PRIOMEDCHILD Joint Call Final report 2014	ERA-NET PRIOMEDCHILD

COORDINATION REPORT

A. IDENTIFICATION

Project Title	Rare disease: use of clinical trial simulation for the choice and optimization of study design
Acronym	CRESim
Period of the project (start date-end date)	
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Date	

B. PUBLIC SUMMARY OF THE PROJECT

This summary is aimed at being disseminated to a broad audience (general public, scientists) to disseminate the results of the project. It should therefore not mention any confidential data and should use an adapted vocabulary. The summary should not ***excede one page****. Please, structure the summary as follows:*

Title
Title which summarises and is explanatory of your project.
Paragraph 1: General objective of the project
Specify the objectives and aims of the project; indicate the context, the general objective, the problems that are considered, the solutions that are sought, the perspectives and the impact at technical/societal level.
Paragraph 2: Major results

C. FINAL SCIENTIFIC REPORT

Maximum 10 pages.
The final scientific report should cover the entire project. It should include the objectives, description of accomplished work and results obtained, taking into account the expectations and the state of the art from the beginning of the project. It should reflect the collective character of work. This report should be written by coordinator and be based on the contribution of each partner.

C.1 Short scientific summmary of the project
C.2 Background and state of the art
C.3 Aims and scientific approach (methods)
C.4 Results

C.5 Discussion
C.6 Short-term exploitation of results / Perspectives
C.7 References
C.8 Comments
Please share all comments/remarks that you have about the consortium, functioning, reporting, etc.

D. LIST OF DELIVERABLES

The following table intends to group aims and deliverables corresponding to the initial application. It should include the workpackage number (WP), aim number inside the workpackage (Aim), deliverable number inside the aim (DLV) followed by the partners involved (Partners). In the column "Planned/New" please specify if the Aim or/and Deliverable was initially planned or new (added after the start of the project). In the column "Description" please briefly describe each workpackage (title), aim and deliverable. In the column "Status" please use "C", "I" or "A" to indicate if the Aim or Deliverable was: completed "C"; Incomplete "I" or abandonned "A". The table below is shown as an example.

WP	AIM	DLV	Partner(s)	Planned/New	Description	Status C/I/A
1			P1			
	1.1		P1	planned		C
		1.1.1	P1	planned		C
		1.1.2	P1	planned		C
	1.2		P3, P5	planned		C
	1.3		P7	new		I

EXAMPLE

E. PUBLICATIONS

E.1 Number of publications

Type of publication	**Total N°**
Peer reviewed articles	
Books or book's chapters	
Articles dedicated to general public	
Conferences dedicated to general public	
Policy documents	
Others	

E.2 List of publications and communications

Please list the publications that are result of common work and implicate several (at least 2) partners. Please group them according to the categories presented in the table above.
Please list only the conferences to which you/partners where invited and presented the project.

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B. PUBLIC SUMMARY OF THE PROJECT

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Title

Rare disease: use of clinical trial simulation for the choice and optimization of study design (CRESim)

Paragraph 1: General objective of the Project

General objective of the CRESim ('Child-Rare-Euro-Simulation') project was to develop a platform performing clinical trial mathematical modelling and *in silico* (= in the computer) simulation in order to identify the optimal randomized trial designs to perform in children for the evaluation of (orphan) drugs tailored to different types of rare diseases. The ultimate aim is to allow performing only clinical trials with the best possible chances of success.

For demonstration purposes, three diseases were considered: severe myoclonic epilepsy in infancy (Dravet Syndrome, DS), cystic fibrosis (CF) and lymphoblastic lymphoma (LL), a child lymphoma. Before performing clinical trial simulations, we had to create virtual populations of patients for each disease. Mathematicians of the CRESIM consortium then built a mathematical model of the disease, using published model elements. Virtual populations of patients for the three diseases were built taking into account the natural evolution of each disease, and then a treatment effect was generated for each virtual patient.

We extracted random samples of virtual patients and randomized them into virtual clinical trials. Several (1000 per design) virtual RCTs have been simulated using different experimental designs and the results have been compared in terms of number of patients to include, trial duration (for the patient, the investigator and the sponsor), or precision of the estimation of the treatment effect.

Paragraph 2: Major results

For the three disease/drug combinations we achieved the construction of a model of the disease, and virtual populations were generated. Several experimental designs using qualitative or quantitative endpoints were implemented: parallel, cross over, N-of-1, randomised withdrawal, early escape design and designs with adaptive randomisation (drop the looser, play the winner). The choice of the best design is based on the most precise evaluation of a treatment effect with the highest power, the smallest number of patients and the shortest trial duration. A multidimension graphical approach was used to summarize the results of the clinical trials simulations.

C. FINAL SCIENTIFIC REPORT

C.1 Short scientific summmary of the Project

Background: Rare diseases are defined based on their low incidence, less than one in 2,000. The randomized controlled clinical trial is the "gold standard" to quantify the effect of a new treatment. However, in children, and more particularly in rare diseases, small numbers of patients require optimizing designs due to difficulties to drive sufficient powerful studies.

Objectives of the CRESim ('Child-Rare-Euro-Simulation') project: To develop a platform performing trial modelling and simulation in order to identify optimal trial designs in children for the evaluation of (orphan) drugs tailored to different types of rare diseases.

Methods: *In silico* approach using RCT modelling and Monte Carlo simulation, implemented for different categories of rare diseases in pediatrics. Several RCTs with different experimental designs have been modelled and simulated, and the different designs were compared in terms of trial duration and precision of the estimation of the treatment effect. For this purpose, three diseases were considered: severe myoclonic epilepsy in infancy (Dravet Syndrome, DS), cystic fibrosis (CF) and lymphoblastic lymphoma (LL). For each disease, a pathophysiological model of the disease as well as PK-PD model was used to predict outcomes for each patient. Then, several virtual RCTs were being simulated using different experimental designs and the results were compared in terms of trial duration (for the patient, the investigator and the sponsor) and precision of the estimation of the treatment effect.

Results:

1. **Cystic Fibrosis :**

We proposed a new physiologically based mathematical model of muco-ciliary transport consisting of the two major components of the mucociliary clearance system (i) periciliary liquid layer (PCL) and (ii) mucus layer. We study mucus evacuation under normal conditions and in CF patients. Clearance of airway secretions is a primary therapy for patients with CF. We considered the action of the aerosolized and inhaled medication dornase alfa, which reduces the viscosity of cystic fibrosis sputum. The results of the model simulations stress the potential relevance of the location of the drug deposition in the central or peripheral airways. Mucus evacuation was increased in case the drug was primarily deposited peripherally, i.e. in the small airways.

2. **Dravet Syndrome :**

Dravet syndrome (DS) is a rare pediatric epilepsy syndrome (incidence <1/40,000) characterized by hemi- or generalized (tonic-) clonic seizures appearing from the first year of life and often triggered by fever. SCN1A gene mutations have been found in about 85% children with DS. A mouse model of DS with SCN1A mutation has shown a selective failure of excitability of GABAergic interneurons resulting in epilepsy. Conventional treatments have been disappointing. However, a newer antiepileptic drug, stiripentol, used in association with the benzodiazepine clobazam, has produced a significant (though incomplete) reduction in seizure rate. This drug is known to increase the duration of GABAA receptors opening time and potentiate the antiepileptic action of clobazam.A physiology-based computational model was developed in order to investigate some possible pathophysiological causes of DS. A lumped-parameter approach lying at the level of cortical neuronal population was used to represent the generation of spontaneous EEG activity. The model includes one sub-population of pyramidal cells and two sub-populations of interacting interneurons: somatic-projecting interneurons (basket-like) with fast synaptic kinetics GABAA (fast, I1) and dendritic-projecting interneurons with slow synaptic kinetics $GABA_A$ (slow). Basket-like cells were interconnected to produce mutual inhibition (I1->I1). Parameters of the sigmoid function (representing the firing rate of interneurons) were adapted to mimic the genetic alteration found in DS. In addition, the model accounts for a number of neurobiological hypotheses. Finally, stiripentol by increasing the shunting inhibition reduced seizure-like activity in a dose dependent manner.This model enables to simulate epileptic spikes over a very short time frame (some seconds). More work is required to be able to mimic epileptic crises, and create a reaslistic virtual population.

