Evaluation of the ex vivo effect of AG-348 treatment on pyruvate kinase-deficient cell metabolism and erythroid development

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To extend and strengthen the initial pilot observations on PKD red blood cells, and thereby further establish the activity of AG-348 treatment for PKD, UMC Utrecht and Agios Pharmaceuticals will collaborate in this pre-clinical study. In particular…

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Blood and lymphatic system disorders congenital
Study type	Observational invasive

Summary

ID

NL-OMON42151

Source
ToetsingOnline

Brief title
Ex vivo effect of AG-348 on PKD

Condition

- Blood and lymphatic system disorders congenital

Synonym
anemia, red blood cell disease

Research involving
Human

Sponsors and support

Primary sponsor: Agios Pharmaceuticals Inc.

Source(s) of monetary or material Support: Ministerie van OC&W,Agios Pharmaceuticals Inc.,Agios Pharmaceuticals Inc., 38 Sidney Street Cambridge, MA, USA

Intervention

Keyword: Cell metabolism, Pyruvate kinase deficiency, Red blood cell, Small activator molecule

Outcome measures

Primary outcome

Stage 1.

The primary endpoints will be: PK activity, PK protein stability, ATP levels,

and measures of cell deformability. To determine whether AG-348 treatment

results in objective differences compared to vehicle control samples will be

split, and part of the sample will be incubated with AG-348, whereas the other

part will not be exposed to treatment. In doing so, each patient sample will

serve as its own control to determine whether AG-348 treatment results in

objective differences compared to vehicle control, and compared to a measured

baseline value for each endpoint.

Stage 2.

Erythroid differentiation will be monitored by the examination of erythroid

cell morphology, and the measurement of erythroid differentiation markers, e.g.

glycophorin A and transferrin receptor, in cells from PKD patients and wild

type controls in the presence or absence of AG-348. Quantitation will be

assessed by FACS. At specific time points PK activity and stability will be

measured.

We will also monitor the PK isozyme switch that takes place during normal red

blood cell development. This isozyme switch concerns the gradual replacement of the PK-M2 isozyme (transcribed from the PKM2 gene) to the PK-R isozyme (transcribed from the PKLR gene) during red cell development. Both isozymes are highly homologous on the amino acid level, and PK-M2 could therefore represent an (additional) target of AG-348 during PKD erythropoiesis.

Secondary outcome

A major secondary study goal is to define broadly differences in cellular metabolite profiles, red cell deformability, and erythroid differentiation in PKD red cells compared to wild type controls (PKD baseline values). For instance, it is well known that PKD red cells are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the PK-R substrate phosphenolpyruvate (PEP) and deficiency in the PK-R product adenosine triphosphate (ATP). We would like to identify further biomarkers that might shed more light on the mechanism of disease and could inform future treatment.

Study description

Background summary

Pyruvate kinase deficiency (PKD) is an autosomal recessive enzymopathy that is the most common cause of hereditary nonspherocytic hemolytic anemia (HNSHA). It is a rare disease characterized by a life-long chronic hemolysis with severe co-morbidities, in particular iron overload. The mature red blood cell is completely dependent on glycolysis for it*s energy supply. Red blood cell pyruvate kinase (PK-R) is a key regulatory enzyme of red cell glycolysis. PKD red cells are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the PK-R substrate phosphoenolpyruvate (PEP) and deficiency in the PK-R product adenosine triphosphate (ATP). It is hypothesized that insufficient energy production affects red cell membrane

homeostasis, thereby promoting premature removal of the PK-deficient red blood cell from the circulation. Clinical symptoms of PK deficiency are usually limited to patients who are compound heterozygous or homozygous for a mutation in PKLR. The phenotypic expression is highly variable, and we and others have found that there is a poor correlation between the genotype and the phenotype. Treatment of PKD patients is generally supportive, focusing on the resultant anemia and iron overload state, and there are no approved drugs that directly target mutated pyruvate kinase. Agios Pharmaceuticals recently developed and reported on the mechanism of action and in vitro cellular effects of AG-348, an allosteric activator of PK-R. They demonstrate that AG-348 can potently activate a spectrum of recombinantly expressed PK-R mutant proteins, including mutations that span distinct subdomains of the enzyme. The binding of AG-348 attenuates the thermostability defect of several mutant alleles of PK-R, including the commonly observed p.(Arg510Gln) mutant.

