

## REVIEW

# Geranylgeranyl diphosphate synthase: Role in human health, disease and potential therapeutic target

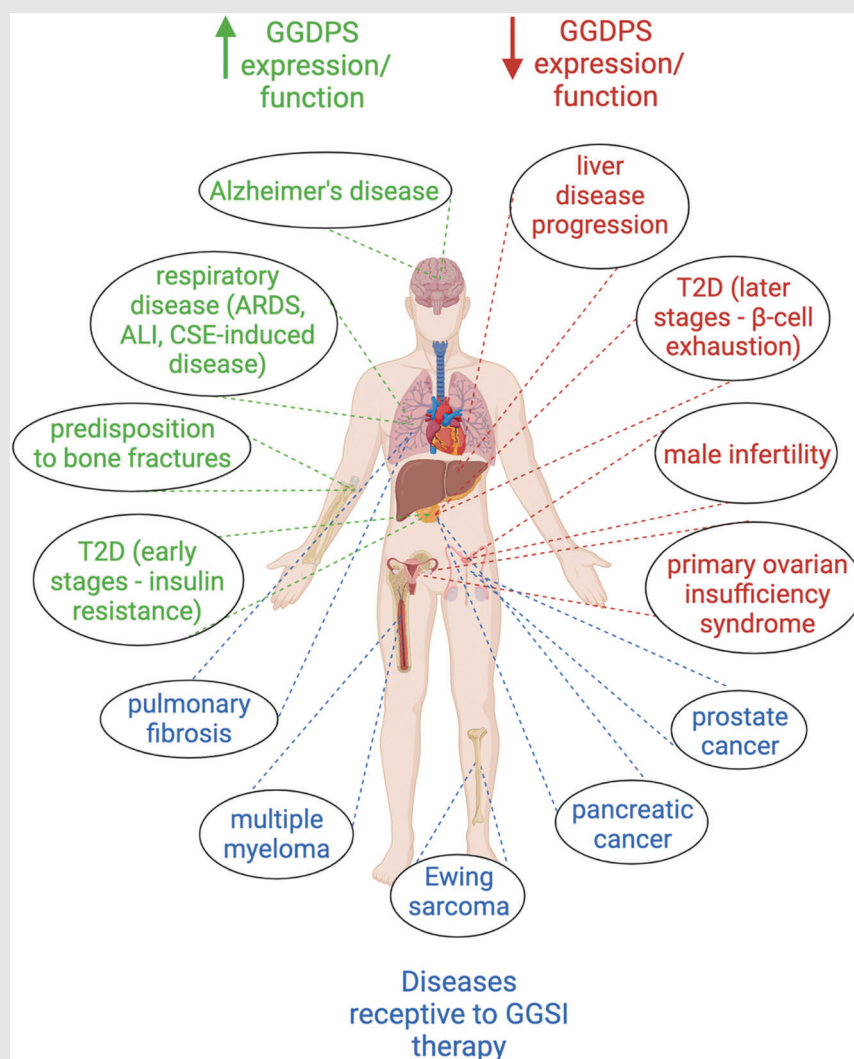
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**Graphical Abstract**

- Geranylgeranyl diphosphate synthase (GGDPS), an enzyme in the isoprenoid biosynthesis pathway, is responsible for the production of geranylgeranyl pyrophosphate (GGPP) used for protein geranylgeranylation.
- Dysregulation of GGDPS expression and/or activity is implicated in the pathophysiology of type 2 diabetes, liver disease, pulmonary disease and several malignancies.
- GGDPS inhibitors have promising efficacy in preclinical models of malignancy and pulmonary fibrosis.

## REVIEW

# Geranylgeranyl diphosphate synthase: Role in human health, disease and potential therapeutic target

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**Abstract**

Geranylgeranyl diphosphate synthase (GGDPS), an enzyme in the isoprenoid biosynthesis pathway, is responsible for the production of geranylgeranyl pyrophosphate (GGPP). GGPP serves as a substrate for the post-translational modification (geranylgeranylation) of proteins, including those belonging to the Ras superfamily of small GTPases. These proteins play key roles in signalling pathways, cytoskeletal regulation and intracellular transport, and in the absence of the prenylation modification, cannot properly localise and function. Aberrant expression of GGDPS has been implicated in various human pathologies, including liver disease, type 2 diabetes, pulmonary disease and malignancy. Thus, this enzyme is of particular interest from a therapeutic perspective. Here, we review the physiological function of GGDPS as well as its role in pathophysiological processes. We discuss the current GGDPS inhibitors under development and the therapeutic implications of targeting this enzyme.

**KEYWORDS**

geranylgeranyl diphosphate synthase, geranylgeranylation, isoprenoid biosynthesis pathway, small GTPases

## 1 | INTRODUCTION

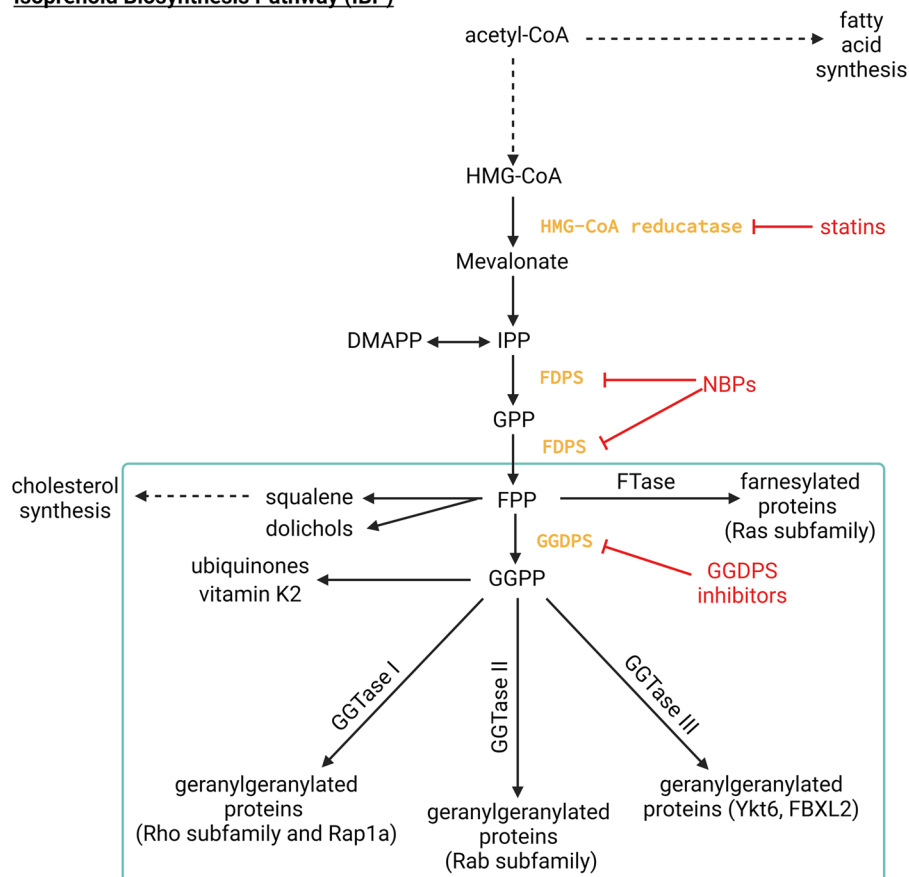
Geranylgeranyl diphosphate synthase (GGDPS) is an essential enzyme in the isoprenoid biosynthesis pathway (IBP) (Figure 1). It facilitates the production of the 20-carbon isoprenoid geranylgeranyl pyrophosphate (GGPP), which acts as a substrate for the post-translational modification of proteins (geranylgeranylation) as well as a precursor of vitamin K2 and ubiquinone. Protein prenylation enables proper localisation, and thus function, of proteins that play key roles in signalling pathways, cytoskeletal regulation and intracellular transport. While other reviews have focused on topics such as inhibitors of

the IBP and protein prenylation, less attention has been paid to the key roles of GGDPS in human health and disease and the implications this has for the implementation of novel therapeutic strategies.