3. **Lymphoblastic lymphoma**

Modeling was performed to allow in silico simulation and identify the best clinical trial to test the possibility to reduce the duration of treatment in order to reduce its toxicity without increasing the

number of relapses. The possible reduction of the duration was applied to pairs of virtual patients developed from a mathematical model of the thymus, the disease and its treatment. Multi-Scale hybrid model is used to simulate the ontogenesis of T cells, the disease at a cellular level and the action of medications. In this model, the fate of T cell is determined by system of ordinary differential equations (intracellular regulation) and reaction-diffusion equations (extracellular regulation).To model the individual behavior of patients, the cellular level and the whole patient have been simulated. T cells have properties (parameters) that can be inherited from the mother cells or be randomly chosen for each new cells. Cancer cells can develop resistance to treatment due to the selection and Darwinian evolution. In the virtual population created by the model, we obtained different responses (cured or relapsed) of patients with the same dose of drug and the same duration of maintenance treatment. We observed that the number of relapses decreases (and number of healings increases) as a function of duration of "short" treatment.
In conclusion, the results of the simulations show that there are no relapses after 12 months of maintenance treatment. However, the standard protocol implies 24 months of treatment. So we can expect that the duration of treatment can be reduced without influence on its results.

4. **Clinical trial modelling / simulation**

Using the virtual population generated for each of the 3 mathematical models described below, randomized clinical trials were simulated by randomly selecting patients to constitute the included virtual population and to predict oucome depending on the treatment arm attributed (active or placebo arm) and the design considered. Seven different randomized clinical trials were simulated: *parallel, cross-over, randomized withdrawal, early escape, adaptive randomization (play the winner and drop the looser) and N of 1 design.* For each simulated design, estimates of the design performances were calculated. The most appropriate design should allow the most precise evaluation of a treatment effect with the highest power, the smallest number of patients and the shortest trial duration. Results were presented on the same figure using a multivariate graphical method in order to compare designs on a specific parameter (Duration of the trial, Number of patient to include) or on combinaison of them.

Overall Conclusions:

The project provided a new environment for more effective drug evaluation, i.e. RCTs simulation in rare diseases. This allowed investigating new compounds for treating rare diseases with the following advantages: (i) reduction of human experimentation in children by simulating the effect of the compound before starting any clinical trial, (ii) reduction of time and expenses on unsuccessful drug clinical trials, and (iii) in fine stimulation of the orphan-drug development process in pharmaceutical industry.

C.2 Background and state of the art

RCTs in rare diseases

The most common clinical trial design is the **parallel-group** design, in which participants are randomized to one of two or more arms, active control(s) or a placebo. Alternative designs (Evansand Ildstad, 2003) have been used for small clinical trials: cross-over, factorial, randomized withdrawal and early-escape designs.
The **cross-over** design compares two or more interventions by randomly assigning each participant to receive the interventions being tested in a different sequence. Once one intervention is completed, participants are switched to another intervention.
In a **factoria**l design, two or more treatments are evaluated simultaneously with the same participant population through randomization in various combinations of the treatments.
In a **withdrawal** design, individuals who respond positively to an experimental intervention are randomized to continue receiving that intervention or to receive a placebo.
The **early-escape** design is another way to minimize an individual's duration of exposure to a placebo: participants are removed from the study if they fail to respond to a defined extent.
Some trial designs have been especially developed for small size studies, including: single subject (*N* of-1), sequential, and adaptive designs.
An ***N-of-1* trial** design is a randomized multi-crossover study of an individual patient's responses to set of (often two) treatments. Treatments are randomly assigned individually or within paired periods and blindly applied to a patient. At set time intervals, corresponding to these different

treatment periods, measurements of the patient's disease status are taken. After several crossover periods, comparisons of the outcomes obtained during the periods for the (two) drugs are made.
In a ***sequential design***, participants are sequentially enrolled in the study and are assigned a treatment (usually at random). The probabilities that participants will be assigned to any particular treatment are modified as they become available. The aim is to improve the efficiency, safety, or efficacy of the experiment while in progress by changing the rules of treatment allocation.
An ***adaptive* design** seeks to skew assignment probabilities to favor the better-performing treatment in a trial that is under way. A major advantage of adaptive design (e.g. 'play the winner' rule) is that over time more patients will be assigned to the more successful treatment.
Clinical trials modelling and simulation
In silico simulations (Boissel et al, 2005) have become a powerful tool for the researcher. Simulation consists in building a virtual model of reality in order to perform experiments by changing the values of the model parameters. RCT simulations can be defined as the generation of a treatment effect for a virtual patient obtained by modeling trial design, human behavior, disease progress and drug effects using numerical methods and specific mathematical models (e.g. pharmacokinetic and pharmacokinetic-pharmacodynamic models for drug behavior and effect).
The CRESim project belongs to the framework of an in silico approach, with the use of RCT modelling and Monte Carlo simulation (Kimko and Duffull, 2003) in order to evaluate and compare various clinical trial designs in different rare diseases.

Dravet syndrome: Dravet syndrome (DS) is a rare pediatric epilepsy of early life onset characterized by pharmacoresistant hemi- or generalized (tonic)-clonic seizures and severe cognitive prognosis. The voltage-gated sodium channel SCN1A gene is mutated in 85% of the patients. Transgenic mice models of DS suggest that the mutation specifically involves GABAergic interneurons and leads to selective loss of sodium current affecting their firing properties. However the mechanisms leading from interictal activity to seizure generation and the subtype of interneurons involved are not known. Computational modeling of neuronal network is currently used to analyze these mechanisms. The inconsistency of firing rate in GABAergic interneurons can be used as a starting point to build a computational model of DS.
To find parameters and connections in the model that reproduce EEG patterns of DS patients. We first made a quantitative and qualitative analysis of EEG recorded in DS patients during interictal, preictal and ictal periods. We next used a lumped-parameter approach (mean-field model) lying at the level of neuronal population and able to represent the generation of spontaneous EEG. This computational model includes one sub-population of pyramidal cells and two sub-populations of interneurons (mediating fast and slow GABAergic currents). EEG signals were characterized by slow background activity (1-4Hz), multifocal interictal epileptic spikes and, as far as hemi-clonic seizures were concerned, by fast oscillations at the onset of seizures. In order to mimic the effect of a SCN1A mutation, the firing rate of GABAergic current was modified in slow interneurons. Preliminary results show that appropriate alterations in the strengths of GABAergic and glutamatergic connections, and in the amplitudes of average EPSPs/IPSPs in the model successfully lead to slow background activity (1-4Hz), generation of interictal epileptic spikes, and fast onset activity and seizure-like activity. Conclusion: Our computational modeling of DS is therefore promising. Further optimization is needed.

Cystic fibrosis: Cystic fibrosis (CF) is a genetic disease that is characterized by thick, tenacious and dehydrated airway secretions. In CF, mutations in CFTR gene, the cystic fibrosis transmembrane conductance regulator, encoding a Cl^- channel, result in a decreased ability to secrete Cl^- and therefore enhance Na^+ absorption. This reduces the volume of periciliary fluid and results in thick mucus that cannot be cleared from the lung by the mucociliary clearance system. Due to relatively little autofeedback between the removal of mucus from airway surface and the secretion of mucin by goblet cells, production of mucus components is continued. In order to approach the physiological process of mucociliary clearance mucus production from goblet cells and submucosal glands as well as mucus transport from daughter sections are taken

into account. Moreover, in our simulations we consider 15th generations of bronchial tree, including their physiological properties, such as, length, diameter of each generation as well as number of tubes in it. Age factor is introduced in the model by means of formula used to scale trachea and bronchi of children in relation to adults, depending on their body height. We also introduce into the model different viscosity of mucus in each generation, which is also important due to heterogeneity of medical deposition in bronchial tree. We consider the action of aerosol spray, called DNAse, which reduces the viscosity of cystic fibrosis sputum.

Lymphoblastic lymphoma

Lymphoblastic lymphoma (LBL) is a neoplasm developing from immature T- or B precursor cells [Schmidt and Burkhardt 2013]. LBL are postulated to arise from precursor B in the bone marrow (BM) or thymic T cells at varying stages of differentiation [Cortelazzo et al. 2011]. Although T-LBL represents around 25% of all NHL in children, it is considered as a rare disease. Most patients with T-LBL typically present with mediastinal tumor. Other manifestations are lymphadenopathy, frequently with cervical and supraclavicular bulky disease. Pleural or pericardial effusions are also common. The presence of a predominantly anterior mediastinal mass can cause respiratory symptoms from coughing, stridor, dyspnea, edema, elevated jugular venous pressure to acute respiratory distress. The median age of diagnosis for T-LBL is 8.8 years and it is 2.5 times more often diagnosed in male patients [Burkhardt et al. 2005, Schmidt and Burkhardt 2013].