Pilot ex vivo studies on PKD red cells exposed to AG-348 showed that these cells had increased PK-R enzyme activity (up to 4-fold over control) and showed consistent dose and time-dependent metabolic responses, including sharp reductions in PEP (up to 70% compared to control) and increases in ATP levels (up to 100% over control). In these ex-vivo settings, ATP levels in AG-348 treated cells can reach levels that are typical of normal, non-PKD red cells.

Altogether, these data support the hypothesis that drug intervention with AG-348 in PKD red cells may restore glycolytic pathway activity and normalize red cell metabolism in vivo. This therapeutic approach may therefore be an effective way to correct the underlying pathology of PKD and, potentially, provide clinical benefit to PKD patients. In this respect, Agios Pharmaceuticals recently initiated two Phase 1, single-center, randomized, double-blind, placebo-controlled clinical trials to assess the safety and tolerability of AG-348 through dose escalation in healthy adult men and women (http://www.agios.com/pipeline-pkr.php).

Study objective

To extend and strengthen the initial pilot observations on PKD red blood cells, and thereby further establish the activity of AG-348 treatment for PKD, UMC Utrecht and Agios Pharmaceuticals will collaborate in this pre-clinical study. In particular, the goal is to test the activity of AG-348 in restoring red blood cell metabolism and cellular function in a range of different genetic backgrounds. This is of great importance as there are only few relatively common mutations in PKD. Hence, most patients are compound heterozygous for a unique combination of two rare mutations. Depending on the type and location of the mutations the response to AG-348 treatment may differ. The primary objective of this study will therefore focus on the question if and how the PKD genotype influences the response to AG-348 treatment. UMC Utrecht is a national and international acknowledged Center of Expertise for PKD. We have diagnosed more than 100 PKD patients in the last two decades, and have studied many of

them on the biochemical and genetic level. Our cohort of PKD patients represents one of the biggest PKD cohorts in the world. All PKD patients have been genotyped. This enables efficient selection of a variety of genotypes to be study with regard to the primary objective.
In addition to studying these direct effects of AG-348 on PKD red blood cells, the secondary objective of this study comprises the investigation of the effect of AG-348 on ex vivo erythropoiesis in a PK-deficient background. Currently, it is not known if, and how, AG-348 affects erythropoiesis in PKD patients. This is of considerable importance, because ineffective erythropoiesis represents one of pathophysiological features of PKD. It seems reasonable to assume that any activation of mutant PK proteins by AG-348 may also occur on the level of the developing red blood cell. Alternatively, PKD patients whose red blood cells fail to respond to AG-348 treatment may show an effect of treatment on the level of erythropoiesis, because ineffective erythropoiesis in PKD seems not related to lack of metabolic energy but, rather, to increased levels of apoptosis in PKD erythroid progenitor cells.

Ultimately, this pre-clinical study will provide a broad dataset by which to evaluate the question of which aspects of AG-348 ex-vivo activity translate to clinical efficacy. This study will substantially increase the understanding of the mode of action of AG-348 treatment for PKD red blood cells, both on the cellular level, as well as on the level of erythropoiesis. We anticipate that the results will support the hypothesis that AG-348 treatment represents an effective and attractive way to correct the underlying pathologies of PKD, ultimately providing clinical benefit to PKD patients.

Primary Objective: To evaluate the ex vivo effect of AG-348 on cell metabolism and erythropoiesis in red cells from PKD patients.

Secondary Objective(s): To establish baseline parameters in PKD patients compared to healthy control individuals.

Study design

This study will be conducted as an observational case-control study, consisting of 2 stages.

