sampling data is completed, a list of concomitant medication possibly interfering with the Pk of the patient should be filled in, marking the research day as completed.

Discussion

Molecular tumor boards, only upsides?

Registering cases in a centralized entity like the iLTB benefits the patient, the fundamental scientific community, and the clinical scientific community. The patient benefits because his/her treating physician receives a prioritized treatment advice from experts in the field of (r/r) pediatric oncology, while the physician will have dealt with pediatric oncology only a handful of times in his/her career. The experts will have an overview of open clinical trials available and accessible for the patient. For fundamental science, an allocation study like the iLTB helps in numerous ways. Because of the extensive molecular profiling used by the iLTB (a drug-sensitivity screen, surface marker analysis, DNA sequencing, and RNA sequencing either performed locally (DNA- and RNA analysis, flow cytometry analysis) or centrally (drug response profiling)) a large amount of data is gathered about the patient. Data that is also available for research on r/r hematological malignancies. Genes of unknown significance, reasons for drug sensitivity, prognostic flow markers, et. can be easier elucidated by

increasing the amount of patient data available. The same goes for the detection of rare genetic aberrations and prognostic relapse dynamics. The iLTB contributes to clinical research because of the extensive profiling. Patients with rare genetic aberrations are identified and are therefore able to enrol into the proper clinical trial. By enrolling more patients, the trial is accelerated, and results are available quicker. The necessity for the speeding up of clinical trials is demonstrated by the results of the PIPseq study: physicians preferred not to prescribe targeted therapies to pediatric patients because of the absence of safety and efficacy data.[19] Assessing why patients were not enrolled into clinical trials is one of the secondary objectives of the iLTB. By gaining insight in the reason for a physician not to enrol their patient into a clinical trial, action can be undertaken to either increase the number of patients enrolled, or to open a clinical trial if a gap in current trials is observed. For example, the information gathered can be shared with a centre running a master protocol, with the request of opening a new arm. If no master protocols or other trials are already investigating an interesting observation, a new study can be started.

Drawbacks to molecular tumor boards

Opposed to the positive note presented above, there are certain drawbacks to molecular tumor boards. In the LEAP consortium profiling speed was the main bottleneck. The RNA-based fusion panel took approximately 16.6 (between 4 and 35) days.[5] Since patients with r/r leukaemia's are subject to especially aggressive forms of cancer, the time from enrolment to profiling to discussion to start of treatment is vital.[5] To ensure the fastest turnover time, from diagnosis to profiling to discussion, the iLTB will work in a different manner. Instead of waiting for patient samples to arrive and profiling in a central location, molecular profiling data from the local site will be used in the patient discussion. While Small Nucleotide Variants (SNVs), deletions, or insertions can be detected on local data as well, expression data needs an extra batch correction step before local data can be compared with one another. Batch effect correction will be discussed below.

Another way to speed up the time from enrolment to meeting is to have the local site enter data themselves. By giving local sites access to (a restricted version of) the database, the local site can fill in all details about a patient themselves and indicate when a patient needs to be discussed. To ensure the privacy of patients enrolled in the database, clear hierarchical roles are implemented in the database (figure 10). Local site data managers will only have access to patient enrolled by the specific site, national initiative data managers have access to patients from that nationality, and sponsor site data managers have access to all patients. By letting enrolling sites themselves enter the data into the database, a big liability is introduced. The local site needs to fill in all the data correctly. To minimize wrongful entry of data, as many questions as possible have become dropdowns and questions not applicable for a specific patient are hidden. The concern of not filling in all data also applies to the follow-up section. Patients are followed for 2 years after enrolment. A follow-up form must be filled every 3 months.

Future prospects for the iLTB

During this internship, the basis of the database has been built and enrolment into the study can start. Apart from the basis, other innovative improvements to a regular database have been started. The first project is the mapping of the database to trials/initiatives that are closely related to the iLTB both upstream (local profiling initiatives, INFORM) and downstream (r/r leukemia trials, e.g. the HEM-iSMART, figure 3). Currently, two different approaches are suggested to implement. A fully automatic approach and a semi-automatic approach. Data managers from both upstream and downstream initiatives are already contacted to help with implementation. An overview of how the two methods would work is provided in figure 11.