Molecular analysis of minimal residual disease. Detection of minimal residual disease (MRD) could be successfully introduced into ALL-treatment plans. Clonal TCR-gene arrangements and aberrant immunophenotypic profiles are used to monitor the disease. Attempts have been made to translate this method for LBL using a flowcytometry-based approach to detect submicroscopic systemic disease [Coustan-Smith et al. 2007, Stark et al. 2009]. To date, the position of MRD and minimal disseminated disease (MDD)-monitoring in risk stratification or relapse/progression prediction is not clear. To evaluate the possible benefit of MDD/MRD diagnostic prospective studies are necessary

Prognostic factors. The pEFS (probability of event-free survival) in LBL is high, whereas survival in relapsed patients is very poor. As the 5-year EFS (event-free survival) rates are acceptable, the possibility that patients with favorable risk profiles might be “over treated” is considered. However, there are currently no validated parameters to identify patients with a favorable risk profile. It is of special importance to detect the 10%-30% of patients with a high risk of relapse in order to adapt therapy regimen early. Age older than 14 years [Tubergen et al. 1995], CNS involvement [Abromowitch et al. 2008] and unresponsive to therapy [Uyttebroeck et al. 2008] are identified as possible unfavorable prognostic factors, but this needs further validation.

Treatment strategy. Probability of EFS in pediatric T-LBLB is currently about 80%[Bonn et al. 2013]. Based on historical studies, the current treatment approach of LBL uses therapy similar to that for childhood acute lymphoblastic leukemia [Termuhlem et al. 2013], and EFS is actually more than 80% in children and adolescent. The important drugs are l-asparaginase, vincristine, anthracyclines, cytarabine, cyclophosphamide, Methotrexate, purinethol and corticosteroids. Successive studies demonstrated the importance of initial intensive treatment, secondary intensification of chemotherapy, prolonged maintenance therapy and prophylaxis of CNS relapse. Even though the development of therapy protocols meant a major step toward curing pediatric patients from LBL, questions remained unanswered. Various trials and protocols have been developed on the Berlin-Frankfurt-Muenster (BFM) or LSA2-L2 (American group) backbone to increase EFS as well as overall survival (OS) and to reduce toxicity within the established therapy regimens [Schmidt and Burkhardt 2013]. The therapy of LBL is rather long with its total duration of 24 months in most protocols including induction, consolidation and maintenance therapy [Schmidt and Burkhardt 2013].

C.3 Aims and scientific approach (methods)

Aims

Various experimental designs will be modelled and simulated for each considered disease for comparison purpose in terms of: (i) trial duration, this criterion represents the 'burden' of the trial for the patient, the investigator and the sponsor, and (ii) precision of the estimation of the treatment effect, a criterion evaluating the 'performance' of the trial.

Methodology

Dravet syndrome modelling

We used EEG as a biomarker and we developed a neuronal population model of EEG activity. A mean-field approach lying at the level of cortical neuronal population is used to represent the generation of spontaneous EEG features. Our integrative disease modeling approach combined the already existing mathematical models of EEG activity [Molaee-Ardekani et al 2010] with some (patho)physiological mechanisms discovered in Dravet syndrome [Yu FH et al 2006, Quilichini et al 2006], brain development [Ben-Ari et al 1990] and epilepsy [Ben-Ari et al 1990, Cohen et al 2002, Angulo et al 2004]: (i) voltage-gated sodium channel SCN1A gene mutation, (ii) GABAergic depolarization, (iii) shunting inhibition, (iv) astrocytes impact and (v) pharmacological impact on neuronal activity. Therefore, the model is composed by the following sub-populations: (i) pyramidal cells (P), receiving excitatory feedback from other pyramidal cells and inhibitory feedback signals from (ii) soma- and proximal dendrite-targeting cells (I1: basket cells and chandelier cells mediating GABAA,fast currents), and (iii) dendrite-targeting cells (I2: neurogliaform cells mediating GABAA,slow currents). The set of 6
differential equations that govern the population model of Dravet Syndrome is established.

$$\vartheta_P(t) = C_{PP}\, y_0(t) - C_{I1P}\,(y_1(t) - \alpha_{I1P}S_{I1}^0 + \beta_{I1P}S_{I1}^0/g) - C_{I2P}\,(y_2(t) - \alpha_{I2P}S_{I2}^0 + \beta_{I2P}S_{I2}^0/b) + q(t) + p(t),$$

$$\vartheta_{I1}(t) = C_{PI1}\, y_0(t) - C_{I1I1}\,(y_1(t) - \alpha_{I1I1}S_{I1}^0 + \beta_{I1I1}S_{I1}^0/g) - C_{I2I1}\,(y_3(t) - \alpha_{I2I1}S_{I2}^0 + \beta_{I2I1}S_{I2}^0/b) + q(t),$$

$$\vartheta_{I2}(t) = C_{PI2}\, y_0(t) - C_{I1I1}\, y_1(t) + q(t).$$

Where yX(t) is the average membrane potential of sub-population X. A,G,B,D and a, g, b, d determine amplitude and rate constant of the average receptor-mediated postsynaptic potential, respectively, A, a in the excitatory case (AMPA), B, b (GABAA,slow), G, g (GABAA,fast) and D, d (GABAB) in the inhibitory case. Integrating action that takes place at the soma in each given subpopulation X is modeled by a nonlinear function of sigmoid shape SX(X) = Qmax X /(1+erX(X−)) to represent saturation and threshold effects taking place in the soma. X are sums of average postsynaptic potentials at the level of each subpopulation, described as follows: We denote by S0 X = SX(0 X), 0 X = X(t0). Interactions between cells are summarized in the model by connectivity constants CXX. In our model we study GABA-mediated excitation due to both fast (SI1()) and (SI2()) slow interneurone action potential firing, whereparameters XX are used to adjust the sensitivity to accumulate intracellular Cl−. Parameters. XX are used to adjust membrane potential values to "physiological" range during shunting inhibition. The influence from neighboring or distant populations is modeled by an excitatory input p(t) = ¯p + N(0,), represented by a Gaussian noise. The release of molecules from astrocytes that modulate cortical slow oscillations is taken into account by means of function q(t) = Fsin(2ft), where F and f represent the amplitude and frequency, respectively, of gliotransmission.
Different morphologies of interictal spikes and patterns of seizures observed in patients were reproduced in the model by tuning the amount of depolarizing GABAA postsynaptic potential (XX). For fixed parameters of the computational model, slowly increasing of the proportion of depolarizing GABAA mediated IPSP (I1→I1 and I1→P) is sufficient to switch the activity from

background to interictal epileptic spikes, a fast onset activity decreasing in frequency with time (shirp-like), seizure like activity and seizure termination. Finally, some specific configurations of the model also reproduced the pharmacological effects of stiripentol other antiepileptic drugs. This new model seems therefore a promising tool to explain the nature of polymorphism of EEG seizure types, the role of genetic mutations in seizure appearance as well as to understand the mechanism of why some drugs are likely to worsen seizures and other ones decrease epileptic activity. As announced previously that was the first step for developing a mathematical model which could be used in the future to optimize new drug trials in Dravet syndrome [Janson and Rit et al 1995]

Cystic fibrosis modelling

Cystic fibrosis (CF) is a genetic disease that is characterized by thick, tenacious and dehydrated airway secretions. In CF, mutations in CFTR gene, the cystic fibrosis transmembrane conductance regulator, encoding a Cl− channel, result in a decreased ability to secrete Cl− and therefore enhance Na+ absorption. This reduces the volume of periciliary fluid and results in thick mucus that cannot be cleared from the lung by the mucociliary clearance system. We considered the model of a viscoelastic traction layer of muco-ciliary transport. In our model we consider the lung up to fifteen generation, where ciliated and goblet cells are situated.The inner surface of lung in each generation i (i = 0..15) is calculated by multiplying the total width (i) of its generation on its length li. The total width of one generation is equal to nd, where d is diameter of the tube and n is the number of tubes in its generation. The lower layer represents the PCL, is modelled by a Newtonian fluid of viscosity μP . The traction layer region h < y < hf , representing the region in which the cilia penetrate the mucus and exert its force. The upper layer hf < y < H, representing the mucus above the penetration region. We obtain a formula to calculate mucus velocity. Mucus in every part of lung mix with the mucus coming from the goblet cells and from the previous generations. Therefore, the viscosity of mucus changes. We use this model to study treatment of mucoviscidosis. The mucus of patients becomes thick and viscous, that decrease velocity of mucus releasing. Increased residence time of mucus in lung causes bacterial overgrowth and inflammation as a result. Inflammation attracts white blood cell, which die and release their genetic material, sticky DNA, into the mucus. This DNA aggravates the already excessive stickiness of the mucus, setting up a cycle of further airway obstruction, inflammation and infection. Aerosolized medications, called DNase, breaks down DNA in the sputum, thus decreasing its viscosity. We introduce in the model the quantitative effects of DNase on the viscosity of cystic fibrosis sputum and we study different modes of aerosol particles deposition in lung. The model shows that aerosol particles deposited in peripheral parts of lung better increase the mucus clearance compared to aerosol particles deposited mostly in trachea and proximal regions.