Stage 1.
Blood samples (36 mL) will be collected from 15 PKD patients, and an equal number of healthy human volunteers as control. They latter will be recruited by the Minidonordienst of the UMC Utrecht. After collection, baseline values will be obtained from both patients and controls. This includes the measurement of general haematological laboratory parameters (e.g. hemoglobin, hematocrit, reticulocyte count), red blood cell morphology, osmotic gradient ektacytometry (to measure red blood cell deformability: a measure of overall cellular integrity and functionality), metabolite profile (including 2,3-DPG and ATP

levels), PK antigen levels, and levels of PK-R and PK-M2 protein expression. After baseline evaluation, samples will be incubated either with or without AG-348 for a total of 24 hours. At specific time points measurements will be performed to study the effect of AG-348 on red blood cell metabolism, red cell integrity, and the PK protein itself. Certain experiments will use whole blood whereas the more in depth analysis of red blood cell PK and its characteristics will require purified erythrocytes as a source because of the presence of pyruvate kinase activity in white cells.

Stage 2.
Based on their response to AG-348 treatment, 5-10 PKD patients will be selected for stage 2 of this study. Patients whose red blood cells strongly respond to AG-348 treatment, as well as PKD patients that show little or no response at all will be included in this stage which comprises the investigation of any effect AG-348 treatment may have on erythropoiesis in PKD. Both patient groups are of interest for this part of the study, because AG-348 treatment may have an effect on red blood development in PKD that is independent (and perhaps different) from the direct effect AG-348 treatment has on PKD red blood cells. Furthermore, it is unclear which aspects of AG-348 ex-vivo activity will ultimately correlate with in vivo clinical efficacy. For stage 2, a single amount of blood (70 mL) will be collected on a separate occasion from selected patients as well as an equal number of healthy human volunteers. Peripheral blood mononuclear cells will be isolated and cultured ex vivo into erythroid cells (i.e. reticulocytes) in presence or absence of AG-348. Erythroid development will be monitored by FACS analysis of erythroid differentiation markers. After completion of the culture (approximately 21 days), PK activity and stability will be measured, and PK-M2 and PK-R levels will be evaluated by Western blot analysis.

The duration of the study is approximately 18 months. It will be conducted by the Laboratory for Clinical Chemistry and Haematology of UMC Utrecht. All measurements will be performed at this Department, except for red cell metabolite analyses. These will be performed at the laboratory of Agios Pharmaceuticals (Cambridge, MA, USA). For this, concerning aliquots of processed blood samples of PKD patients will be shipped to Agios Pharmaceuticals for the purpose of being analysed and used for this study only.

Study burden and risks

Patient participation comprises the donation of a limited amount of blood. According to the EMA (European Medicines Agency) and WHO (World Health Organization) guidelines and standards it is safe to withdraw a general maximum of 2.4 ml of blood / kg. Even when considering very conservative mean average weights of adult males and females (64 and 51 kg, respectively) the associated maximum volumes (154 and 122 ml, respectively) are well above the maximum volume of blood to be collected for this study (36 ml in stage 1 and 70 ml in stage 2). Physical discomfort is limited but may include bruising.

Due to the fact that participation in this study requires only either a single donation of blood, or two donations of blood on separate occasions (separated by at least 2 months) we consider the participation of patients and human healthy volunteers in this study to be without risks.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

1. Adult patients with biochemically and genetically diagnosed PK deficiency, i.e. patients should be compound heterozygous or homozygous for mutations in PKLR, the gene that encodes red blood cell PK.
2. The participant is willing and able to give written informed consent.

Exclusion criteria

Patients who have been recently transfused with packed red blood cells (*Recently* is defined as less than 3 months before blood collection).

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	19-05-2015
Enrollment:	30
Type:	Actual

Ethics review

Approved WMO Date:	25-02-2015
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Utrecht (Utrecht)
Approved WMO Date:	07-09-2015
Application type:	Amendment
Review commission:	METC Universitair Medisch Centrum Utrecht (Utrecht)
Approved WMO	

Date:	03-05-2016
Application type:	Amendment
Review commission:	METC Universitair Medisch Centrum Utrecht (Utrecht)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL50963.041.14