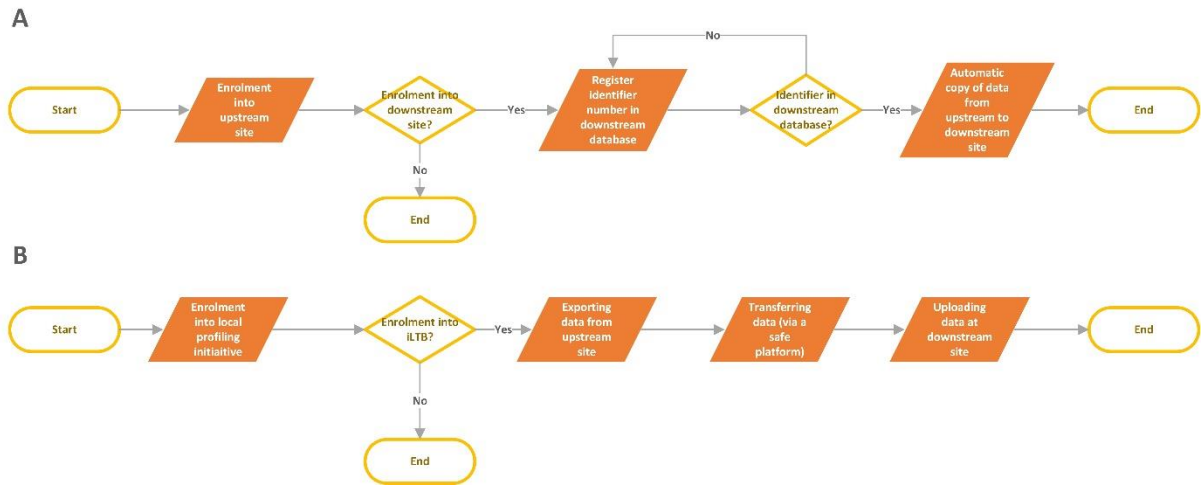

Figure 7: flow diagrams of automatic and semi-automatic data transfer from upstream initiative (local profiling initiative or iLTB) to downstream initiative (iLTB or r/r clinical trial). ***A:*** *automatic data copying depends on an identifier being present in both the upstream and downstream databases. If the identifier is present, data will be copied. By tying together fields from both databases containing the same information, data can be copied literally.* ***B:*** *semi-automatic copying means data exchange and human work during copying. Data should be exported, transferred to the other data manager, and then imported again. Lay-outs of the database must be the same, like in the automatic approach, but don't have to be tied together.*

By relating the fields in the iLTB database to the fields of clinical trials, into which many patients in the iLTB are referred, enrolment from the iLTB into the clinical trial will be faster. Both methods have upsides and downsides. The fully automatic method means more initial work. Databases would need to communicate non-stop. Different database systems would need to be tied together, requiring close collaboration between sponsors. Small changes in one database would have impact on all other databases connected, so maintenance is labour intensive. On the upside, inclusion of patients would be fast and seamless, saving time when quick decisions need to be made for the sake of the patient. The semi-automatic approach is less IT-intensive but requires more work for the data management/research nurse department. Data exchange would need to be coordinated to assure quick transfer of exported data. The semi-automatic approach seems the easiest to maintain with multiple centres as this approach is lower maintenance, and more adaptable to changes and inclusion of new studies.