T-cell Lymphoblastic Lymphoma Modeling

Very few mathematical models have been developed for T cell lymphoblastic lymphoma. The number of malignant cells in an organ or in the whole organism has already been studied with systems of differential equations (Cai et al. 2007). But this approach cannot easily take into account the inherent variability in patients and do not provide predictions of patient survival. Hybrid discrete-continuous models are widely used for the investigation of dynamics of cell populations in biological tissues and organisms (Aihara et al.2010) that involve processes at different scales (Kurbatova et al. 2013, Eymard et al. 2014). In off-lattice models cell shape can be explicitly modelled and cell interactions with neighbouring cells and surrounding medium can be investigated. Cellular automata are applied to model tumour invasion (Anderson et al. 1998), where tumour is considered as a collection of many individual cancer cells (Jeon et al.2010). Multi-scale models of cell dynamics take into account biological processes at different levels, e.g. cell population, intracellular (molecular) level, extracellular matrix, and other organs. These different levels interact with each other and determine the behaviour of the whole system (Volpert et al. 2013). In hybrid models, cells can be considered as individual objects (discrete aspect of the model) which can divide, differentiate, die by apoptosis, move and interact mechanically with each other. To model the individual behaviour of cells and also that of patients, properties are assigned to each newly born cell. These properties determine cell self-renewal capacity and

malignant potential. Some specific values of these properties are assigned at the birth of cells (cell position, type of cells, life expectancy, and threshold of sensitivity to treatment, threshold of sensitivity to the external environment and initial intra and extra concentrations of proteins). Some other values are computed at each Δt (intra and extra concentration, coordinate of cells (x,y), radius of cells). These characteristics can be transmitted to daughter cells or they can be random variables. Cell fate is determined by intra- and extra- cellular regulatory networks, which can depend on some extracellular factors (nutrients, hormones, growth factors, other cells, extracellular matrix). Those factors can be produced or consumed by cells of this given population or they can come from other organs, as it is the case for hormones and nutrients (Volpert et al. 2013). The intracellular regulation (intracellular variable denoted u) is described by ordinary differential equations (continuous aspect of the model) :

$$\frac{du}{dt} = c_0 + kG$$

And can be influenced by some substances (extracellular variable denoted G) in the extracellular matrix described by partial differential equations (continuous aspect of the model) :

$$\frac{\partial G}{\partial t} = D\Delta G + W - \gamma G$$

Stochasticity due to random events (cell cycle duration, orientation of the mitotic spindle at division) and small population effects also plays an important role. When cells divide and increase their number, they can push each other resulting in cell displacement. In order to describe mechanical interaction between cells, we restrict ourselves here to the simplest model where cells are presented as elastic spheres (compressible) with an incompressible core. Cell motion is determined by the sum of the mechanical forces acting on that cell from other cells. Under the assumption of small deformations, we can express the mechanical contact force acting between them as a function of the distance between their centers. Such discrete-continuous models are called hybrid models, because they consist in the coupling of discrete and continuous models (Alarcon et al. 2003, Anderson et al. 2007, Anderson and Chaplain 1998, Dallon 2007, Deutsch 2007, Eymard et al. 2014, Kurbatova et al. 2013, A.A. Patel et al. 2001, J.M. Osborne et al. 2010). Hybrid models allow a more detailed description of the disease, such as considering the microenvironment and the cell-to-cell communication of healthy or malignant cells. This reproduces more accurately the individual behaviour of patients (predisposition to the disease and response to a given treatment) and allows obtaining, for example, different responses for the same treatment. Similarly, we will study the toxicity of treatment on healthy cells exposed to treatment. This model simulates lymphoma from its detection (increase of the thymus volume), to the different phases of treatment, i.e. induction and maintenance after the acute phase. Methotrexate and purinethol (Orgungbenro et al.2014) are common drugs used in maintenance treatment of lymphoma. Ordinary differential equations (ODE) of physiologically based pharmacokinetics (PBPK) will be integrated in the model in order to provide the effects of therapy administered to the patient: cure or relapse depending for example on the duration of treatment. Relapse will be related to residual disease due to malignant cells remaining in reservoirs, lymphoid tissues. We assume that similar equations as for the PBPK model can be used at the cellular level to compute the quantity of drug (variable p) inside cells.

$$\frac{dp}{dt} = k^{+}p_0(t) - k^{-}p$$

Validation of the model will be based on the choice of parameters and on the comparison between the results and available biological and medical databases. Some of the parameters are already known: cell number, cell cycle duration, some pharmacokinetic parameters. Parameters of intracellular and extracellular regulations will be fitted in order to obtain correct proportions of different cell types, correct rates of apoptosis and differentiation. We will compare the results in terms of number of relapses of virtual patients with results given by the EuroLB database. Statistical comparison between samples of virtual population and samples of real population will

allow to validate the numerical estimation of unknown parameters. The implementation of the full system is done in the computer language C++ (OS: Ubuntu) and will use the computing cluster of University Lyon 1.

In silico trial Modelling

Modelling in silico trials requires mathematical models for representing the disease, the drug / patient interaction, and the experimental design (Chabaud et al, 2002). Such models already exist and are published, but they usually address a restricted aspect of the problem, e.g. PK and PK/PD models in adults for a given drug, pathophysiology and evolution of the disease (such as receptor function, biomarker action, and genetic aspects). Published models will be primarily searched for in bibliographic data bases. However, additional models will be specifically developed.

Simulating clinical trials requires to set-up a *simulation plan*, detailing a series of linked models (Bajard et al, 2009, Blesius et al, 2006). Each simulation model is categorized into one of three sub models:

- The input-output (IO) sub-model predicts outcomes for each patient. It includes a pathophysiological model of the disease if any, and the PK-PD drug properties (Nony and Boissel, 2002). The model structure and parameters must be based on existing data from clinical studies, to adequately reproduce the reality regarding drug (and metabolite) concentrations, biomarkers of therapeutic or toxicological response, or the incidence of a clinical outcome (e.g. death, occurrence of a seizure or improvement in cognitive scales in epilepsy) or an adverse event (Wang et al, 2009, Duffull et al, 2000).
- The covariate distribution sub-model describes the characteristics of the patients and is built fromexisting patient databases;
- The execution submodel describes the characteristics of experimental design and of protocol deviations (i.e. deviation(s) from the protocol either patient-related or investigator-related) (Bajard et al, 2009). All protocol deviations are unexpected by nature, and therefore only probabilistic models can be used to simulate them (Nony et al, 1998).

The full model will express the quantitative therapeutic effect as the sum of the IO sub-model and the execution sub-model (Boissel et al, 2008).

Simulation process: In each disease, the simulation process proceeds in two steps: the simulation of a virtual population of patients, and the simulation of RCTs following specific experimental designs.

Simulation of a virtual population: A population of 'N' virtual patients is generated. Several covariate values are attributed randomly to each patient, parameters of the IO sub model, both for therapeutic and for adverse effects, parameters of the execution sub model, and covariates characterizing investigator (center) or patient (inclusion/exclusion criteria, baseline characteristics). Samples of patients are randomly drawn from this population for inclusion in each further clinical trial.

Simulation of RCTs*:* Random samples of patients drawn from the virtual population are included in *in silico* clinical trials. The diversity of drug-patient interactions is simulated using the variance of each parameter distribution of the IO sub-model. Thus in one disease, a given number (e.g. 1000) virtual trials may be simulated independently for each experimental design.

Statistical analysis of each RCT results: Depending on each study design, different statistical methods are considered.

Analysis of final results: The final analysis must determine the most appropriate design for a given pair 'disease-drug'. This analysis is mostly descriptive. For each situation, i.e. [trial design, pair 'rare isease-drug'], the number of times each trial result appears significant (significance level: 5%) is considered for the final hierarchy. The final hierarchy is presented taking into account both the precision of the estimation of the treatment effect (cf. above), and the trial duration.

C.4 Results

During the active time period of the project following research results were presented and submitted for publication. We show here the abstracts of the articles. The full text is available in annexes.

