

**Repositioning of TAK-475 in Mevalonate Kinase Disease: translating theory into practice**

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IMPRESSIONS

## **Abstract**

Mevalonate Kinase Deficiency (MKD, OMIM #610377) is a rare autosomal recessive metabolic and inflammatory disease. In MKD, defective function of the enzyme mevalonate kinase (MK) due to mutation in the MVK gene, leads to the shortage of mevalonate-derived intermediates, which results in unbalanced prenylation of proteins and altered metabolism of sterols. These defects are reflected in a complex multisystemic inflammatory and metabolic syndrome. Although biologic therapies blocking the pro-inflammatory cytokine interleukin -1, can significantly reduce inflammation, they cannot completely control the clinical symptoms, in particular those affecting the nervous system. For this reason, MKD has been designed as an orphan drug disease. Of note, zaragozic acid, an inhibitor of squalene synthase, has been proven to inhibit the hyper-inflammatory response in cellular models of MKD, by diverting mevalonate intermediates toward geranylgeranylation rather than to the synthesis of squalene. A similar action might be obtained by Lapaquistat (TAK-475, Takeda), a drug that underwent extensive clinical trials as cholesterol lowering agent 10 years ago with a good safety profile. Here we describe the preclinical evidence supporting the repositioning of TAK-475 from its originally intended use to the treatment of MKD and discuss its potential to modulate the mevalonate pathway in inflammatory diseases.

### **Keywords:**

Cholesterol, Statin, Squalene synthase inhibitor, Lapaquistat acetate, Mevalonate Kinase Deficiency, Hypercholesterolemia, Inflammation

## 1. The cholesterol pathway and its regulation

Cholesterol is a fundamental molecule for the proper functioning of animal cells, playing several and different roles. It is essential for cell membranes, regulating their integrity and fluidity, as well as their permeability, by affecting the phospholipidic bilayer [1].

Being embedded in the cellular membrane, cholesterol may also modify the behavior of proteins that are in the membrane as well. For example, it seems to be essential for the formation of rafts, an insoluble complex containing cholesterol, sphingolipids and membrane proteins [2]. These rafts have been shown to be associated with different second messenger proteins, such as src-like tyrosine kinases and heterotrimeric G proteins [3]. Different studies on lymphocytes have demonstrated that rafts help bringing together membrane receptors, thus building and stabilizing protein signalling complexes [4-6]. Cholesterol is also directly involved in the activation state of some protein, such as Hedgehog, ion channels, receptors and enzymes [7, 8].

Cholesterol metabolism, presence and activity is of fundamental importance even in neurons and neuronal transmission, as the brain is the most cholesterol rich organ, and the 23% of the cholesterol present in a human body is found in the central nervous system [9, 10]. At the synaptic level, cholesterol is involved and required in the vesicle supply processes [11-13] and the  $Ca^{2+}$  channel mobility [14], thus playing an important role in the synaptic transmission and regulation. It is also essential for the insulation of neuronal axons, being the rate limiting component for the development of myelin, both in central and peripheral nervous system [15, 16]. Indeed, an altered metabolism of cholesterol is linked to several diseases known to affect the nervous system, and to neurodegeneration. The clearest evidence of this link is shown by Niemann-Pick type C (NPC) disease. In patients suffering from NPC, a mutation in *NPC1* or *NPC2* causes a disrupted trafficking of cholesterol, resulting in its lack both in membranes and in the steroid biosynthetic pathway [17]. However, cholesterol metabolism is linked also to other neurodegenerative diseases [18], such as Alzheimer's disease [19, 20], Huntington disease [21, 22] and Parkinson's disease [23]. Particularly, high levels of cholesterol in the brain, are supposed to trigger some amongst the main inflammatory pathways that take place in the brain: i) oxidative stress ii) mitochondrial dysfunction and iii) excitotoxicity, by increasing pro-inflammatory cytokines and the generation of ROS, depleting the amount of antioxidant molecules (GSH, SOD) and inhibiting the mitochondrial complex I [24-27]. Another fundamental biological function exerted by cholesterol is its role as a precursor for other biological molecules, such as hormones, vitamins and gall components. The so called steroid hormones are synthesized either in the adrenal glands or in the gonads (corticosteroids or sex steroids, respectively) starting from cholesterol and they regulate a great variety of physiological functions, ranging from inflammation, electrolyte balance, immune response to sexual development and characteristics. Because of their very different fields of action, synthetic corticosteroids are used since decades as drug therapy for a wide range of disorders, although the treatment might come with adverse reactions [28]. Increases in cholesterol levels, and consequently in sex steroids levels, are also linked with urological diseases [29], up to be considered a risk factor in prostatic cancer in men [30] and breast cancer in women [31]. As said before, cholesterol is also the main precursor for vitamin D synthesis. This molecule is essential for calcium homeostasis and for bones proper structure, but it is also linked to hypertension, cancers, metabolic syndrome, autoimmune and infectious diseases [32]. Vitamin D deficiency is considered a worldwide problem affecting countries all over the world [33, 34]. Finally, cholesterol is needed as a component of bile, where it is found solubilized together with bile salt and phospholipids. Here too, an unbalanced cholesterol homeostasis might lead to the formation of cholesterol gallstones, a common disorder in countries with a high total calories diet [35].