The iLTB is revolutionary in it's study design because profiling is not performed by the iLTB, but by the local initiative enrolling the patient. By having the local site performing the analysis the iLTB saves time in two ways: not having to wait on arriving samples, and not doing double work by profiling both locally and internationally. The main drawbacks in not profiling in the same center but having data from different centers, are batch effects. Batch effects arise from differences in data preparation protocols and experimental conditions. For the iLTB, a method of correcting for these different batches is necessary, without complex statistical methods. For this reason, the iLTB proposes a normalization and correction method using an internal control from the sample itself, in the form of four pre-selected housekeeping genes. Housekeeping genes are genes that are expressed at a baseline level throughout all cells and are unaffected by a change in biological conditions. These genes can be used as a reference, to determine relative expression changes compared to them. Expression of known oncogenes relative to the housekeeping genes is compared to expression of the oncogenes relative to the housekeeping genes in a reference cohort that is diagnose specific. By comparing diagnose-specific expression of a known oncogene in the sample to expression of the same gene in the reference cohort, assumptions can be made about the (over)expression of the oncogene in the

form of a Z-Score.[34] Based on the Z-Score a treatment recommendation can be made for a targeted therapy targeting the (over)expressed oncogene (figure 12).

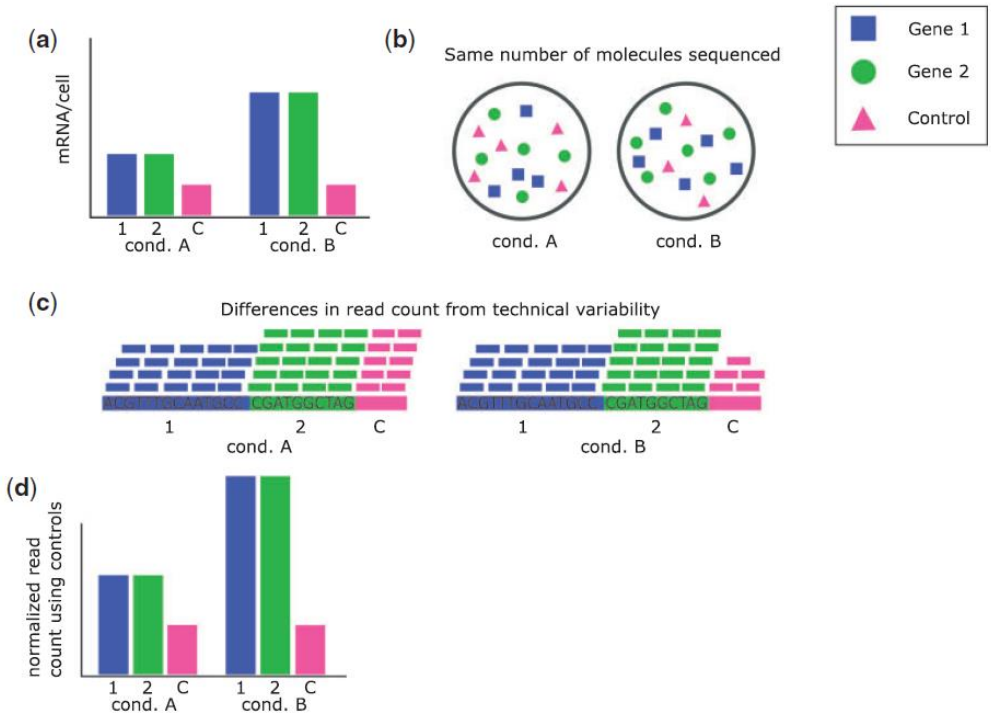

Figure 8: Normalization via housekeeping genes. ***A:*** *in two different conditions A and B, genes 1 and 2 are differentially expressed. in condition B the genes are upregulated more than in condition A.* ***B:*** *when sequencing, roughly the same number of reads are sequenced.* ***C:*** *When analyzing read count, no assumptions can be made about expression relative to one another. Only when looking at the control assumptions can be made because we know the control gene is expressed at the same level in both conditions.* ***D:*** *By normalizing genes 1 and 2 relative to the control, the actual differences in expression are observed. Figure by Evans et al.*[34]

To implement the overexpression determination tool mentioned above, a reference cohort needs to be established. The reference cohort should include 30 samples per diagnosis (r/r AML, r/r ALL, r/r Lymphoma, or if not available, initial diagnosis samples) for accurate calculation of Z-Scores. Apart from the reference cohort samples, housekeeping genes used for the internal control need to be selected.