The present review provides a comprehensive overview and synthesis of the current literature examining the role of GGDPS in human disease. This review takes a mechanistic approach, exploring the role of GGDPS in modulating disease processes such as insulin resistance and development of type 2 diabetes (T2D), liver disease, pulmonary disease and others. Also explored are the pharmacological agents that impact GGDPS activity, as these agents have not only provided insight into the sequelae of

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**Isoprenoid Biosynthesis Pathway (IBP)**

**FIGURE 1** The mammalian isoprenoid biosynthesis pathway. Inhibitors (shown in red) include statins used most commonly for treatment of hypercholesterolemia, nitrogenous bisphosphonates (NBPs) used for treatment of various bone diseases and GGDPs inhibitors which have not yet been approved for clinical use.

disrupting GGDPs activity, but also have potential therapeutic relevance for a variety of malignancies and other human diseases.

## 2 | THE IBP

The IBP, also referred to as the mevalonate pathway, is responsible for the production of all mammalian isoprenoids (Figure 1). The pathway begins in the endoplasmic reticulum (ER) with the rate-limiting conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate via the enzyme HMG-CoA reductase (HMGCR). Through further phosphorylation and decarboxylation, mevalonate is converted to isopentenyl pyrophosphate (IPP). IPP and its isomer dimethylallyl pyrophosphate (DMAPP) then undergo subsequent condensation reactions via farnesyl diphosphate synthase (FDPS) to form first the 10-carbon geranyl pyrophosphate (GPP) and then the 15-carbon farnesyl pyrophosphate (FPP). FPP can then be utilised in the ER for sterol and

dolichol synthesis. In the cytosol, the enzyme GGDPs utilises FPP along with IPP to catalyse the formation of the 20-carbon GGPP. GGPP can also be used for other purposes, such as the synthesis of vitamin K2 and ubiquinone which is utilised during oxidative phosphorylation.<sup>1,2</sup> Finally, following production of mammalian isoprenoids FPP and GGPP, prenyltransferases, such as farnesyl transferase (FTase) and geranylgeranyl transferases (GGTase I, GGTase II and GGTase III), catalyse the addition of the isoprenoids to target proteins.<sup>3-5</sup> This post-translational modification is known as protein prenylation.

Prenyltransferases catalyse the addition of isoprenoids FPP or GGPP to a cysteine residue near the C-terminus of the target substrate. Substrates for prenylation include Ras, Rho and Rab families of small GTPases, nuclear lamins, as well as certain kinases and phosphatases. It is the motif encompassing the C-terminal cysteine residue that confers substrate specificity for prenyltransferase enzymes.<sup>6</sup> For FTase and GGTase I, the cysteine is found in the consensus sequence referred to as the CAAX box, in which case the C refers to the Cys residue while A represents an aliphatic

residue. Variation amongst the identity of the X residue is what confers specificity for either FTase or GGTase I: FTase targets substrates with a Met, Ser, Gln, Ala or Cys while GGTase I targets those with a Leu or Glu.<sup>7</sup> However, these enzymes are not mutually exclusive with regards to substrate specificity. K-Ras and N-Ras, which are usually farnesylated, can be geranylgeranylated by GGTase I when FTase is inhibited.<sup>8,9</sup> Similarly, RhoB has been identified as a substrate of both FTase and GGTase I.<sup>7</sup> This crossover may be due to the fact that FTase and GGTase I share a common  $\alpha$ -subunit.<sup>10</sup>

GGTase II, also referred to as Rab GGTase because of its specificity for small GTPases of the Rab sub-family, targets a different recognition motif including XXCC, XCXC, CCXX, CCXXX or CXXX with the X residue varying depending on the substrate. Similar to FTase and GGTase I, the enzyme is a heterodimer. However, its  $\alpha$ -subunit only has 27% identity with that of the FTase/GGTase I  $\alpha$ -subunit, while the  $\beta$ -subunit shows 29% identity to FTase.<sup>11</sup> GGTase II also differs from FTase/GGTase I in that it requires the assistance of the Rab escort protein (REP1) which recruits substrate proteins to the enzyme, binds to GGTase II and facilitates the trafficking of the Rab proteins post-prenylation.<sup>12,13</sup> In addition, GGTase II substrate targets are often doubly geranylgeranylated.

GGTase III is a recently discovered prenyltransferase enzyme that catalyses the double prenylation of the ubiquitin ligase FBXL2, as well as Golgi SNARE<sup>14</sup> protein Ykt6 in combination with FTase.<sup>5,15</sup> Similar to GGTase II, GGTase III requires a chaperone protein (SKP1) for geranylgeranylation. It has been reported to share an identical  $\beta$ -subunit with GGTase II but has a distinct  $\alpha$ -subunit.

Discovering the identity and function of prenylated proteins remains an active area of investigation. Use of GGPP probes and alkynyl C15 pyrophosphate derivatives through metabolic labelling have enabled identification of substrates of the prenylome.<sup>6</sup> One study discovered 80 substrates, 64 of which were identified for the first time at an endogenous expression level.<sup>16</sup> Further clarification of the prenylome will permit identification of potential targets of therapies that disrupt protein prenylation.