### 1.1. Synthesis of cholesterol

As mentioned in the previous paragraph, cholesterol is a very important biomolecule for the human body, as it affects a great number of physiological functions and pathways. It has also been said, how

any disruption in the cholesterol homeostasis might result in a disorder that could affect a different district of the body. For this very reason, cholesterol levels must be regulated in a tight manner, keeping a proper balance between *de novo* biosynthesis, dietary intake and efflux. As very well explained in the article published by Sharpe and Brown in 2013, the biosynthetic pathway that lead to endogenous cholesterol is a very complex one, with more than 20 enzymes and intermediates and different branches that originate by the pathway itself [36]. However, what is more interesting to the aim of this review, is that cholesterol and mevalonate share the first part of their synthetic pathway. Actually, the so called mevalonate pathway starts from Acetyl CoA and leads to lanosterol, branching point for two different pathways to cholesterol (Figure 1). Indeed, mevalonic acid is the fourth intermediate on the way to cholesterol, and it is synthesized by the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). This enzyme, although acting quite early along the pathway, is considered to be the rate-limiting step in the synthesis of cholesterol and isoprenoids and it is physiologically finely regulated at transcriptional, translational, post-translational modification levels, as well as by degradation triggered by sterol and/or non-sterol accumulation. In this way, the over accumulation of a single end-product should be avoided, leading to a major balance between sterol and non-sterol production. This feedback inhibition is also due to the fact that the total amount of cholesterol in the body is a combination between endogenous biosynthesis and exogenous dietary intake [37, 38]. As we will discuss later, the ability of HMGR to regulate the mevalonate/cholesterol pathway has been fundamental for scientists in the discovery, study and development of drugs able to affect the rate of the pathway and, bottom line, the production of endogenous sterols and isoprenoids, amongst other molecules.

## 1.2 Hypercholesterolemia and cholesterol lowering therapies

So far, scientists established that cholesterol exerts various vital functions and it is linked to a certain numbers of disorders. Indeed, an unbalanced amount of blood cholesterol is one of the most common risk factor, for cardiovascular diseases (CVD), including atherosclerosis, stroke or myocardial infarction, especially in the western countries.

In particular, elevated levels of low-density lipoprotein cholesterol (LDL-C), in combination with high plasma triglycerides or low plasma high-density lipoprotein cholesterol (HDL-C), represent an evident risk of mortality due to CVD [39, 40].

The pathological condition characterized by high levels of cholesterol in the blood is called hypercholesterolemia, also known as dyslipidaemia. Elevated levels of this sterol may be a consequence of diet (food rich in saturated fat), obesity, inherited genetic diseases or the presence of other disorders, such as diabetes and nephrotic syndrome [41-43].

The inherited forms of hypercholesterolemia (called familial hypercholesterolemia) occur less frequently but are more severe than cases of non-genetic hypercholesterolemia. The diagnosis is based primarily on the presence of very high circulating cholesterol levels (above 300mg/dl in adults and 250mg/dl in children) in the absence of secondary causes of hypercholesterolemia [44].