Currently, the first steps in implementing the overexpression calculation into the database have already been done. 4 housekeeping genes were selected by a bio-informatician, and in the database a location for the reference cohort was created. A biobank submission requesting the data necessary for the reference cohort (30 AML and 30 lymphoma cases) has been drafted and is under internal review before submission.

After the reference cohort data is received, the data needs to be prepared for inclusion in the reference cohort (calculation of mean expression and SD of the oncogenes in the genelist). After calculation of the needed values, the values can be uploaded into the database, and the calculation can be implemented (figure 13).

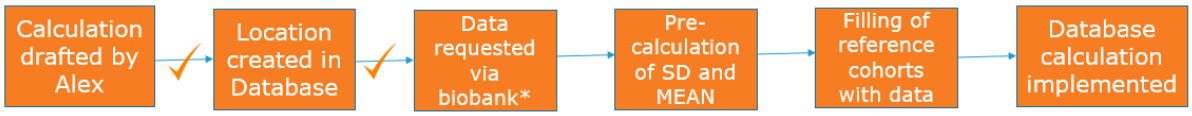

Figure 9: future direction of the overexpression project before implementation into the iLTB can be finalized. THe calculation has been drafted and a location (reference cohort data) has been created in the database. As soon as biobank data is received, data can be precalculated and inserted not the reference cohort. After integration of the data in the reference cohort, the calculation can be programmed in Alea, and Z-Score calculation can be done from within Alea.

Pinocchio

The amount of approved TKIs in adults has been expanding rapidly, and treatment of pediatric patients with TKIs is also increasing, as mentioned in the introduction and supplementary data table 1. Gathering more Pk data is essential to the effective usage of TKI treatment in pediatric patients, because of the high inter-patient differences in absorption, distribution, metabolism, and excretion. By amending the Pinocchio protocol to include children receiving TKIs we hope to elucidate more efficient and precise dosing regimens and gain insight into how maturation of the human body might alter exposure. TKIs are classifiable by the target they inhibit. Compounds inhibiting the same target are still under development and new compounds will keep entering clinical research over the coming years. To ensure that the protocol was robust and future-proof, the decision was made to not include a prespecified list of TKIs to the protocol but work on a target-based approach. By choosing for the target-based approach, new compounds that fall into one of the 8 classes of TKIs can be added to the study without amending the protocol, and therefore without resending all documentation to the METC.

One major concern with the Pinocchio protocol is the inclusion of enough patients. As mentioned before, subgroups of patients are small because of genetic differences. The problem with the Pinocchio study stratum 2 is that for every genetic aberration, there is multiple compounds available, making subgroups even smaller. By opening the study in multiple countries and centres in the future, more patients can be included. The protocol is written to easily facilitate opening in other centres. No Dutch-specific institutions are mentioned. Another challenge might be that most patients receiving a TKI will receive the therapy in the context of a clinical trial. Recent literature has been trying to determine how common off-label prescription of drugs to pediatric patients is. Around 40% of hospital prescriptions in children aged 0-18 year are off-label.[31,35] While prescribing drugs off-label might in many cases be good for the patient because they are receiving the most fit therapy, there are some problems with off-label prescription of drugs. One of the problems is that no data is gathered on pharmacokinetics, toxicities, and possible responses to the therapy, creating a risk for toxicities in the patient. Another problem is that clinical trials that might be applicable for patients receiving the off-label drug miss an inclusion, and therefore are 'slowed down'. Especially when looking at the small subsets of patients expected to enrol in some clinical trials (like the HEM-iSMART, figure 3). Missing an inclusion possibly sets back the specific treatment arm expecting only a handful of patients a year for months. On top of that, off-label prescribing of drugs leads to a higher incidence of adverse events. Adverse events that are not documented in a clinical trial report and therefore the scientific community doesn't learn from.[35] the higher chance of adverse events currently discourages physicians to prescribe targeted therapy to pediatric patients, because of a lack of safety and efficacy data.[5,16,19] With the assessment of Pk data of targeted therapies in pediatric oncology we hope to provide especially these discouraged physicians with a framework, for them to confidently and safely prescribe TKIs to pediatric patients that need them.