(i) Experimental designs for small randomised clinical trials: an algorithm for choice

Background: Small clinical trials are necessary when there are difficulties in recruiting enough patients for conventional frequentist statistical analyses to provide an appropriate answer. These trials are often necessary for the study of rare diseases as well as specific study populations e.g. children. It has been estimated that there are between 6,000 and 8,000 rare diseases that cover a broad range of diseases and patients. In the European Union these diseases affect up to 30 million people, with about 50% of those affected being children. Therapies for treating these rare diseases need their efficacy and safety evaluated but due to the small number of potential trial participants, a standard randomised controlled trial is often not feasible. There are a number of alternative trial designs to the usual parallel group design, each of which offers specific advantages, but they also have specific limitations. Thus the choice of the most appropriate design is not simple.
Methods: PubMed was searched to identify publications about the characteristics of different trial designs that can be used in randomised, comparative small clinical trials. In addition, the contents tables from 11 journals were hand-searched. An algorithm was developed using decision nodes based on the characteristics of the identified trial designs.
Results: We identified 75 publications that reported the characteristics of 12 randomised, comparative trial designs that can be used in for the evaluation of therapies in orphan diseases. The main characteristics and the advantages and limitations of these designs were summarised and used to develop an algorithm that may be used to help select an appropriate design for a given clinical situation. We used examples from publications of given disease-treatment-outcome situations, in which the investigators had used a particular trial design, to illustrate the use of the algorithm for the identification of possible alternative designs.
Conclusions: The algorithm that we propose could be a useful tool for the choice of an appropriate trial design in the development of orphan drugs for a given disease-treatment-outcome situation **(Appendix 1)**

(ii) A Methodological Framework For Drug Development In Rare disease

Introduction: Developing orphan drugs is challenging because of their severity and the requisite for effective drugs. The small number of patients does not allow conducting adequately powered randomized controlled trials (RCTs). There is a need to develop high quality, ethically investigated, and appropriately authorized medicines, without subjecting patients to unnecessary trials.
Aims and Objectives: The main aim is to develop generalizable framework for choosing the best-performing drug/endpoint/design combinations in orphan drug development using an in silico modeling and trial simulation approach. The two main objectives were (i) to provide a global strategy for each disease to identify the most relevant drugs to be evaluated in specific patients during phase III RCTs, (ii) and select the best design for each drug to be used in future RCTs.
Methodological approach: In silico RCT simulation will be used to find the optimal trial design and was carried out in two phases: (i) statistical analysis of available clinical databases and (ii) integrative modeling that combines mathematical models for diseases with pharmacokinetic-pharmacodynamics models for the selected drug candidates.
Conclusion: There is a need to speed up the process of orphan drug development, develop new methods for translational research and personalized medicine, and contribute to European Medicines Agency guidelines. The approach presented here offers many perspectives in clinical trial conception. **(Appendix 2)**

(iii) Model of mucociliary clearance in cystic fibrosis lungs

Mucus clearance is a primary innate defense mechanism for human airways. Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encod- ing the Cystic Fibrosis

Transmembrane Conductance Regulator (CFTR) pro- tein. Cystic fibrosis is characterized by dehydration of airway surface liquid and impaired mucociliary clearance. As a result, there is difficulty removing microor-ganism from the airways of the lung, and patients experience chronic pulmonary infections and inflammation. We propose a new physiologically based mathe- matical model of muco-ciliary transport consisting of the two major components of the mucociliary clearance system (i) periciliary liquid layer (PCL) and (ii) mucus layer. We study mucus evacuation under normal conditions and in CF patients. Clearance of airway secretions is a primary therapy for patients with CF. We considers the action of the aerosolized and inhaled medication dornase alfa, which reduces the viscosity of cystic fibrosis sputum. The results of the model simulations stress the potential relevance of the location of the drug de- position in the central or peripheral airways. Mucus evacuation was increased in case the drug was primarily deposited peripherally, i.e. in the small airways. **(Appendix 3)**

(iv) Mathematical model for T lymphoblastic lymphoma

This study focuses on the evolution of a T lymphoblastic lymphoma during a period of two years. This is the time required to study the onset of the disease, the treatment of the acute phase and the maintenance treatment. The disease starts in the thymus and causes an increase in volume. It is a rare type of lymphoma : 25 occurrences treated in France, with a good prognosis: approximately 85% of patients recover. Patients can be definitely cured or relapsed during or after treatments. Relapses usually occur early after remission of the acute phase. Median time of relapse was 1 year after complete remission (range 0.2 − 5.9 years). The purpose of this work is to test the possibility to reduce the duration of treatment in order to reduce its toxicity without increasing the number of relapses. The possible reduction of the duration is applied to pairs of virtual patients developed from a mathematical model of the thymus, the disease and its treatment. **(Appendix 4)**

(v) Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 1: methotrexate.

Methotrexate is an antimetabolite and antifolate drug that is widely used in the treatment of malignancies and auto-immune disorders. In childhood acute lymphoblastic leukaemia, methotrexate is often combined with 6-mercaptopurine and both of them have been shown to be very effective for maintenance of remission. Large variability in the pharmacokinetics of methotrexate has led to increasing use of therapeutic drug monitoring in its clinical use to identify patients with high risk of toxicity and optimise clinical outcome. A physiologically based pharmacokinetic model was developed for methotrexate for oral and intravenous dosing and adults and paediatric use. The model has compartments for stomach, gut lumen, enterocyte, gut tissue, spleen, liver vascular, liver tissue, gall bladder, systemic plasma, red blood cells, kidney vascular, kidney tissue, skin, bone marrow, thymus, muscle and rest of body. A mechanistic model was also developed for the kidney to account for renal clearance of methotrexate via filtration and secretion. Variability on system and drug specific parameters was incorporated in the model to reflect observed clinical data and assuming the same pathways in adults and children, age-dependent changes in body size, organ volumes and plasma flows, the model was scaled to children. The model was developed successfully for adults and parameters such as net secretion clearance, biliary transit time and red blood cell distribution and binding parameters were estimated from published adult profiles. A relationship between fraction absorbed and dose using reported mean bioavailability data in the literature was also established. The model also incorporates non-linear binding in some tissues that has been described in the literature. Predictions using this model provide adequate description of observed plasma concentration data in adults and children. The model can be used to predict plasma and tissue concentrations of methotrexate following intravenous and oral dosing in adults and children and therefore help to improve clinical outcome **(Appendix 5).**

(vi) ***Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 2: 6-mercaptopurine and its interaction with methotrexate***

6-mercaptopurine (6-MP) is a purine antimetabolite and prodrug that undergoes extensive intracellular metabolism to produce thionucleotides, active metabolites which have cytotoxic and immunosuppressive properties. Combination therapies involving 6-MP and methotrexate have shown remarkable results in the cure of childhood acute lymphoblastic leukaemia (ALL) in the last 30 years. 6-MP undergoes very extensive intestinal and hepatic metabolism following oral dosing due to the activity of xanthine oxidase leading to very low and highly variable bioavailability and methotrexate has been demonstrated as an inhibitor of xanthine oxidase. Despite the success recorded in the use of 6-MP in ALL, there is still lack of effect and life threatening toxicity in some patients due to variability in the pharmacokinetics of 6-MP. Also, dose adjustment during treatment is still based on toxicity. The aim of the current work was to develop a mechanistic model that can be used to simulate trial outcomes and help to improve dose individualisation and dosage regimen optimisation. A physiological based pharmacokinetic model was proposed for 6-MP, this model has compartments for stomach, gut lumen, enterocyte, gut tissue, spleen, liver vascular, liver tissue, kidney vascular, kidney tissue, skin, bone marrow, thymus, muscle, rest of body and red blood cells. The model was based on the assumption of the same elimination pathways in adults and children. Parameters of the model include physiological parameters and drug-specific parameter which were obtained from the literature or estimated using plasma and red blood cell concentration data. Age-dependent changes in parameters were implemented for scaling and variability was also introduced on the parameters for prediction. Inhibition of 6-MP first-pass effect by methotrexate was implemented to predict observed clinical interaction between the two drugs. The model was developed successfully and plasma and red blood cell concentrations were adequately predicted both in terms of mean prediction and variability. The predicted interaction between 6-MP and methotrexate was slightly lower than the reported clinical interaction between the two drugs. The model can be used to predict plasma and tissue concentration in adults and children following oral and intravenous dosing and may ultimately help to improve treatment outcome in childhood ALL patients **(Appendix 6)**

(vii) ***A physiologically based pharmacokinetic model for clobazam and stiripentol in adults and children.***

Purpose: To develop a physiologically based pharmacokinetic model in adults and children for clobazam, its active metabolite norclobazam and stiripentol and to account for significant clinical interaction that has been reported when clobazam and stiripentol are co-administered.

Methods:A PBPK model with ten compartments was developed. An in vitro-in vivo extrapolation technique was used to scale clearance in children for clobazam and norclobazam and clearance parameters for stiripentol were obtained from fitting. Other drug and system parameters were obtained from the literature.

Results: The tissue/blood partition coefficients adequately predict observed volume of distribution for clobazam and stiripentol. In a clinical study in children where clobazam was administered alone and co-administered with stiripentol, the predicted and observed minimum concentration at steady state (mean and 95% confidence interval) during clobazam monotherapy were 0.19 (0.05-0.49 mg/L) and 0.20 (0.17-0.23 mg/L), respectively, and predicted and observed norclobazam concentrations were 0.49 (0.16-1.38 mg/L) and 0.95 (0.91-0.99 mg/L), respectively. From an interaction study with stiripentol the predicted stiripentol concentration was 10.12 (2.51-39.36 mg/L) and the observed concentration was 10.0 (8.3-11.7 mg/L); the predicted clobazam concentration was 0.29 (0.07-1.05 mg/L) and the observed concentration was 0.31 (0.24-0.38 mg/L); and the predicted norclobazam concentration was 2.30 (0.45-5.53 mg/L) and the observed concentration was 4.32 (3.77-4.87 mg/L).