### 3 | GGDPS STRUCTURE

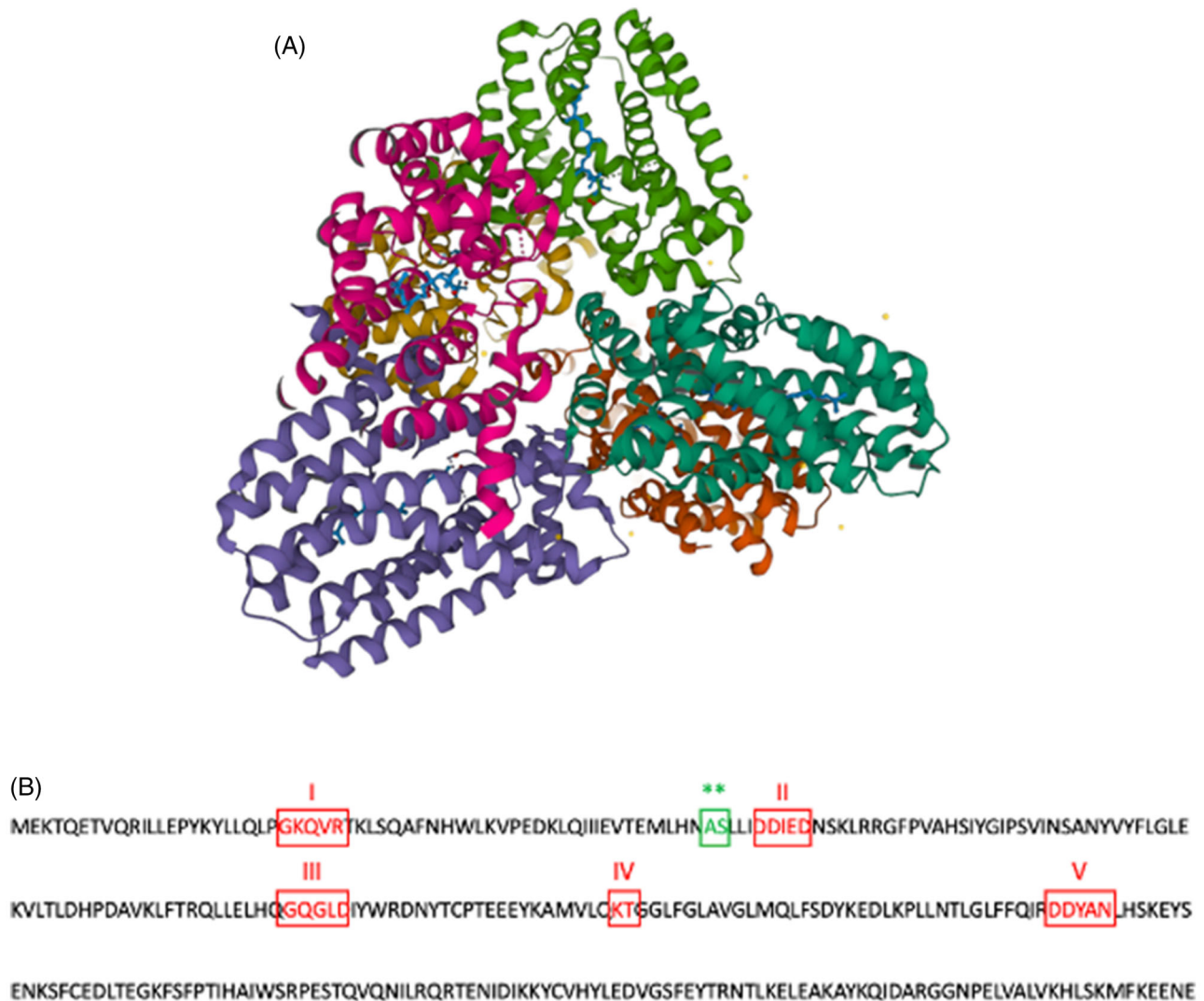
Human GGDPS was first characterised by Kavanagh et al. in 2006.<sup>17</sup> Characterisation of its paralogue FDPS as well as orthologues in species such as *S. cerevisiae*, *T. thermophilus*, *P. horikoshii* and *B. taurus*, have provided further structure-function information. Kavanagh et al. reported that GGDPS is a complex homohexamer made up of three alpha-helical dimers forming a three-blade propeller-like structure (Figure 2).<sup>17</sup> They found each of the six

monomers to associate with two  $Mg^{2+}$  ions and one GGPP molecule. However, more recent studies suggested that three  $Mg^{2+}$  are required for substrate binding.<sup>18</sup> Such discrepancies are most likely due to the low resolution at which the enzyme was characterised.<sup>18,19</sup> Miyagi et al. also reported GGDPS to form an octamer in its active form, proving the need for more information on the quaternary structure of this enzyme.<sup>20</sup> Interestingly, sequence analysis found that this complex quaternary structure is specific to mammalian and insect GGDPS.<sup>17</sup> Other plant, fungal, archaeal and bacterial orthologues lack the conserved residues that form the enzyme's inter-dimer region.<sup>17</sup> This is because in eukaryotes, GGPP production follows the addition of 5-carbon IPP to FPP while the plant, fungal, archaeal and bacterial orthologues catalyse the condensation reaction of three IPP substrates to the allyl head of DMAPP.<sup>21,22</sup>

With different quaternary structures and only 17% sequence identity, FDPS and GGDPS have significantly similar tertiary structures.<sup>17,23</sup> Both enzymes contain five conserved regions (I–V) maintained amongst all transprenyltransferases (Figure 2). Regions II and V are made up of aspartate-rich motifs (DDXXD/N) involved in ligation of  $Mg^{2+}$  ions and association with the pyrophosphate on the allylic substrate. Region III (GQXXD) contains a Gln185 which also facilitates this process by providing a polar contact for the allylic tail and  $Mg^{2+}$  ions. Region I (GKXXR) contains basic residues, specifically Arg28, His57, Arg73 and Arg74, responsible for pyrophosphate binding of IPP in the homoallylic subpocket. Region IV (KT) provides a Thr152 and Lys151 that stabilise the carbocation intermediate.

Conservation of these key residues may explain the near-identical catalytic mechanism of both FDPS and GGDPS. This mechanism is proposed to be a three-step ionisation–condensation–elimination reaction.<sup>24</sup> First, the enzyme binds IPP and the allylic substrate in their respective pockets. Then, the allylic carbocation is formed by removal of the IPP tail, facilitated by the three  $Mg^{2+}$  ions bound in the catalytic cavity. The C1 carbon on the carbocation electrophilically attacks the C4 carbon on the IPP substrate forming a C–C bond. The final product is formed from stereospecific elimination of a proton.

Variation between these two isozymes arises when comparing the hydrophobic channel for the allylic substrate isoprenyl tail. FDPS contains two capping phenyl residues in this cavity (Phe89/99) which are expected to limit chain length (Figure 2). GGDPS instead contains Ala59 and Ser60 which allows for the larger C20 GGPP product.<sup>17</sup> Interestingly, GGDPS has a second hydrophobic cavity below its active site. Kavanagh et al. determined this second site to represent an inhibitory binding site upon realisation that the aliphatic GGPP product tail does not extend into the



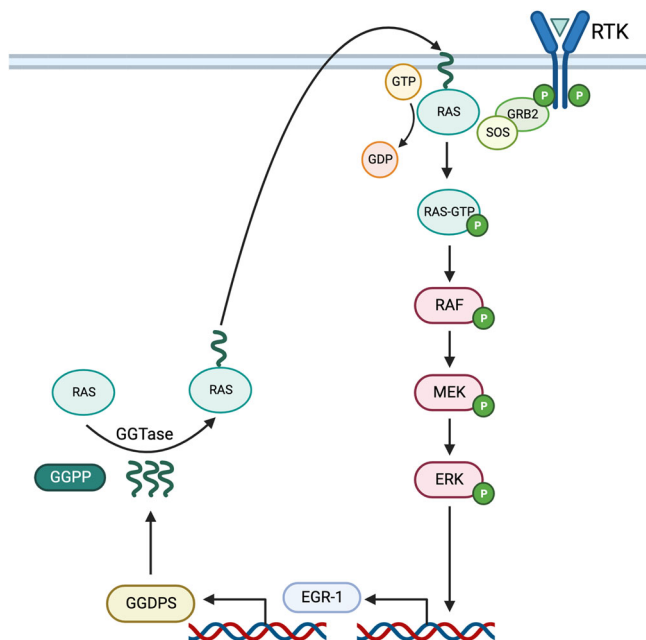
**FIGURE 2** Human GGDPs. (A) Protein structure of human GGDPs with bound GGPP (blue), water molecules (yellow) and  $Mg^{2+}$  ions (red). (B) Amino acid sequence for human GGDPs. Conserved sequence motifs are shown in red and labelled (I–V). Residues unique to GGDPs (not present in FDPS) that allow for chain length elongation are shown in green. Images created from the RCSB Protein Data Bank ([www.rcsb.org](http://www.rcsb.org)) of PDB I<https://doi.org/10.2210/pdb2Q80/pdb> (Kavanagh et al.<sup>17</sup>).