In the protocol a couple of challenges arose compared to stratum 1. In stratum 1 all patients receive a form of chemotherapy. The main difference between administration of chemotherapeutic agents

and TKIs is that chemotherapeutic agents are administered intravenously, and TKIs orally. If a patient is receiving chemotherapy, he/she will visit the hospital for every administration and will have a central line inserted from which the blood samples can be taken for the study, resulting in minimal extra burden for the patient. To minimize burden on patients in stratum 2, several steps were taken. First, a dynamic sampling schedule was proposed (Table 2). Since patients take TKIs for a longer period, a sampling schedule was created in which sampling could take place on the same day as regular patient visits to the hospital. By combining sampling and regular hospital visits, patients would not have to come to the hospital for additional visits. Secondly, since patients will rarely have a central line present at the time of sampling, a finger prick per sample or a venipuncture from which all three samples can be taken were added to the sampling methods. Sampling should be combined with routine blood sampling on the day of hospital visits if possible. The patient would receive a maximum of one extra venipuncture or three finger pricks per research day when participating in the study.

One option that might be interesting to explore as an alternative to sampling in the hospital, is sampling via finger prick by the parents at home. The VASCO study by the RIVM (Vaccination Study Corona, investigating vaccine efficacy over time) has a similar sampling method.[36] Participants receive a sampling kit at home and via medical postal services the samples are delivered to the RIVM lab for analysis. In research similar approaches are already implemented for TDM of patients suffering from inflammatory bowel syndrome.[37] Sampling at home would mean patients and parents would not have to stay in the hospital 6 hours post dose but would introduce more uncertainties. Precise sampling time would need to be recorded. The risk of losing participants would also be larger. Parents would simply forget to sample, or even refuse to sample their child themselves. Combining the at-home-sampling with patient visits would still be beneficial in this case. By doing the pre-dose sampling with the research nurses while in the Princess Máxima Center, and then going home with a home sampling kit, the parents might feel more at ease, having seen the research nurse perform the sampling and only having to do 2 samples themselves. The risk of forgetting to do the samples also decreases because the first sample has already taken place. One challenge is that sampling at home currently is that home samples are whole blood, and for TKI PK analysis blood plasma is used. The lab division of the Princess Máxima Centre is working on a solution to gain access to blood plasma sampled at home.

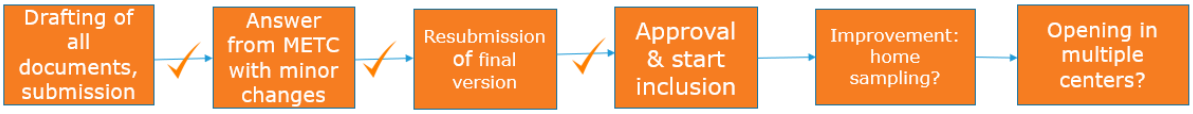

Figure 10: timeline of the Pinocchio study. All documents have been finalized and the study is awaiting the final approval by the METC. as soon as the final approval is given, inclusion of patients can be started. At the same time a possibility for improvement of the protocol is already thought of. Together with the home sampling improvement, the process of opening in multiple centers can be started.

Overall, the Pinocchio study is awaiting final approval by the METC Rotterdam before inclusion can start. In figure 14 the current timeline including prospects are noted down.