Conclusion: The PBPK model adequately described observed data and the extent of interaction between clobazam/norclobazam and stiripentol (**appendix 7)**

(viii) A physiologically based pharmacokinetic model for valproic acid in adults and children.

Valproic acid is an anti-convulscant drug that is widely used in the treatment of different types of epilepsy and since its introduction the clinical use has increased rapidly both as a sole agent and in combination therapies. The mechanism of action has been linked to blockade of voltage-dependent sodium channels and potentiation of GABAergic transmission. The most widely used route of administration of Valproic acid is oral, although it can also be given intravenously and rectally and its pharmacokinetics has been studied extensively. The aim of this work was to develop a physiologically based pharmacokinetic model for plasma and tissue/organ prediction in children and adults following intravenous and oral dosing of Valproic acid. The plasma/tissue concentration profile will be used for clinical trial simulation in Dravet syndrome, a rare form of epilepsy in children where the combination of Valproic acid, stiripentol and clobazam has shown remarkable results. A physiologically based pharmacokinetic model was developed with compartments for gut lumen, enterocyte, gut tissue, systemic blood, kidney, liver, brain, spleen, muscle and rest of body. System and drug specific parameters for the model were obtained from the literature from in vitro and in vivo experiments. The model was initially developed for adults and scaled to children using age-dependent changes in anatomical and physiological parameters and ontogeny functions for enzyme maturation assuming the same elimination pathways in adults and children. The results from the model validation showed satisfactory prediction of plasma concentration both in terms of mean prediction and variability in children and adults following intravenous and oral dosing especially after single doses. The model also adequately predicts clearance in children. Due to limited distribution of Valproic acid into tissues, the concentration in plasma is about 8-9 times higher than tissues/organs. The model could help to improve clinical outcome in the treatment of Dravet syndrome through dose optimization **(appendix 8)**

(ix) Depolarizing GABA : A keyplayer in DRAVET syndrome ?

Introduction: Dravet syndrome (DS) is a rare pediatric epilepsy syndrome (incidence <1/40,000) characterized by hemi- or generalized (tonic-) clonic seizures appearing from the first year of life and often triggered by fever. SCN1A gene mutations have been found in about 85% children with DS. A mouse model of DS with SCN1A mutation has shown a selective failure of excitability of GABAergic interneurons resulting in epilepsy. Conventional treatments have been disappointing. However, a newer antiepileptic drug, Stiripentol, used in association with the benzodiazepine clobazam, has produced a significant (though incomplete) reduction in seizure rate. This drug is known to increase the duration of GABAA receptors opening time and potentiate the antiepileptic action of clobazam.

Methods: A physiology-based computational model was developed in order to investigate some possible pathophysiological causes of DS. A lumped-parameter approach lying at the level of cortical neuronal population was used to represent the generation of spontaneous EEG activity. The model includes one sub-population of pyramidal cells and two sub-populations of interacting interneurons: somatic-projecting interneurons (basket-like) with fast synaptic kinetics GABAA (fast, I1) and dendritic-projecting interneurons with slow synaptic kinetics $GABA_A$ (slow). Basket-like cells were interconnected to produce mutual inhibition (I1->I1). Parameters of the sigmoid function (representing the firing rate of interneurons) were adapted to mimic the genetic alteration found in DS. In addition, the model accounts for a number of neurobiological hypotheses. First, we implemented the mechanism referred to as “dynamic depolarizing $GABA_A$” mediated post-synaptic potential in the model, as a number of studies reported that the chloride reversal potential can switch from negative value to more positive value depending on interneuronal activity. Second, “shunting inhibition” promoted by $GABA_A$ receptor activation. Finally, the dose dependent effect of benzodiazepine and Stiripentol on the generated field potential was also considered.

Results: For fixed parameter configurations of the computational model, the slow increase of the proportion of depolarizing GABAA mediated IPSP (I1->I1 and I1->P) is sufficient to switch the activity from background to interictal isolated epileptic spikes, to fast onset activity with decreasing frequency with time (shirp-like activity), seizure like activity and seizure termination.

Different morphologies of interictal spikes and patterns of seizures observed in patients were reproduced in the model by tuning the amount of depolarizing GABAA postsynaptic potential. Finally, stiripentol by increasing the shunting inhibition reduced seizure-like activity in a dose dependent manner.
Conclusion: Our computational modeling of DS is therefore promising. Further optimization is needed. **(Appendix 9)**

(x) How to select the most appropriate randomized controlled clinical trial design: a new approach using modelling and simulation

Objective: *In silico* simulations of clinical trials have become powerful tools for drug development. Therefore, the main objective of this study is to compare in silico different RCTs experimental designs in terms of power, accuracy of the estimation of treatment effect or number of patients receiving active treatment
Methodology: A virtual population of patients was simulated before their randomization in virtual clinical trials. Treatment effect was modelled using a dose-effect relation. Different experimental designs were considered and performances between the different designs were compared. One thousand clinical trials were simulated for each experimental design with an example of modelled disease.
Results: According to simulation results the number of patients needed by each design to reach 80% power was 50, 60, 65 and 70 for cross-over, parallel or randomized withdrawal designs, drop the loser, early escape or play the winner, respectively. The median of treatment effect estimations for the 1000 RCT simulations was similar for all experimental designs, both for quantitative and qualitative endpoints.
Conclusion: Our approach could be helpful to investigate for the best experimental design that could be considered for future RCTs in order to optimize drug evaluation particularly in rare diseases, theragnostic approaches or in specific patients. **(Appendix 10)**

C.5 Discussion

Despite the short time period and the the diversity of diseases, we were still able to achieve important results. Thanks to disease modelling which permitted us the developing of “Virtual populations” generation and this was sucessfully achieved in all 3 diseases (Dravet Syndrome (DS), cystic fibrosis (CF) and T-cell lymphoblastic lymphoma (LBL). We were also able to connect drug PK/PD models on the disease models, with two drugs and their interaction in LBL (methotrexate and purinethol), three drugs in DS (levetiracetam, clobazam, stiripentol), and Dornase alpha in CF. Several trial designs could be modelled and simulated, and results could be compared.
Disease modelling is a difficult step in this overall framework. We found very few published models for all three diseases, and had to develop our own models. How much the model gets into details of the disease pathophysiology is a challenging issue. It should be simple enough for the overall scheme to be feasible, but detailed enough to provide a realistic approach of the disease. Modelling can always be further developed. We need here to use databases for identifying patient characteristics to be added to the existing models. The model of T-LBL provides as outcome the clinical expected outcome, relapse (yes/no). However, we need to achieve modelling of relevant clinical outcomes for CF, e.g. the current output is mucociliary clearance, and it should be translated into FEV1 or CT Scan outcome); for Dravet syndrome the output consists in a very short time scale (some seconds), which does not represent the reality of the clinical context, and cannot provide as outcome a number of epileptic seizures.
We modelled only efficay outcomes, and intentionally skipped side effects / toxicity in this first part of the Project. This should be added to the model when further developped. Our models also need further validation against clinical data. Databases are available for all the three studied diseases and this is a next step to be considered.For trial simulation, we first modelled the different trial designs, and randomised virtual patients from the cohorts of CF, DS, and LBL. A thousand samples of patients were randomly selected from a virtual population. For each of them, all the designs were applied in order to obtain sufficiently accurate results. Developped programs

were used on Cresim mathematical model but papers describing results obtained are not yet available for publication 'only poster for CF simulations').Seven designs have been modelled but other designs could be added. Orthogonal approach such as Bayesian or meta-analysis could also be studiedin the future.

C.6 Short-term exploitation of results / Perspectives

With the end of the CRESim project the scientific exploitation of the results is by far not ended. Vast amount of new data has been gathered. The approach proposed in CRESim may be applied to several different situations by combining three aspects (diseases-drugs-endpoints) and could be integrated into a translational research process. Existing databases could be analyzed to identify prognostic and predictive biomarkers, potential treatments of interest, and for the validation of models. This approach could be implemented in a larger perspective combining different diagnostic strategies (i.e. theranostic strategies). This approach could contribute to the development of European Medicines Agency guidelines. We anticipate this approach will be useful for:

(i) The greater orphan disease research community and provide funding organizations and patient advocacy groups with suggestions for the best way forward. In addition to enabling academic clinical research, strategies such as this may also help start-up companies obtain funding, as well as increase the pharmaceutical industry's commitment to orphan drug development. However, this proposed in *silico* approach for rare diseases would need a final and consistent validation of the treatment effect using an in vivo clinical trial carried out in real patients. **Our approach could be considered as a potential way to foster reflection on orphan drug development**.