The majority of cases of familial hypercholesterolemia are due to defects in three different genes, coding for LDL receptor, apolipoprotein B and proprotein convertase subtilisin/kexin type 9 (although other possible genotypes are not excluded). Since these autosomal mutations affect proteins involved in the clearance of LDL-particles, LDL cholesterol remains in the blood stream, accumulating on the arterial wall (plaque), eventually damaging the tissue and/or blocking the lumen [45].

Although it is an estimated number, basically due to differences in the screening methods between countries, the prevalence of familiar hypercholesterolemia is supposed to be in the order of 1 in every 200, which roughly means 35 million subjects worldwide [46].

Proper control of cholesterol levels is very important in prevention or treatment of atherosclerotic diseases and other CVD. The development of atherosclerotic cardiovascular disease is a rapid process that begins early in life [47]. Since first signs of endothelium damage have been found already in children, the early identification of risk factors and the early treatment are a priority, already in childhood [48].

One of the first therapies aimed at lowering cholesterol levels goes back to the 40s and consisted in a simply low fat/low cholesterol diet. Little was known about endogenous cholesterol metabolism biology, and the general belief was that circulating levels of cholesterol were affected only by dietary intake. However, although the total cholesterol level is a combination of endogenous production and exogenous intake, the dietary therapy brought through a couple of decades only little results, and never striking [49]. Thus, the diet as the only cholesterol lowering therapy was slowly abandoned and more often coupled with a drug therapy.

Therefore, different drugs have also been tested in clinical trials in order to reduce the amount of triglycerides, LDL or cholesterol.

The main compounds used were clofibrate, niacin, both acting on the LDL/HDL ratio, and cholestyramine, a bile acid sequestrant. In the 1975, though, the report published by Coronary Drug Research Product asserted that the use of the first two compounds did not affect total mortality and cause-specific mortality [50].

Meanwhile, in the 50s, the cholesterol biosynthetic pathway had been studied, and HMGR had been shown to be the rate-limiting step of the pathway. In the 70s, Endo and colleagues found that a fungus produced a compound called citrinin, a natural substance able to inhibit HMGR [51]. Following citrinin, other natural and synthetic compounds were discovered: all these molecules fall now under the name of statins.

The statins, being structurally similar to the HMG-CoA, are competitive inhibitor of HMGR and bind to the active site of the enzyme. This competition reduces mevalonate biosynthesis and slows down the subsequent serial steps to produce cholesterol. According to Williams [52], statins have greater affinity for HMGR than HMG-CoA, therefore they are particularly efficient in controlling the cellular cholesterol contents [53].

To date, a number of statins (such as rosuvastatin, atorvastatin, simvastatin, pravastatin or lovastatin) are on commerce, they are among the most sold drugs in USA and Europe and surely are the first choice drug for lowering cholesterol levels in patients suffering from dyslipidaemia (for an extensive review on cholesterol lowering drugs see) [54].

Current treatment guideline regarding the blood lipid control [55] suggests a therapeutic target goal of less than 100 mg/dl for LDL-C in high risk patients.

Depending on dosage and type, statins are able to reduce serum cholesterol by 30-58% [56]. This target is achieved by hepatic upregulation of LDL receptors and increased clearance of serum cholesterol, both triggered by statins mechanism of action. However, most statins required a maximum dosage to reach a noticeable cholesterol reduction, at the expense of high therapy cost and side effects: although considered a relatively safe treatment [57], during these years several adverse effects were noticed, often causing the therapy discontinuation or even the withdrawn of the drug, as happened for cerivastatin in 2001 [58].

The majority of the statin intolerance cases showed muscular pains, which could vary from myalgia to severe rhabdomyolysis [59], increased risk of diabetes [60], liver damage and neuropathy. Although rhabdomyolysis and hepatotoxicity are rare, they can be fatal.

These unwanted adverse effects may be due to the activity of statins in blocking the cholesterol synthesis: by inhibiting an early stage enzyme at the beginning of such complicated pathway, statins affect not only cholesterol levels and all the physiological functions already mentioned, but cause an important reduction of downstream non-sterol products too, in particular the mevalonic acid, coenzyme Q10, heme-A and, subsequently, the isoprenoid intermediates [61]. Isoprenoids, including farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP) and dolichol, are important and critical for the regulation of protein synthesis, glycosylation and post-transcriptional modifications, and mitochondrial respiration. Their depletion leads to an alteration of metabolic pathways and cell growth.