Conclusion

While both projects discussed above sound different from one another, both projects come back to a central problem in pediatric oncology: the gathering of enough patient data to get novel drugs approved as quickly as possible in pediatric patients. The iLTB plans to pool pediatric r/r hematological malignancy patients without a standard of care together and uniform the treatment advice given to them. A result from this might be the standardization of treatment of these r/r hematological malignancies, and the consecutive acceleration of pediatric trials that include these patients. The Pinocchio study is a first step towards TDM implementation but will for now serve as a method of

gaining insights in the variable Pk of TKIs in pediatric patients. In the best case the iLTB will lead to standardization of treatment advice in Europe. With the database we created we hope to give this study a rigid foundation from which the study can be expanded. The database is almost finished and ready for implementation. Working in an international setting with many different stakeholders was valuable and intriguing to say the least. The Pinocchio protocol was a completely different project to work on. Designing the new stratum 'from scratch', discussing possible improvements in the study design, submitting to an ethics committee (responding to their concerns), and setting up logistics for enrollment created valuable insights in how clinical research design works in (one of the) leading pediatric oncology centers.

Aknowledgements

I want to thank Uri Ilan, for the supervision in the iLTB project during the full 9 months of the internship. For the calm and steady feedback he has given me over time, and for taking my presentation skills and academic writing at least 5 levels higher than they were when starting this internship. For inviting me to come for a winter BBQ, and for the great conversations. I want to thank Edoardo Pennesi for the supervision in the Pinocchio study. For the conversations about stocks, NFTs, skiing and life in general. I want to thank prof. dr. Michel Zwaan for giving me the opportunity to be an intern in his research group in the Princess Máxima Center, in the middle of the COVID pandemic and a lockdown, and prof. dr. Alwin Huitema for being my second reviewer. I want to thank Sae, Saurabh, Valeria, and Rana for the endless lunches, Dutch lessons I gave and the laughs that came from them, and the company in the statistician room where I was seated for the most time. Alex for the insights in the wonderful world of bioinformaticians, and dr. Judith Boer for allowing me to participate in the weekly oncogenomics meeting. I want to thank Danny for showing me how database design works, and for the time he kept making for me even with his incredibly full schedule.

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Supplementary data

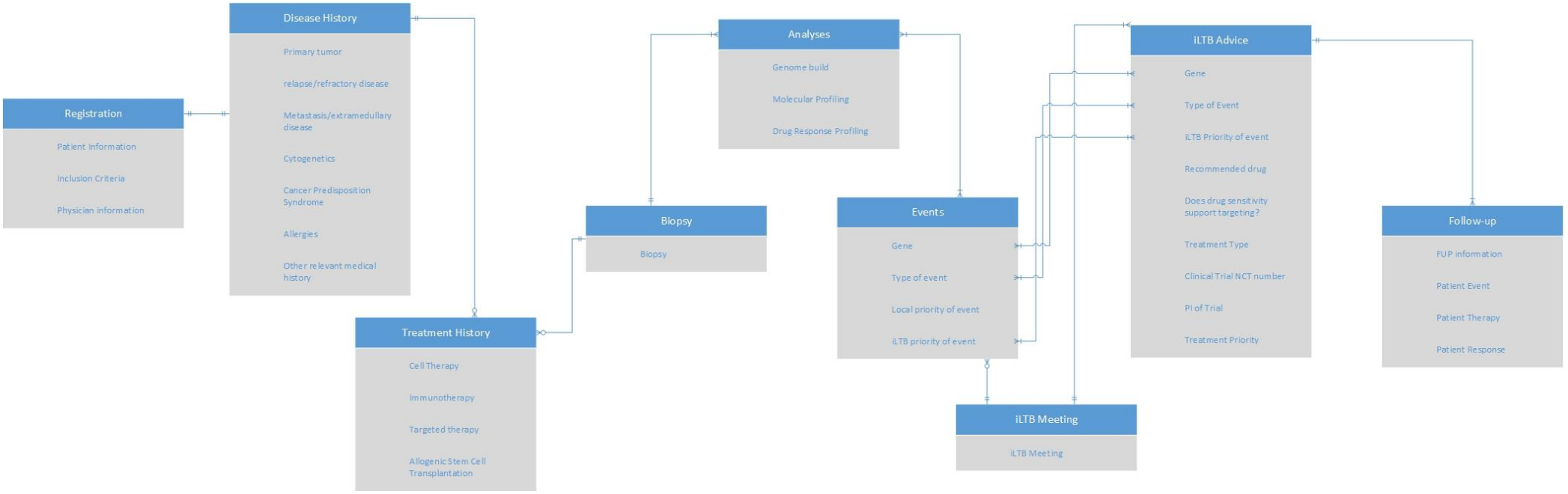
Supplementary Table 1: an overview of different TKIs their target, and their indications in both adults and children