(ii) The algorithm that we proposed seems to be a useful tool in the case of rare diseases and the development of orphan drugs as well as for specific populations where recruitment could be difficult. **Use of this algorithm will facilitate the choice of the most appropriate design for a given disease-treatment-outcome situation.**

(iii) CRESim project has allowed development of a **physiologically based model of mucociliary clearance in patients with CF**. The model output of tracheal mucus velocity showed good correlation with previous clinical data. This approach can be used to model the effects of drugs in virtual clinical trials, in order to efficiently optimize multiple aspects of a trial (e.g. protocol of drug administration, medication dose, dosing interval, trial design, trial duration, sample size), before performing a clinical trial in real patients.

(iv) **Our in silico approach** offers many perspectives even if the definitive choice of a one given design remains difficult, depending on numerous factors. In a given (drug-disease) situation, our approach may be helpful to investigate for the best future RCT design to be selected. Moreover, simulation approach will be more and more used by large pharmaceutical companies to complete their regulatory filings, collaboration between patient associations, investigators, sponsors, methodologists and statisticians remains clearly mandatory.

(v) In our **computational model approach**, at the level of neuronal network is promising regarding its application to Dravet Syndrome. Some parameters of the model were identified which permitted to adequately reproduced EEG patterns of Dravet patients, decreased inhibition due to SCN1A mutation, depolarizing GABA, GABAergic effect of STP. This model can ultimately be considered as a potential useful tool for simulating the effects of new therapeutic targets in Dravet syndrome. The same approach could be extended to other rare epilepsies of genetic origin with a known physiopathological effect.

Our challenge is to find funding for further developing this approach. Several attempts were performed, two are pending : PRTK call were we submitted the LBL project continuation, and an application to H2020 call on systems biology, with our European partners in CF and PK/PD modelling, and other consorrtia.

C.7 References

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C.8 Comments

We encountered some difficulties with funding reduction by 20%, and a very ambitious program. We had to abandon the platform project since we have no guaranty of funding to allow the maintenance of the platform. Future developpement of this strategy towards enriched trials in non rare diseases, and ongoing collaboration with partners is very encouraging.

D. LIST OF DELIVERABLES

The following table intends to group aims and deliverables corresponding to the initial application. It should include the workpackage number (WP), aim number inside the workpackage (Aim), deliverable number inside the aim (DLV) followed by the partners involved (Partners). In the column "Planned/New" please specify if the Aim or/and Deliverable was initially planned or new (added after the start of the project). In the column "Description" please briefly describe each workpackage (title), aim and deliverable. In the column "Status" please use "C", "I" or "A" to indicate if the Aim or Deliverable was: completed "C"; Incomplete "I" or abandonned "A". The table below is shown as an example.

Deliverables were identified for the project overall, and attributed to WP1, even though they are the result of a collaborative work.

WP	AIM	DLV	Partner (s)	Planned/ New	Description	Status
WP1– (P. Nony, C. Cornu, B. Kassai, Lyon, FRANCE):	**1. Methodology, scientific coordination and management**	**D1.1** Kick-off meeting	P1 & P7	Planned	up-date bibliographic search for available experimental designs	C
		D1.2 Website and communication tools		Planned		C
		D1.3 Organization of scientific meetings		Planned		C
		D1.4 Periodic internal reports		Planned		C
		D1.5 Mid-term and final reports		Planned		C
		D1.6 Platform of in silico simulation		Planned	**(i)** Development of virtual population of pediatric patients **(ii)** Modelling of various RCTs with specific designs	A

E. PUBLICATIONS

E.1 Number of publications

Type of publication	Total N°	
Peer reviewed articles	10	☑
Books or book's chapters		
Articles dedicated to general public		
Conferences dedicated to general public		
Policy documents		
Others		

E.2 List of publications and communications

Please list the publications that are result of common work and implicate several (at least 2) partners. Please group them according to the categories presented in the table above.
Please list only the conferences to which you/partners where invited and presented the project.

	Publication
	1. Experimental designs for small randomised clinical trials: an algorithm for choice. Cornu C, Kassai B, Fisch R, Chiron C, Alberti C, Guerrini R, Rosati A, Pons G, Tiddens H, Chabaud S, Caudri D, Ballot C, Kurbatova P, Castellan AC, Bajard A, Nony P; CRESim & Epi-CRESim Project Groups, Aarons L, Bajard A, Ballot C, Bertrand Y, Bretz F, Caudri D, Castellan C, Chabaud S, Cornu C, Dufour F, Dunger-Baldauf C, Dupont JM, Fisch R, Guerrini R, Jullien V, Kassaï B, Nony P, Ogungbenro K, Pérol D, Pons G, Tiddens H, Rosati A, Alberti C, Chiron C, Kurbatova P, Nabbout R. ***Orphanet J Rare Dis. 2013 Mar 25;8:48. doi: 10.1186/1750-1172-8-48. Review*** ***2.*** Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 1: methotrexate. Ogungbenro K, Aarons L; CRESim & Epi-CRESim Project Groups. J Pharmacokinet Pharmacodyn. 2014 Apr;41(2):159-71. ***doi: 10.1007/s10928-014-9354-4. Epub 2014 Mar 21*** ***3.*** Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 2: 6-mercaptopurine and its interaction with methotrexate. Ogungbenro K, Aarons L; CRESim & Epi-CRESim Project Groups. ***J Pharmacokinet Pharmacodyn. 2014 Apr;41(2):173-85. doi: 10.1007/s10928-014 9355-3. Epub 2014 Mar 21.*** **4.** Ogungbenro k, Aarons l; and the Cresim & Epi-cresim project groups. A physiologically based pharmacokinetic model for clobazam and stiripentol in adults and children**. pharm res. 2014 jul 23. [epub ahead of print]** **5.** Ogungbenro k, Aarons l; Cresim & Epi-cresim project groups. a physiologically based pharmacokinetic model for valproic acid in adults and children. **eur j pharm sci. 2014 oct 15;63:45-52.**
	Manuscripts submitted for publication
	1. A Methodological Framework For Drug Development 1 In Rare Diseases.Patrice Nony; Polina Kurbatova; Agathe Bajard ; Salma Malik; Charlotte Castellan; Sylvie Chabaud;Vitaly Volpert; Nathalie Eymard; Behrouz Kassai; Catherine Cornu and the CRESim and Epi-CRESim study

	groups. ***Submitted Orphanet J Rare Dis.*** 2. A new approach using modelling and simulation to choose the best appropriate design for randomized clinical trials. Agathe Bajard , Sylvie Chabaud, Catherine Cornu, Anne-Charlotte Castellan, Salma Malik, Roland Fisch, Corinne Alberti, Patrice Nony and the CRESim and Epi-CRESim project groups. ***Submitted Journal of clinical epidemiology*** 3. Mathematical model of T-cell lymphoblastic lymphoma: disease, treatment, cure or relapse of a virtual cohort of patients. N.Eymarda, V.Volperta,b,c, N. Bessonovd, CRESim Project Groups, Leon Aarons; Agathe Bajard; Clément Ballot; Yves Bertrand; Frank Bretz; Daan Caudri; Charlotte Castellan; Salma Malik; Sylvie Chabaud; Catherine Cornu; Frank Dufour; Cornelia Dunger-Baldauf; Jean-Marc Dupont; Roland Fisch; Renzo Guerrini; Behrouz Kassaï; Patrice Nony; Kayode Ogungbenro; David Pérol; Gérard Pons; Harm Tiddens; Anna Rosati. ***(Manuscript submitted to Journal of Theoretical Biology).*** 4. P. Kurbatovaa,b, , N. Bessonovc, V. Volpertd, H.A.W.M. Tiddense, C. Cornua,f,g, P. Nonya,f, D. Caudrie, on behalf of the CRESim working group, Leon Aarons; Agathe Bajard; Clément Ballot; Yves Bertrand; Frank Bretz; Daan Caudri; Charlotte Castellan; Salma Malik; Sylvie Chabaud; Catherine Cornu; Frank Dufour; Cornelia Dunger- Baldauf; Jean-Marc Dupont; Roland Fisch; Renzo Guerrini; Behrouz Kassaï; Patrice Nony; Kayode Ogungbenro; David Pérol; Gérard Pons; Harm Tiddens; Anna Rosati. Model of mucociliary clearance in cystic fibrosis lungs ***(Manuscript submitted to Journal of Theoretical Biology).*** 5. P.Kurbatova, F.Wendling, F. Mina, C.Cornu, S Malik, R.Guerrini, A.Kaminska, O.Dulac, G.Pons, R.Nabbout, C.Chiron, P.Nony, P.Benquet and the CRESIM/EpiCRESIM study Group Depolarizing GABA : A keyplayer in DRAVET syndrome ?, ***(Manuscript to be submitted)***
	Oral Presentations
	1. Catherine Chiron, Polina Kurbatova and the CRESim project. The Use of Modelling and simulating to design new trials,exemple of Dravet syndrome, **Orphan Drug & Rare Disease Seminar "ACCELERATING ACCESS TO THERAPEUTIC INNOVATION"17th-18th October 2013 Marseille.** 2. P. Nony, C. Cornu. Rare diseases: use of clinical trial simulation for the choice and optimization of study design.**Grip-Priomedchild meeting, Bilbao, 29th of October 2012** 3. Dravet syndrome physiology-based pharmacodynamic modeling attempt (Cresim project). Presented by P.Kurbatova as an oral presentation at 14th **European Society for Developmental Perinatal and Pediatric Pharmacology (ESDPPPP) congress, Salzburg, June 2013** P.Kurbatova, P.Benquet, F.Wendling, C.Cornu, R.Guerrini, A.Kaminska, O.Dulac, G.Pons, R.Nabbout, C.Chiron, P.Nony and the CRESIM/EpiCRESIM study Group 4. Rare disease: use of clinical trial simulation for the choice and optimization of study design (CRESim), presented by Patrice Nony **Journée maladies rares Groupement Hospitalier Est(GHE), 28th Feb 2014.** 5. C. Ballot, C Cornu, Yves Bertrand,partner 1 & 4 Cresim project . Lymphobalstic lymphoma model, **Orphan Drug & Rare Disease Seminar 2012, Lyon.** 6. Agathe BAJARD. Randomized Clinical Trial simulation, CRESim Project. **Evaluation of drugs for rare diseases seminar, Lyon, March 1-2 2012**