former elongation site, but instead into this second binding cavity. This GGPP molecule was found to bind the aspartate motifs in the allylic site rather than the basic residues in the homoallylic site, suggesting an inhibitory feedback mechanism for product regulation. This was confirmed by crystallography studies showing a GGPP analogue, 3-azaGGPP, acting as a competitive inhibitor with respect to FPP.<sup>25</sup>

#### 4 | GGDPS REGULATION

Regulation of GGDPs activity primarily stems from product inhibition.<sup>17,26–28</sup> As mentioned earlier, GGDPs has an inhibitory binding site which binds GGPP and ren-

ders the enzyme inactive. This creates a negative feedback loop to maintain homeostatic GGPP levels. Recent studies have also implicated regulation of the enzyme at the transcriptional level. Activation of the extracellular signal-regulated kinase (ERK) pathway initiates translocation of transcription factor early growth response gene 1 (EGR1) which promotes expression of GGDPs as well as expression of other cholesterol biosynthesis enzymes such as HMGCR<sup>29–33</sup> (Figure 3). Activation of the ERK pathway and subsequent EGR1-initiated GGDPs expression can occur in response to changes in insulin levels, consistent with the insulin-dependent induction of cholesterol biosynthesis that is found in the liver. Recent evidence also suggests a role of EGR2 in regulation of GGDPs expression.<sup>34</sup> Transcriptional regulation of GGDPs is also



**FIGURE 3** Ras/ERK/EGR1 pathway regulation of GGDPS gene expression. GGDPS has recently been identified as a target gene for EGR1. Activation of the MAPK signalling pathway initiates EGR1 expression and GGDPS expression. GGDPS expression allows for production of GGPP, the substrate utilised by GGTases for geranylgeranylation of target proteins (ex: K-Ras). Prenylation of K-Ras promotes Ras membrane localisation allowing for the reactivation of the ERK pathway in the presence of insulin or other pathway activators, creating a positive feedback loop. Note: K-Ras is usually farnesylated by FTase, however in regard to this pathway promoting GGDPS expression, it was shown to be primarily geranylgeranylated by GGTase I.

supported by the finding of two different GGDPS mRNAs in 16 different human tissues, as well as several partial cDNA sequences, suggesting the existence of additional mRNAs.<sup>23,35</sup> The two identified mRNAs were most abundant in the heart, skeletal muscle and testis and the shorter mRNA was the major species out of the two.<sup>23</sup> However, the functional consequences of the different mRNAs have not yet been delineated.

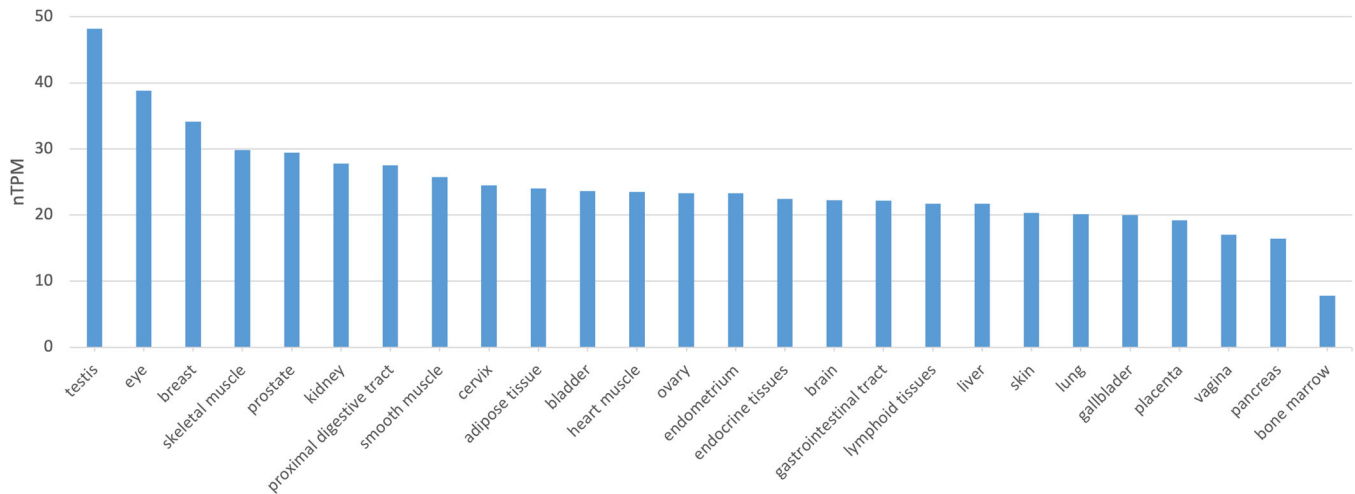
Regulation of cholesterol biosynthesis enzymes, specifically HMGCR, result from binding of sterol regulatory binding element (SREBP) transcription factors.<sup>36–39</sup> SREBPs operate in a negative feedback loop which, when cholesterol levels are high, leads to the degradation of HMGCR.<sup>40</sup> A SRE has not been identified in the GGPS1 gene, which encodes GGDPS, suggesting GGDPS expression is not dependent on SREBPs. This is supported by the finding that while sterol accumulation leads to decreased mRNA expression of enzymes such as HMGCR and FDPS, GGDPS mRNA levels are unaffected by changes in cellular sterol levels.<sup>35</sup>