Evidences suggest that statins predispose to mitochondrial defects in a dose-dependent manner, by affecting the production of coenzyme Q10, also known as ubiquinone (component of the mitochondrial respiration chain) and heme-A (involved in the mitochondrial electron transport) and therefore the proper functionality of mitochondria, highly present in muscle tissue [62]. Indeed, the interference with the synthesis of these two mitochondrial elements may play a role in the pathogenesis of statin-induced myopathy, the primary adverse effect that might limit the use of statins [63, 64]. Some *in vitro* studies suggested that this myotoxicity could be alleviated by the addition of farnesol and geranylgeraniol: these results indicate that depletion of isoprenoid metabolites is the primary cause of HMG CoA reductase-induced myotoxicity [65]. Coenzyme Q10 deficiency increases also the risk of heart attack and high blood pressure. Still, coenzyme Q10 protects the body against oxidative stress, a strong predisposing factor for diabetes, metabolic syndromes and CVD.

Regarding the pediatric patients, the first line treatment for familial hypercholesterolemia is the diet, low in cholesterol and saturated fat and high in complex carbohydrates. Bile acid-binding resins (including Cholestyramine and colestipol) have also been found to be effective and safe in childhood, but are poorly tolerated and results unpalatable. Furthermore, they can not be administered in patients with high triglyceride levels and they lead to a decline in serum folate, carotinoid, and 25-hydroxyvitamin D concentrations (multivitamins and folic acid supplementation recommended [66]). The statins, commonly used and highly effective in adults, are not the first choice drug in childhood, for little reliable evidence about their safety. Although the safety is not well established, they are also administered to high-risk children, affected, for example, by familiar hypercholesterolemia (high cholesterol) and familial combined hyperlipidemia (high cholesterol and high triglyceride) [67, 68]. To evaluate the statin outcome in pediatric dyslipidemic patients, different clinical trials were conducted: the studies showed that statins are effective at lowering LDL levels (reduction from baseline: 17% to 50%; more effective than the dietary approach and the use of bile sequestering resins) and are well tolerated, with the common adverse effects representing by the headache, gastrointestinal distress and myalgia [69, 70]. Some of these studies also assessed the children's safe use of statins in terms of height-weight growth, pubertal development and hormone levels. Even though statins seem to be efficacious, safe and well tolerated in children, the short duration of drug application appears strictly limiting to extrapolate efficacy data and long term safety.

## **2. From HMG-CoA inhibitors to squalene synthase inhibitors**

Although statins are the first-line treatment, they resulted enable to achieve a good LDL-C target levels (target goal of less than 100mg/dl [55]) in about half patients due to efficacy limitations, to non-response, to appearance of undesired side effects and to drug-drug interaction, especially when used in combination with other lipid-lowering drugs [71, 72]. For these disadvantages, there is a need for additional lipid-lowering compounds (safe, effective and well tolerated), especially for high-risk patients.

Another class of drug has been developed in order to affect the activity of the squalene synthase (SQS), an enzyme working more downstream in the cholesterol biosynthesis pathway (Figure 1). SQS, localized in the endoplasmic reticulum membrane, is a monomeric protein that can be found in animals, fungi and plants. It catalysed the two-step reaction in which two identical molecules of FPP are converted and dimerized into squalene, with the consumption of NADPH [73]. The first reaction results in the formation of presqualene diphosphate, a stable cyclopropylcarbinyl diphosphate intermediate, while the second reaction involves the heterolysis, isomerization, and reduction of presqualene diphosphate to form squalene.

SQS activity, protein level, and gene transcription are strictly regulated by alterations in cellular cholesterol content, decreasing with cholesterol excess and increasing with cholesterol deficit [74].

Whereas the block of HMG-CoA (e.g. by statins) also interferes with the synthesis of essential non-sterol isoprenoids, squalene (a natural 30-carbon molecule, C<sub>30</sub>H<sub>50</sub>) serves as the exclusive biochemical precursor to the steroids family, included cholesterol (C<sub>27</sub>H<sub>46</sub>O).