	7. Kurbatova P, Cresim project . The Use of Modelling and simulating to design new trials, exemple of Dravet syndrome, **Orphan Drug & Rare Disease Seminar** 8. Dravet Syndrome modeling, Seminar in Research Unit U663 "Infantile Epilepsies and Brain Plasticity" **Paris, France, 9 September, 2014**
	Posters Presentations
	1. C. Ballot, C Cornu, Yves Bertrand,P Nony and Cresim project . Lymphobalstic lymphoma model, P2T 7eme congrès de physiologie, de pharmacologie et thérapeutique Dijon 4-6 April 2012. 2. C. Ballot, C Cornu, Yves Bertrand,P Nony and Cresim project . Lymphobalstic lymphoma model, **6ème Conférence Francophone d'Épidémiologie Clinique (EPICLIN),19èmes Journées des Statisticiens des Centres de Lutte contre le Cancer 9 au 11 mai 2012 – Lyon** 3. Modélisation et simulations de différents plans expérimentaux d'essais cliniques randomisés: application aux maladies rares (projet CRESim). A. Bajard, S. Chabaud, C. Cornu, C. Ballot, P. Kurbatova, C. Castellan, et P. Nony pour le groupe CRESim. **EPICLIN, Lyon, Mai 2012** 4. C Cornu, B Kassai, R Fish, C Chiron, C Alberti, R Guerrini, A Rosati, G Pons, H Tiddens, S Chabaud, D Caudri, C Ballot, P Kurbatova, C Castellan, A Bajard, P Nony, and the CRESim & Epi- CRESim Project Groups. Experimental designs for small randomised clinical trials: an algorithm for choice,***P2T, VIII congrès de physiologie, de pharmacologie et thérapeutique, 22-24 avril 2013***, 5. Modelling and simulation of different experimental designs for randomized clinical trials (CRESim Project). ***P2T, VIII congrès de physiologie, de pharmacologie et thérapeutique, 22-24 avril 2013***, 6. P.Kurbatova, F.Wendling, F. Mina, C.Cornu, R.Guerrini, A.Kaminska, O.Dulac, G.Pons, R.Nabbout, C.Chiron, P.Nony, the CRESIM/EpiCRESIM study Group and P Benquet.Computational Model of Dravet syndrome; presented by P.Benquet, at ***the 6th International Workshop on Seizure Prediction (IWSP6), San Diego) in October 2013***, 7. Computational modeling of Dravet syndrome. P.Kurbatova, P. Benquet, F. Wendling, C. Cornu, R. Guerrini, A. Kaminska, O. Dulac, G. Pons, R. Nabbout, C. Chiron, P. Nony as a ***poster at 11th International Congress of European Association for Clinical Pharmacology & Therapeutics: (EACPT), Geneva, Aug 2013***. 8. Model of mucociliary clearance in cystic fibrosis lungs (CRESim European Project) Kurbatova P, Bajard A, Malik S, Tiddens H, Volpert V, Cornu C, Bessonov N, Kassai B, Chabaud S, Nony P, Caudri D. SFP PC-29, ***Congrès des sociétés de Pédiatrie, Lyon France 2014*** 9. Modelling and simulation of experimental randomized controlled trial designs in a rare disease: cystic fibrosis. P. Kurbatova, A. Bajard, H. Tiddens, V. Clopert, C. Cornu, N. Bessonov, B. Kassai, S. Chabaud, P. Nony, D. Caudri. **European Respiratory Society Congress, Munich, September 2014.** *10.* Modeling and simulation of experimental designs to optimize clinical trials for cystic fibrosis, Bajard A, Kurbatova P, Chabaud S, Salma Malik, Nony P, Caudri D, Cornu C, Volpert V, Bessonov N, Kassaï B, Tiddens H, and the CRESIM group. ***The International Conferences for Rare Diseases and Orphan Drugs (ICORD) 9th Annual Conference , October 7-9, 2014, The Netherlands.*** *11.* Mathematical model of T-cell lymphoblastic lymphoma: disease,

	treatment, cure or relapse of a virtual cohort of patients. Nathalie Eymard, Vitaly Volpert, Nicolai Bessonov, CRESim Project Groups. ***The International Conferences for Rare Diseases and Orphan Drugs (ICORD) 9th Annual Conference , October 7-9, 2014, The Netherlands.*** 12. A Methodological Framework For Phase III Drug Development In Rare Diseases. Patrice Nony; Polina Kurbatova; Agathe Bajard ; Salma Malik; Charlotte Castellan; Sylvie Chabaud; Vitaly Volpert; Nathalie Eymard; Behrouz Kassai; Catherine Cornu and the CRESim and Epi-CRESim study groups. ***<u>The International Conferences for Rare Diseases and Orphan Drugs (ICORD) 9th Annual Conference , October 7-9, 2014, The Netherlands.</u>*** 13.
	National conferences, seminars, miscellaneous
	1. P2T, VIII congrès de physiologie, de pharmacologie et thérapeutique, 22-24 avril 2013 2. 6ème Conférence Francophone d'Épidémiologie Clinique (EPICLIN),19èmes Journées des Statisticiens des Centres de Lutte contre le Cancer 9 au 11 mai 2012 – Lyon 3. 14th European Society for Developmental Perinatal and Pediatric Pharmacology (ESDPPPP) congress, Salzburg, June 2013 4. P2T, Congrès de physiologie, de pharmacologie et thérapeutique, 22-24 avril 2013. 5. 6th International Workshop on Seizure Prediction (IWSP6), San Diego) in October 2013, 6. 11th International Congress of European Association for Clinical Pharmacology & Therapeutics: (EACPT), Geneva, Aug 2013. 7. Congrès des sociétés de Pédiatrie, Lyon France 2014 8. 9th International Conference for Rare Diseases and Orphan Drugs (ICORD), October 7-9, 2014, The Netherlands.
	Awards **Best Poster Prize 8. 9th International Conference for Rare Diseases and Orphan Drugs (ICORD), October 7-9, 2014, The Netherlands.**

G. PUBLICATIONS

Appendix 1

Experimental designs for small randomised clinical trials: an algorithm for choice

Appendix 2

A Methodological Framework For Drug Development In Rare disease

Appendix 3

Model of mucociliary clearance in cystic fibrosis lungs

Appendix 4

Mathematical model for T lymphoblastic lymphoma

Appendix 5

Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 1: methotrexate.

Appendix 6

Physiologically based pharmacokinetic modelling of methotrexate and 6-mercaptopurine in adults and children. Part 2: 6-mercaptopurine and its interaction with methotrexate

Appendix 7

A physiologically based pharmacokinetic model for clobazam and stiripentol in adults and children.

Appendix 8

A physiologically based pharmacokinetic model for valproic acid in adults and children.

Appendix 9

Depolarizing GABA : A keyplayer in DRAVET syndrome ?

Appendix 10

How to select the most appropriate randomized controlled clinical trial design: a new approach using modelling and simulation

Appendix 11

Scientific Report Partner 3: H. Tiddens, Erasmus University Medical Centre -Sophia Children's Hospital, Netherlands

Appendix 12

Scientific Report Partner 4: L. Aarons, University of Manchester, United Kingdom

Appendix 13

Scientific Report Partner 5: S. Chabaud, Centre Léon Bérard, France

Appendix 14