## 5 | GGDPS EXPRESSION AND KNOWN MUTATIONS

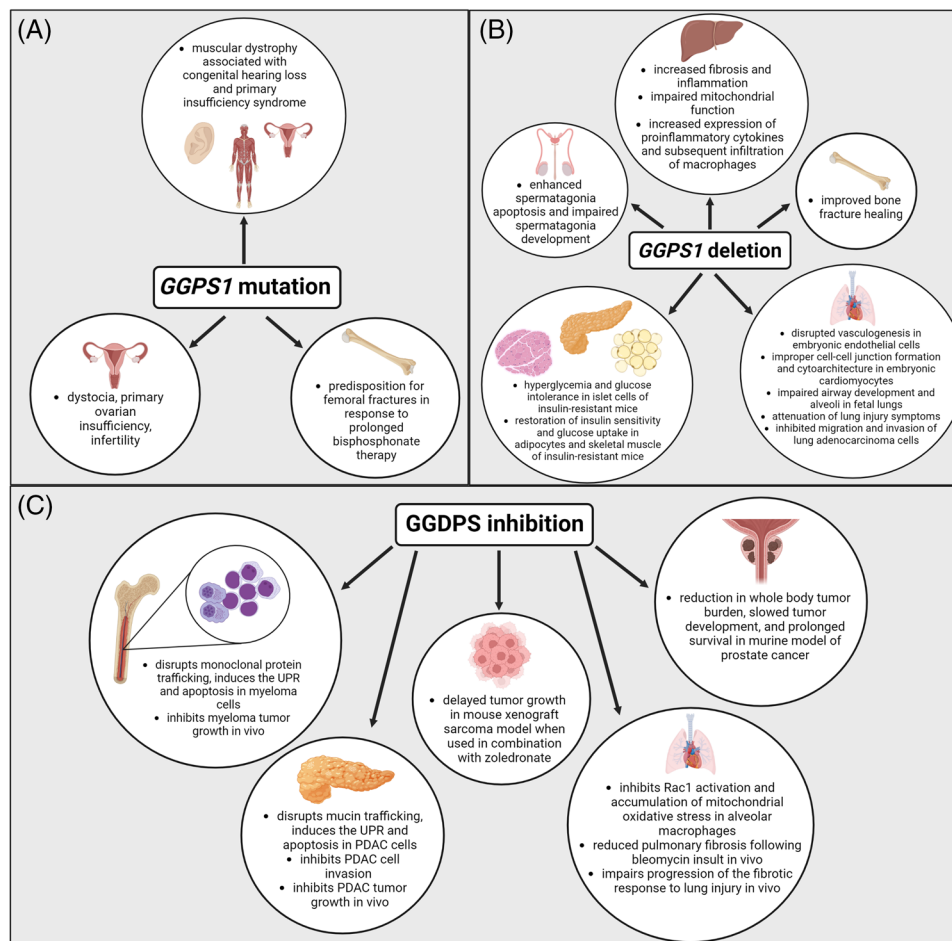
Due to GGDPS's role in isoprenoid biosynthesis and metabolism, it is ubiquitously expressed in all tissues (Figure 4). Based on data from the Human Protein Atlas, *GGPS1* expression is highest in the testis which may be related to the essential role of GGDPS in spermatogenesis.<sup>35,41,42</sup> Expression levels also appear elevated in the eye, skeletal muscle and breast tissue, suggesting that GGDPS may play an important role in these tissues as well. While previous studies have found elevated levels of geranylgeranylated proteins in the human heart, brain, skeletal muscle and testis, variation between geranylgeranylated protein levels and *GGPS1* tissue expression may indicate that expression of the synthase does not necessarily correlate with levels of geranylgeranylated proteins.<sup>23</sup>

Recent studies have shown a mutation in the *GGPS1* gene was associated with a unique case of muscular dystrophy associated with congenital hearing loss and primary ovarian insufficiency (Figure 5A).<sup>43</sup> Multiple instances of biallelic missense mutations were found to cause this syndrome.<sup>43,44</sup> Functional assays revealed that the activity of the mutated GGDPS was only moderately impaired (~50%), which would not be expected to elicit a phenotype. Because of this, the investigators hypothesised that the described phenotype was perhaps a consequence of a more subtle change in GGDPS function, such as affecting subcellular localisation of the enzyme for cell-type specific processes.<sup>43</sup> Interestingly, attempts to knock-in this mutation resulted in embryonic lethality, potentially through underdevelopment of the placental/embryonic vascular unit.<sup>43</sup> This finding is consistent with the observation that *GGPS1* is essential for folliculogenesis and oocyte maturation, as *GGPS1* deficiency in oocytes impacts female fertility in a stage-specific manner.<sup>45</sup> It is theorised that *GGPS1* deficiency or single nucleotide polymorphisms (SNPs) in somatic cells of the uterus may be responsible for dystocia, or difficulty during the delivery process. However, this has not been confirmed since most females with *GGPS1* mutations suffer from primary ovarian insufficiency and infertility.<sup>46</sup> Decreased *GGPS1* expression in the testis has also been associated with infertility in men, suggesting a role for this enzyme in both male and female reproductive organs.<sup>41,42,47,48</sup>

*GGPS1* SNPs have also been identified in connection with bone remodelling. Multiple studies have found various *GGPS1* mutations to contribute to predisposition of femoral fractures in response to prolonged bisphosphonate therapy.<sup>49–52</sup> The mechanism underlying this predisposition is not fully understood, but data suggest the fractures may result from the combination of impaired FDPS



**FIGURE 4** GGPS1 expression across tissues. Expression values are shown in nTPM (normalised transcripts per million) created by combining the HPA and GTEx transcriptomics datasets using the Human Protein Atlas normalisation pipeline. Data were obtained from the Human Protein Atlas (<https://www.proteinatlas.org/ENSG00000152904-GGPS1/tissue>).



**FIGURE 5** Pathophysiological processes and disease states influenced by GGPS1 mutations, tissue-specific GGPS1 deletions and GGPS1 inhibitors. (A) GGPS1 mutations have been associated with a unique syndrome of muscular dystrophy associated with congenital hearing loss and primary ovarian insufficiency. GGPS1 SNPs have also been associated with bone disease and theorised to cause dystocia and infertility issues in women. (B) Tissue-specific KO models for spermatogenesis, cardiovascular and thoracic development, lung injury, insulin-resistance, liver disease and bone health have uncovered possible roles of GGPS1 in disease. (C) GGPS1 inhibitors show potential benefit in models of multiple myeloma, pancreatic ductal adenocarcinoma (PDAC), sarcoma, pulmonary fibrosis and prostate cancer.

activity (secondary to the bisphosphonate) and GGDPS activity (secondary to the mutation).<sup>51</sup>

## 6 | KNOCKOUT MODELS OF GGDPS

Various knockout models have elucidated a critical role of GGDPS in embryonic development (Figure 5B). A knock-in mouse model was generated to study the effect of the Y259C missense mutation known to contribute to muscular dystrophy associated with congenital hearing loss and primary ovarian insufficiency which was mentioned above.<sup>43</sup> This mutation led to embryonic lethality due to improper embryonic vascularisation and cell–cell junction formation. A similar effect resulted from *GGPSI* deletion in mouse embryonic cardiomyocytes. The enzyme proved to be essential for proper cardiac cytoarchitecture and cell–cell junction formation.<sup>53</sup> Similarly, knockout of GGDPS in mouse embryonic endothelial cells disrupted vasculogenesis causing embryonic lethality, while deletion of the gene in foetal lungs was shown to impair proper development of airways and alveoli.<sup>54</sup>

The role of GGDPS in fertility has also been explored using knock-out models. Loss of GGDPS in Sertoli cells was found to enhance spermatogonia apoptosis and block further spermatogonia development.<sup>47</sup> Deletion in myometrial cells was found to impair uterine contractions resulting in dystocia and disrupted embryonic placing, while deletion in oocytes was associated with ovarian dysfunction and infertility.<sup>44,45</sup>

Connections between GGDPS and non-alcoholic fatty liver disease (NAFLD) have been identified. Liver-specific deletion of GGDPS was found to reprogram hepatic metabolism toward glycolysis, resulting in fibrosis and inflammation.<sup>55</sup> In addition, liver-specific GGDPS deletion impaired mitochondrial function by disrupting modification of essential mitochondrial proteins such as Rab7, which is important for mitophagy and mitochondrial fission, further contributing to conversion toward a glycolytic phenotype and liver fibrosis.<sup>55</sup> GGPP also acts as a precursor for coenzyme Q (CoQ) which functions as an electron transporter in the mitochondrial electron transport chain. Therefore, loss of GGDPS and depletion of GGPP may disrupt CoQ synthesis and oxidative phosphorylation.