The inhibition of SQS causes a direct down-regulation exclusively in the cholesterol production and thus a subsequent fall only in plasma cholesterol levels. SQS inhibitors are believed to have potential advantages over statins, since the blockade at this point may avoid the side effects associated with decrease formation of isoprenilate intermediates and metabolites: hence, these agents are expected to have hypo-lipidemic effects without myotoxicity or hepatotoxicity.

Furthermore, SQS inhibition is less affected by positive regulatory feedback mechanisms than HMG-CoA blockade: SQS inhibitors are less likely to induce an activation of enzyme in the cholesterol pathway, and thus is less likely that they reduce efficacy over time, compared to statins. Indeed, plasma cholesterol, initially reduced by a HMG-CoA reductase inhibitor such as pravastatin, increases again on long-term administration [75].

### **3. Lapaquistat acetate (TAK-475) as lipid-lowering agent**

Among SQS inhibitors (zaragozic acids, dicarboxylic acid and quinuclidine derivatives, 4,1-benzoxazepine as well as substituted morpholine derivatives) there are several members that exhibit different structural profile and therapeutic potential [76].

The most advanced SQS inhibitor is the newly discovered lapaquistat acetate (also known as TAK-475), which has been evaluated in advanced clinical trials: it is the only agent progressed into phase III clinical trials in Europe and the United States [77].

It is developed by Takeda Pharmaceutical Company Ltd (Osaka, Japan) from a series of 4,1-benzoxazepine-3-acetic acid derivatives: it is the most orally bioavailable member in this class [78]. The alkyl group at the position C-1 makes TAK-475 a potent competitive inhibitor compared to the other benzoxazepine compounds [79]. The synthetic route of TAK-475 was reported in a patent application by Takeda [WO-09710224]. *In vivo*, TAK-475 is rapidly absorbed and metabolized into TAK-475 M-I (known as T-91485), the major active deacylated metabolite (Figure 1). Indeed, after oral administration to rats, only this metabolite has been detected in the blood and liver of animals [78].

Different pre-clinical studies have tested the effect of TAK-475 (compared to statins) in several animal species and *in vitro* models [78, 80].

In Wistar rats (characterized by obesity, hyperlipidemia and insulin resistance), lapaquistat acetate, administered orally at different concentrations, showed a dose-dependent inhibition of hepatic cholesterol biosynthesis, measured by the conversion of intravenously injected [2-<sup>14</sup>C] acetate into

cholesterol. Furthermore, it showed lipid-lowering properties in beagle dogs, marmosets (characterized by a lipoprotein profile similar to that of humans) and cynomolgus monkeys: in particular, in all these animal models, TAK-475 lowered plasma non-HDL cholesterol and plasma triglycerides and reduced the hepatic triglyceride secretion rate. Unlike atorvastatin, this SQS inhibitor led also to an increase of plasma HDL cholesterol, known as the "good" cholesterol, because it is positively associated with a decreased risk of coronary heart disease [78].

In monkeys, TAK-475 and atorvastatin exhibited similar hypolipidemic effects, but while the statins led to a marked increase of plasma alanine aminotransferase (ALT) and aspartate transaminase (AST) levels, TAK-475 did not interfere with hepatic transaminase activities, thus demonstrating a lower hepatotoxicity. Same results were obtained by Hiyoshi et al that reported that atorvastatin increased ALT activity in rhesus monkey whereas the SQS inhibitor used in this study (ER-27856) did not affect this hepatic parameter [81].

These data are consistent with clinical observations obtained by a double-blind, randomized study with 826 hypercholesterolemic patients, demonstrating that HMG-CoA reductase inhibitors have been reported to cause elevation of plasma AST and ALT levels [82].

Regarding the *in vitro* experiments, performed by Nishimoto and colleagues, TAK-475 and its metabolite T-91485 were able to significantly reduced the cholesterol synthesis in HepG2 cell (Hepatocellular carcinoma cell line, suitable *in vitro* model for the study of polarized human hepatocytes): both compounds induced a dose-dependent increase of the binding of <sup>125</sup>I-LDL to the LDL receptors, suggesting an increase of LDL receptors expression [78].