TKI	Target	Indication in adults¥	Indication in children¥	Pediatric Target exposure*
Crizotinib	ALK	mNSCLC ALCL	ALCL	$C_{min} \geq 480$ (ng/mL)
Ceritinib	ALK	mNSCLC ALCL	not approved	$C_{min} \geq 871$ (ng/mL)
Alectinib	ALK	mNSCLC ALCL	not approved	$C_{min} \geq 435$ (ng/mL)
Brigatinib	ALK	NSCLC ALCL	not approved	Na
Lorlatinib	ALK	NSCLC	Not approved	Na
Cobimetinib	MEK	Melanoma	not approved	$C_{min} \geq 127$ (ng/mL)
Trametinib	MEK	NSCLC Melanoma	not approved	$C_{min} \geq 10.6$ (ng/mL)
Binimetinib	MEK	Melanoma	not approved	Na
Imatinib	BCR-ABL	CML, Ph+ ALL, MDS, CEL, DFSP, GIST' HES	CML, Ph+ ALL, GIST	$C_{min} \geq 1000$ - 110 (ng/mL)
Nilotinib	BCR-ABL	CML	CML	$C_{min} \geq 469$ (ng/mL)
Dasatinib	BCR-ABL	CML	CML	$C_{min} \geq 2.6$ (ng/mL)
Ponatinib	BCR-ABL	CML, ALL	not approved	$C_{min} \geq 22.8$ (ng/mL)
Bosutinib	BCR-ABL	CML	not approved	Na
Gefitinib	EGFR	mNSCLC	not approved	$C_{min} \geq 200$ (ng/mL)
Erlotinib	EGFR	mNSCLC, PC	not approved	$C_{min} \geq 500$ (ng/mL)

Lapatinib	EGFR	HER2+ tumors	not approved	$C_{min} \geq 780$ (ng/mL)
Afatinib	EGFR	mNSCLC	not approved	$C_{min} \geq 14.4$ (ng/mL)
Osimertinib	EGFR	NSCLC	not approved	$C_{min} \geq 166$ (ng/mL)
Axitinib	VEGFR	RCC	not approved	$C_{min} \geq 5$ (ng/mL)
Cabozantinib	VEGFR	RCC, HCC	not approved	$C_{min} \geq 1125$ (ng/mL)
Lenvatinib	VEGFR	TC, HCC	not approved	$C_{min} \geq 51.5$ (ng/mL)
Pazopanib	VEGFR	STS; RCC	not approved	$C_{min} \geq 20000$ (ng/mL)
Regorafenib	VEGFR	CRC; GIST; HCC	not approved	$C_{min} \geq 1400$ (ng/mL)
Sunitinib	VEGFR	mRCC; GIST; pancreatic NET	not approved	Na
Vandetanib	VEGFR	TC	TC (>5y/o)	$C_{min} \geq 730$ (ng/mL)
Everolimus	mTOR	NET, CCR, BC	not approved	$C_{min} \geq 10$ (ng/mL)
Quizartinib	FLT3	Not approved	Not approved	Na
Crenolanib	FLT3	Not approved	Not approved	Na
Gilteritinib	FLT3	AML	Not approved	Na
(Midostaurin)	FLT3	AML, ASM, SM-AHN, MCL	Not approved	Na
Entrectinib	NTRK	NSCLC, solid tumors	>12 with solid tumors	Na
Larotrectinib	NTRK	Solid tumors	Solid tumors	Na

Supplementary table 2: list of oncogenes integrated into the iLTB database. Other genes can at any time be added.