GGDPS has also been implicated in T2D through an EGR1/GGDPS/Ras/ERK1/2/IRS-1 pathway-dependent manner.<sup>31</sup> Knockout of GGDPS in the pancreatic  $\beta$ -cells of insulin-resistant mice resulted in hyperglycaemia and glucose intolerance due to  $\beta$ -cell dysfunction.<sup>56</sup> However, deletion of GGDPS in the adipocytes and skeletal muscle of insulin-resistant mice was found to restore insulin sensitivity and glucose uptake.<sup>31,57</sup> Thus, it is evident that there is a complex and tissue-specific rela-

tionship between GGDPS and T2D that requires further investigation.

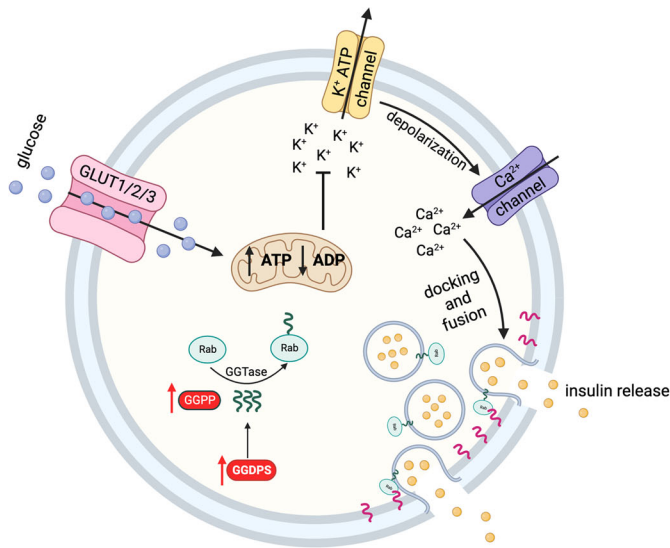
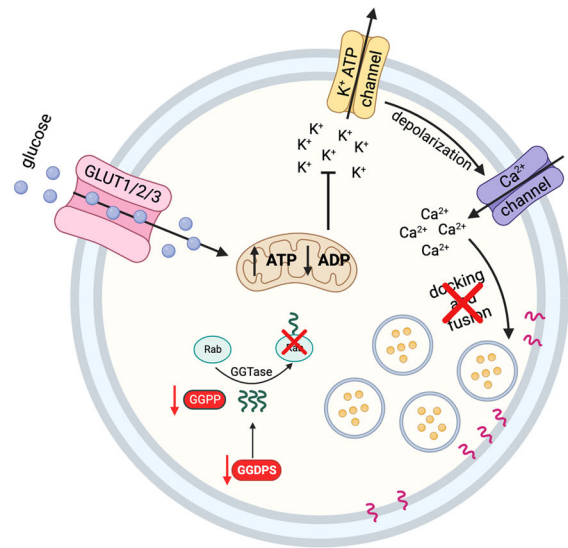
Other GGDPS knockout models have focused on ventilator-induced lung injury and acute lung injury (ALI). In both mouse models, lung-specific deletion of *GGPSI* attenuated disease symptoms.<sup>58–60</sup> Deletion of *GGPSI* reduced Rab10 membrane localisation which led to decreased activation of TLR4–NF- $\kappa$ B signaling.<sup>58</sup> NF- $\kappa$ B is a necessary transcription factor for NLRP3 activation, therefore by inhibiting this pathway, NLRP3 inflammatory transcription was inhibited, attenuating disease-induced lung inflammation.<sup>58</sup> Further studies found that inhibition of NLRP3 inflammasome by GGDPS inhibition resulted in promotion of autophagy, allowing for attenuation of sepsis-induced lung injury.<sup>59</sup> Similarly, knockdown of the enzyme also inhibited migration and invasion of lung adenocarcinoma cancer cells.<sup>61</sup>

Finally, in a mouse model evaluating GGDPS in the context of bone fractures, it was identified that loss of the enzyme improved the fracture healing process.<sup>62</sup> This was due to activation of the Bmp2/Smad-dependent Runx2 pathway. Bmp2 is essential for initiation of the fracture healing process by regulating expression of transcription factor Runx2 which can then initiate expression of genes for terminal differentiation of chondrocytes. Bmp2 initiates the phosphorylation of various Smad proteins such as Smad1/5/8, which initiates terminal differentiation of chondrocytes, or Smad2/3, which inhibits terminal differentiation. *GGPSI* conditional knockdown mice were found to have diminished TGF- $\beta$  signalling within the first 14 days post-fracture. Lack of GGDPS inhibited the Ras/ERK/EGFR pathway leading to decreased expression of TGF- $\beta$ , allowing for terminal differentiation of chondrocytes and accelerated fracture healing. However, Bmp2 signalling was up-regulated in *GGPSI* KO mice within the first 7 days post-fracture leading to increased Smad1/5/8 phosphorylation and Runx2 expression. In addition, it was theorised that lack of TGF- $\beta$  signalling reduced Smad2/3 expression allowing for decreased inhibition of Runx2. The investigators also reported a significant increase in vascular endothelial growth factor A (Vegfa) expression at 7- and 21-days post-fracture, indicating that deletion of *GGPSI* increased vasculogenesis further accelerating the fracture healing process.

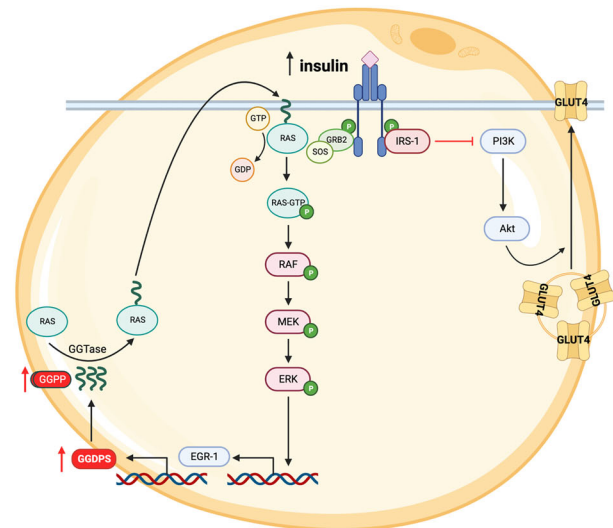
## 7 | GGDPS IN DISEASE STATES

Overexpression of GGDPS has been associated with various clinical pathologies. A positive correlation was found between neurofibrillary tangle (NFT) density, p-Tau levels and mRNA prevalence of both GGDPS and FDPS in the brains of Alzheimer's disease (AD) patients.<sup>63</sup> With