Another small study, conducted on Hartley guinea pigs, demonstrated an additional cholesterol lowering action (evaluated by total blood cholesterol levels) of TAK-475 when administered in combination with ezetimibe, an inhibitor of cholesterol absorption in the small intestine [83].

Moreover, when co-administered with statins, it was able to completely prevent the statin-induced myotoxicity, assessed by creatine kinase levels evaluation [84].

All these observations, obtained by pre-clinical studies, suggested that TAK-475 has marked lipid-lowering effects, through inhibition of hepatic triglyceride secretion rate, upregulation of LDL receptors and through the increase of plasma HDL cholesterol content [78]. Since it acts without depleting cellular levels of isoprenoids, its characterization as a drug showing a better safety profile and minor side effects was expected. Summing up, it has accumulated sufficient data and efficacy for its utilization as a therapeutic agent (as monotherapy or adjunctive therapy) for dyslipidemia.

Even though preliminary reports from Phase II and Phase III trials are available, no clinical trial results have been published in peer-review journals yet. Stein et al. summarized the data from lapaquistat clinical program, obtained from 12 different clinical trials (double-blinded, randomized trials) with more than 6000 dyslipidemic patients [85].

In particular, the effect of lapaquistat (50 or 100 mg/day) with or without statin therapy was tested. Compared to placebo, TAK-475 produced a significant decrease in LDL-C levels in a dose-dependent manner; and when administered in combination with statins, the cholesterol is further reduced (about additional 15% of reduction). Interestingly, also an inflammatory marker, the high-sensitivity C reactive protein (hsCRP), was found to be decreased by lapaquistat acetate in a dose-dependent manner, suggesting that changes in lipid levels may modulate the inflammatory cascade.

Surprisingly, the incidence of adverse effects was quite similar for all analysed groups: contrary to expectations, there was no particular advantage in TAK-475 treatment compared to statin therapy.

Moreover, the higher dosage (100 mg) was associated with liver enzyme elevations (ALT and AST) and a rare bilirubin increase, attesting a potential hepatotoxicity. The lower dosage (50mg), instead, did not exhibit any sign of liver damaging compared to placebo, but did not lead to a sufficient LDL-C reduction for commercial viability either. Indeed, 50 mg TAK-475 showed comparable effects to those obtained by other lipid-lowering compounds already present on market [85]. For these issues, the clinical development of lapaquistat for the treatment of hypercholesterolemia was terminated in 2007.

#### **4. Mevalonate Kinase Deficiency (MKD)**

Hyper IgD Syndrome (HIDS) (OMIM ##260920) is an auto-inflammatory syndrome autosomal recessive described for the first time in 1984 [86]. In 1999 two distinct groups described the gene disease, MVK, [87, 88] and identified this syndrome and the already known mevalonic aciduria (MA) (OMIM ##610377) as the mild and severe extremes respectively, of a phenotypic spectrum, the Mevalonate Kinase Deficiency (MKD) [89].

The MVK gene (12q24.11, NM\_000431) encodes for the enzyme mevalonate kinase (MK) (E.C. 2.7.1.36), that catalyses the phosphorylation of mevalonate to produce 5-phosphomevalonate and is the first enzyme following the highly regulated and flux determining enzyme HMG-CoA reductase in the isoprenoid biosynthesis pathway.

To date, MVK mutations associated with MKD are 204 and they are present in almost all exons without a "hot spot area". The replacement c.1129 G> A (V377I) (1129G> A) is present in the majority of HIDS patients, especially in Northern Europe (> 80%) and was shown to be founder effect, then spreading to the rest of Europe and the USA [90]. The V377I was found in homozygosity in a few subjects, it is usually combined with a second heterozygous mutation associated with HIDS phenotype and/or MA [91].

The mutations in the MVK gene lead to a reduction of the catalytic activity enzyme and/or to a reduction of its protein levels. The residual enzyme activity (REA) of MK is associated with the severity of the disease [92].