Gene List								
ABL1	CACNA1H	CREBBP	FES	IL7R	MS4A1	PHF6	SH2B3	WHSC1
ABL2	CBFA2T3-GLIS2	CRLF2	FGFR1	JAK1	MSH2	PIK3CA	SHROOM3	WHSC1L1
ADGRV1	CBL	CSF3R	FGFR2	JAK2	MSH3	PIK3CD	SMARCA4	WT1
AKT1	CCND1	CTCF	FGFR3	JAK3	MSH6	PIK3R1	SMARCB1	XPO1
AKT2	CCND2	CXXC1	FGFR4	KDM4A	MTOR	PLCG2	SON	ZEB2
AKT3	CCND3	DAB2IP	FGF19	KDM6A	MUTYH	PLXNA4	SOS1	ZFP36L2
ALK	CCNE1	DDX3X	FGR	KDM6B	MYC	PMS1	SPRED1	
APC	CCNI	DICER1	FLT3	KIT	NCOA6	PMS2	SPRY1	
ARAF	CD19	DNMT1	FLT4	KMT2A	NCOR1	PRKCH	SPRY2	
ARID1A	CD22	DNMT3A	FPGS	KMT2B	NF1	PRPS1	SPRY4	
ARID2	CD33	DOT1L	FRG1B	KMT2C	NF2	PRPS2	SRC	
ASH1L	CD38	DUSP1	GATA3	KMT2D	NOTCH1	PTCH1	SRSF9	
ASNS	CD44	DUSP14	GNB1	KMT5B	NPM1	PTEN	STAG2	
ASXL1	CD47	DUX4	GNB2	KRAS	NR3C1	PTPN11	STAT3	
ASXL2	CD52	EBF1	GRB2	LATS2	NR3C2	RAD51C	STAT5A	
ATM	CD7	EED	HCK	LCK	NRAS	RAD51D	STAT5B	
ATRX	CD79B	EGFR	HDAC1	LYN	NRK	RAF1	SYK	
AUTS2	CDK4	EHMT1	HOXA9	MACROD2	NSD1	RASA1	TBL1XR1	
BCL2	CDK6	EP300	HRAS	MAP2K1	NSD2	RASAL1	TCF3-HLF	
BCL2L1	CDK9	EPOR	HSH2D	MAP2K2	NSD3	RB1	TERT	
BCL2L2	CDKN1A	ERCC3	HTR3A	MCL1	NT5C2	RUNX1	TET2	
BLK	CDKN2A	ERG	IDH1	MDM2	NTRK3	SDHA	TP53	
BRAF	CDKN2B	ETV6	IDH2	MED12	PALB2	SDHB	TSC1	
BRCA1	CEBPA	EZH2	IKZF1	MEF2A	PARP1	SDHC	TSC2	
BRCA2	CEP290	FANCA	IKZF2	MEF2D	PARP2	SDHD	TTN	
BRD2	CHD4	FANCC	IKZF3	MITF	PAX5	SETD1A	TYK2	
BRD4	CHEK1	FBXW7	IL17RA	MLH1	PBX1	SETD2	UBAP2	
BTG1	CHEK2	FBXO31	IL3RA	MLH3	PDGFRA	SETD5	USP9X	
BTK	CNTN3	FDFT1	IL6ST	MPL	PDGFRB	SF3B1	VHL	



Supplementary Figure 1: enlarged picture of the database structure of the iLTB database. The arrows indicate the logical order in which ecrfs can and will be filled. Some fields from the events tab are copied to the advice tab, explaining the arrows between these crfs. The start and endpoints of lines indicate the relationship between two forms. || means one and only one. 0< means 0 or more, and |< means 1 or more.