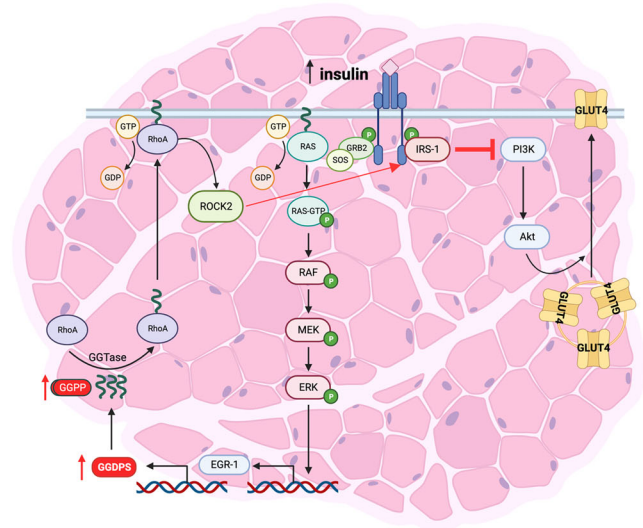


(A) Pancreatic  $\beta$ -cell during hyperinsulinemia(B) Pancreatic  $\beta$ -cell exhaustion following hyperinsulinemia

## (C) Adipocyte in response to hyperinsulinism



## (D) Muscle cell in response to hyperinsulinism



**FIGURE 6** The putative roles of GGDPS in T2D. (A) GGDPS expression is elevated in pancreatic  $\beta$ -cells during chronic hyperglycaemic challenge. Increased blood glucose stimulates release of the insulin granule pool. Docking and fusion of insulin granules is facilitated by Rab proteins which require geranylgeranylation in order to localise and function properly. (B) Resulting hyperinsulinemia and increased secretory demand may be the cause of pancreatic  $\beta$ -cell exhaustion accompanied by decreased expression of GGDPS. Decreased enzyme expression diminishes intracellular GGPP levels, inhibiting geranylgeranylation of Rab proteins and subsequently disrupting the docking and fusion of insulin granules. (C) GGDPS is up-regulated in adipocytes during hyperinsulinemia due to continuous activation of the Ras/ERK/EGR1 pathway. Continuous activation of the pathway leads to phosphorylation of IRS-1 at the inhibitory serine position, disrupting the PI3K/Akt pathway and translocation of GLUT4 to the membrane. (D) GGDPS is up-regulated in skeletal muscle during hyperinsulinemia due to continuous activation of the Ras/ERK/EGR1 pathway. In the skeletal muscle, RhoA is continuously geranylgeranylated allowing for activation of ROCK2. Activation of the RhoA/ROCK pathway contributes to the inhibitory phosphorylation of IRS-1 disrupting GLUT4 translocation.

elevated levels of both FPP and GGPP having been identified in the brains of patients with AD, it has been postulated that elevated GGDPS expression may contribute to NFT and  $\beta$ -amyloid plaque formation.<sup>64–66</sup>

As previously mentioned, aberrant levels of GGDPS have also been implicated in T2D (Figure 6). Interestingly,

GGDPS is overexpressed in the liver, skeletal muscle and adipose tissue of mice with obesity, insulin resistance and hyperinsulinemia.<sup>57,67,68</sup> However, GGDPS expression was noted to be significantly decreased in the islet cells of T2D patients in response to  $\beta$ -cell dysfunction.<sup>56</sup> In normoglycaemic patients, glucose challenge is met with a biphasic

response in which the initial insulin release phase relies on the docked insulin granule pool to facilitate glucose uptake and glycogen synthesis.<sup>69</sup> Patients with T2D have been shown to have a decrease in insulin granules docked at the plasma membrane, diminishing glucose-responsive insulin release.<sup>56</sup>

GGDPS has been shown to be up-regulated in  $\beta$ -cells during the compensatory hyperinsulinemia period in db/db mice.<sup>57</sup> However, GGDPS was found to be down-regulated following prolonged excessive insulin release and subsequent  $\beta$ -cell exhaustion.<sup>56</sup> It is theorised that such  $\beta$ -cell exhaustion is due to increased secretory demand in response to chronic hyperglycaemic challenge.<sup>69</sup> This theory is supported by evidence that GGDPS deficiency contributes to depletion of the docked granule pool due to decreased geranylgeranylation of Rab27a which is required for insulin granule docking.<sup>56</sup> The reason for decreased expression of GGDPS during  $\beta$ -cell exhaustion is not fully understood, but may be due to oxidative stress, ER stress or the result of prolonged  $\beta$ -cell use.<sup>70</sup>

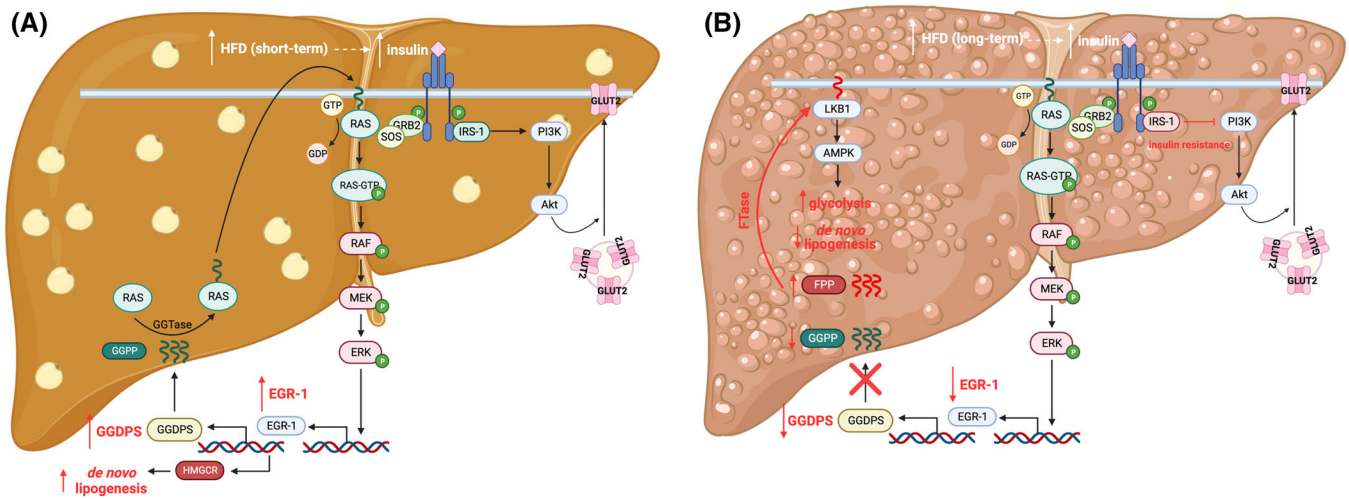
Notably, geranylgeranylation of proteins such as Rab27a, Rab3, Cdc42 and RhoA are essential for proper insulin secretion. During  $\beta$ -cell exhaustion when GGDPS expression is decreased, expression of these small GTPases is also decreased, thereby disrupting insulin trafficking and secretion.<sup>56,67</sup> Decreased levels of GGDPS activity may also impact glucose homeostasis by disrupting proper localisation of Rac1. Rac1 is important in the vesicular trafficking of GLUT4 glucose transporters in skeletal muscle and fat cells and disruption of GLUT4 transport to the membrane results in loss of glucose-stimulated insulin secretion.<sup>31,67,68,71</sup>