In patients with HIDS MK mutations involve only a partial impairment of its activity (REA = 7-20%). The febrile episodes occur every 3-6 weeks and last 3-5 days; almost always they are associated with cervical lymphadenopathy and often with important abdominal pain, including vomit and/or diarrhoea. There can also be other symptoms, such as headaches, aphthae, skin rash, myalgia and arthralgia. Fever rises abruptly and often above 40°C, after which the temperature gradually returns to normal.

In case of greatly reduced or even absent enzymatic residual activity of MK (REA <1%) (MA), patients may also display dysmorphic characters, arthritis, retinal degeneration, ataxia, cerebellar atrophy and developmental delay [93-95]. This difference in REA is also been reflected by mevalonic acid levels in plasma and urine: elevated anytime in MA patients, while quite normal outside inflammatory crisis periods in HIDS patients [89, 96].

The MK enzyme activity is mainly regulated at the transcriptional level. MVK promoter contains indeed an element controlled by sterols (SRE), able to induce gene transcription as a result of a deficit of the cholesterol biosynthesis products. It has been demonstrated that following treatment with compounds that inhibit this biosynthetic pathway (as statins) enzyme activity increases by 3-6 times [97].

MK, along with the most known HMG-CoA synthase and HMG-CoA reductase (HMGR), is involved in the regulation of the biosynthesis of cholesterol and non-sterol compounds, and represents a secondary control point. The farnesyl pyrophosphate (FPP), the geranyl pyrophosphate (GPP) and the geranylgeranyl pyrophosphate (GGPP) to MK downstream products are able to inhibit MK [97]. Clinical onset of MKD disease typically starts within the first years of life, sometimes immediately following childhood vaccinations, a minor trauma or stress, but the inflammatory episodes tend to become less frequent and less severe with growth. In the period between two inflammatory crisis

patients are well and grow normally. Laboratory tests show leucocytosis, increased inflammatory markers, and, often high levels of IgD, although it is not sure whether this last aspect, which for another syndrome gets its name, is only an epiphenomenon or is actually involved in the pathogenesis of the disease [98-100].

The link between the metabolic defect caused by mutations in MK gene and the periodic inflammatory phenotype observed in patients MKD is not fully understood yet.

It was first shown that the decrease of the intermediate isoprenoid levels, rather than the accumulation of mevalonate upstream MK, induces a high secretion of the pro-inflammatory cytokine IL-1 $\beta$  [101]. This finding is supported by previous observations indicating how isoprenoid are highly required in case of inflammatory processes. For example, the administration of LPS, TNF- $\alpha$  or IL-1 $\beta$  in guinea pig produces rapid up-regulation of hepatic HMGR and a down-regulation of squalene synthase (SQS), the first specific enzyme of cholesterol synthesis [102-104]. In addition, statins, a class of drugs that act by inhibiting the HMGR, and then lowering the intermediate levels of isoprenoid and cholesterol, may have pro-inflammatory effects or anti-inflammatory, depending on the experimental context [105-107].

In patients with MKD, reduced MK activity could result in a decrease of isoprenoid compounds, especially those with a high turn-over:

- ubiquinone-10, which shows lowered plasma level in MA patients [108];
- prenylated RAS proteins, which are involved in many cellular processes such as signal transduction and cytoskeleton organization [109-111];
- guanylate-binding prenylated proteins synthesized in response to IFN- $\gamma$  and LPS [112], and somehow associated with activation of the inflammation.

Recently, it has been demonstrated that the blockade of the mevalonate pathway and the decrease of isoprenoids induces high secretion of IL-1 $\beta$  through self-processing of caspase-1. A mechanism that would also explain the increased production of IL-18 (another substrate of caspase-1) and perhaps IL-33, which could have a role in some phenotypic aspects, such as skin erythema, by activating Th2 [101, 109].

The increase of HMGR activity observed especially in leukocytes of MA patients is not the pathogenic event, but rather a positive feedback induced by shortage of products in the enzyme valley and would explain the increased mevalonate plasma levels found in MKD patients. Indeed, the therapeutic attempts with statins, initially considered to be potentially pathogenic and pejorative, as they are considered to trigger the onset of severe episodes of inflammation, worsening the shortage of intermediate isoprenoid levels [113]. Finally, the cyclical nature of the inflammatory episodes, typical of the syndrome, would be adduced to a higher temperature sensitivity showed by the mutated MK. As a result of physical exertion or infection (even trivial), situations in which the body temperature increases, the REA of mutated MK would drop. This even more decreased activity would lead to an increase of the metabolic block and a decrease in the isoprenoid production, eventually resulting in inflammation stimulation. At the same time, the positive feedback triggered by isoprenoid shortage would increase the activity of HMGR. A highly active HMGR and an inactive MK would cause an accumulation of mevalonate, which would eventually somehow restore MK activity and subsequently the levels of downstream compounds, the other would build up in plasma and urine [114].

There is currently no efficacious treatment available for MK deficiency [115, 116]. In individual HIDS cases, clinical improvement has been reported due to treatment with non-steroidal anti-inflammatory drugs, corticosteroid, colchicine, or cyclosporine. In the majority of patients however, these treatments do not have beneficial effects. In a small group of HIDS patients, treatment with the HMG-CoA reductase inhibitor simvastatine had a minor positive effect on the number of days of illness [113], but treatment with similar statins in MA patients led to severe worsening of the clinical symptoms [100]. Also treatment of HIDS patients with etanercept, a soluble p75 TNF alpha receptor-Fc fusion protein, or with anakinra, a recombinant form of IL-1-receptor antagonist, showed inconsistent results [117].

## 5. Repositioning of TAK-475 on MKD

The results obtained *in vitro* studies have demonstrated that the zaragozic acids (ZAs), inhibitors of squalene synthase, has been shown effective in counteracting the effects of a defect of isoprenoid derivatives by acid mevalonic [118].

ZAs is a family of fungal metabolites containing a novel 4,6,7-trihydroxy-2,8-dioxobicyclo[3.2.1]octane-3,4,5-tricarboxylic acid core, produced by fungi. The first three ZAs isolated and characterized were named simply A, B, and C was isolated from fungal culture, *Sporormiella intermedia*, and *L. elatius*, respectively [119]. *In vitro* experiments zaragozic acid A, as well as its two analogs B and C, was reported to be potent competitive inhibitors of cholesterol synthesis in Hep G2 cells. In the same way in the mouse model, zaragozic acid A was found to be an inhibitor of acute hepatic cholesterol synthesis accompanied by an accumulation of organic acids. In summary, these data showed the zaragozic acids were a new class of therapeutic agents with potential for the hypercholesterolemia treatment [118].

The efficacy of zaragozic acid is probably related to a relative accumulation of geranylgeraniol due both to block its conversion into squalene, both to a mevalonate kinase enzyme induction. Preliminary studies also suggest that this action could also help to protect neuronal cells toxic effect that occurs in the isoprenoid deficient conditions.

Taken together, these data suggest that TAK-475, characterized by the same mechanism of action of zaragozic acid, may constitute a drug "orphan" for patients with MKD.

It would be recommended in MKD patients the administration of TAK-475 in association with additional coenzyme Q10 supplementation (as already suggested in MA, in which coenzyme Q10 is administered with vitamin D and E), to reduce the potential adverse effects, especially myopathy, due to the use of TAK-475.

Repurposed drugs, as TAK-475, have the advantage of decreased development costs and reduced time to launch due to previously collected pharmacokinetic, toxicology and safety data [120, 121].

This process of developing new indications for existing drugs as TAK-475 have a pleiotropic role because perturbing multiple biological entities, (on and off-targets) themselves involved in various biological processes .

TAK-475 acts, as described, downstream of statins and allows the production of intermediates of the pathway of the cholesterol (geranylgeranylation) that with statins would be blocked. Following this hypothesis, TAK-475 has conducted preclinical studies in rats and dogs for MKD showed very promising results [122]. To date, there are no data on TAK-475 effects in pediatric patients.

The study of drugs that act on the mevalonate pathway has allowed scheduling control mechanisms that go beyond the cholesterol production.

To date there is an absolute contiguity between different pathogenetic autoinflammatory diseases; it is, therefore, possible that treatment with TAK-475 may also is useful for other autoinflammatory syndromes for which do not exist today completely effective therapies.

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