EGR1 was found to be highly expressed in the adipose tissue of T2D patients, thus providing a mechanism underlying the increased expression of GGDPS.<sup>31</sup> EGR1 responds to insulin stimulation, therefore GGDPS expression is sustained in response to hyperinsulinism eventually leading to insulin resistance.<sup>31</sup> Mechanistically, EGR1 increases in response to hyperinsulinism subsequently activating GGDPS transcription. GGDPS expression then promotes K-Ras membrane association allowing for the reactivation of the ERK1/2 pathway in the presence of insulin. K-Ras is usually a substrate of farnesylation but studies have shown it can also be geranylgeranylated by GGTase I.<sup>8</sup> Through the use of FTase and GGTase I inhibitors respectively, it was determined that it was the geranylgeranylation of K-Ras that was necessary for the continued activation of the pathway.<sup>30</sup> Sustained activation of this pathway during hyperinsulinemia has also been shown to cause desensitisation of the PI3K/Akt pathway.<sup>32</sup> Sustained activation results in phosphorylation of insulin receptor substrate-1 (IRS1) at the inhibitory serine position.<sup>31</sup> This disrupts

PI3K/Akt pathway activation impairing translocation of GLUT4 transporters and exacerbating insulin resistance.<sup>31</sup> A similar mechanism was found in the skeletal muscle of obese and insulin resistant mice albeit through activation of the RhoA/ROCK pathway due to GGDPS-mediated geranylgeranylation.<sup>57</sup>

Progression of NAFLD to hepatocellular carcinoma (HCC) has been associated with aberrant GGDPS expression.<sup>38</sup> Studies have found GGDPS down-regulation to be a possible predictive factor for progression of NAFLD to fibrosis, and lower expression of the enzyme also has been shown to predict recurrence of HCC.<sup>55</sup> Similar to T2D, the mechanism behind GGDPS expression and NAFLD progression seems to be related to the Ras/ERK/EGR1 pathway (Figure 7). Short-term exposure to a high-fat diet (HFD) has been shown to increase EGR1 expression and GGDPS expression. Expression of EGR1 also initiates expression of HMGCR leading to de novo lipogenesis and subsequent fat accumulation. Continuous activation of this pathway due to long-term exposure to HFD leads to decreased insulin sensitivity and down-regulation of EGR1 and GGDPS. Without sufficient production of GGPP, FPP accumulates leading to the farnesylation of liver kinase B1 (LKB1). LKB1 activates the AMP-activated protein kinase (AMPK) pathway leading to mitochondrial dysfunction and metabolic reprogramming to a glycolytic phenotype.<sup>55</sup> This results in hepatic inflammation through increased release of pro-inflammatory cytokines and macrophage infiltration, leading to hepatic fibrosis. This theorised mechanism is supported by biopsies from patients with NAFLD without fibrosis and inflammation which showed increased GGDPS expression whereas decreased expression of the enzyme was found in more advanced NAFLD cases.<sup>55</sup> Non-alcoholic steatohepatitis (NASH) proceeds NAFLD in the progression of HCC and initial characteristics of NASH include insulin resistance resulting from elevated EGR1 expression in response to long-term HFD.<sup>55</sup> Along with the theory of EGR1-induced NAFLD progression, advanced stages of NASH show decreased EGR1 expression due to chronic insulin insult. Therefore, the down-regulation of GGDPS is also associated with the advancement of NASH leading to HCC. Interestingly, with respect to virus-related HCC, GGPS1 mRNA and protein expression levels were found to be up-regulated in HCC tumour tissue compared with adjacent non-malignant tissue.<sup>72</sup> Additionally elevated mRNA and protein expression levels were associated with pathological indicators of advanced disease stage further revealing the complicated and specific role of GGDPS homeostasis in liver disease.

GGDPS has been implicated in inflammatory responses of ALI and acute respiratory distress syndrome (ARDS). The enzyme was found to have significantly increased



**FIGURE 7** The putative roles of GGDPS in liver disease. (A) Short-term high-fat diet (HFD) leads to increased insulin levels and continuous activation of the Ras/ERK/EGR1 pathway. EGR1 induces the expression of GGDPS and HMGCR promoting continuous activation of the Ras/ERK/EGR1 pathway and de novo lipogenesis causing gradual fat accumulation and development of NAFLD. (B) Long-term HFD leads to decreased EGR1 and GGDPS expression. This inhibits the production of GGPP resulting in the accumulation of FPP. FPP is then utilised by FTase for the farnesylation of LKB1 activating the LKB1/AMPK pathway causing a metabolic shift towards glycolysis. This metabolic shift leads to hepatic inflammation and fibrosis. Continuous activation of the Ras/ERK/EGR1 pathway from HFD also leads to insulin resistance which is characteristic of NASH indicating liver disease progression towards HCC.

expression in the alveolar macrophages isolated from patients with ARDS and ALI-induced mice.<sup>73</sup> It was also shown that cigarette smoke extract induces GGDPS expression in an EGR1-dependent manner resulting in constitutive activation of the Ras/ERK/EGR1 pathway.<sup>30</sup> GGDPS expression was increased in the lung tissues of bleomycin-induced lung injury mice following lung injury and fibrosis. Enzyme deficiency was found to augment lung fibrosis suggesting a protective role of GGDPS in pulmonary fibrosis.<sup>74,75</sup> GGDPS expression was significantly increased in lung adenocarcinoma tissues compared with adjacent normal tissues, and expression level was found to correlate with indicators of disease stage such as large tumours, high TNM stage, lymph node metastasis and poor prognosis.<sup>61</sup> Similarly, GGDPS expression was found to be negatively associated with survival in patients with small cell lung cancer.<sup>76</sup>

## 8 | IBP INHIBITORS

Altered metabolism is a hallmark of cancer, making the IBP a target of interest. The IBP is essential for both sterol and non-sterol synthesis and has also been associated with cell survival and proliferation.<sup>77,78</sup> The cellular effects due to IBP inhibition vary depending on the specific enzyme that is targeted. The following classes of IBP inhibitors that have proven useful in understanding the effects of depleting intracellular GGPP levels and globally disrupting protein geranylgeranylation include statins, nitrogenous

bisphosphonates (NBPs) and GGDPS inhibitors (GGSIs). Statin-mediated inhibition of HMGCR disrupts cholesterol synthesis, but also disrupts synthesis of isoprenoid intermediates (FPP and GGPP). Likewise, the NBPs, by virtue of inhibiting FDPS, disrupt not only sterol synthesis but also GGPP synthesis. Finally, direct inhibitors of GGDPS do not impact sterol synthesis, but do impact synthesis of compounds distal to GGPP synthesis in the pathway. While all three classes of drugs have proven useful in elucidating the effects of disrupting isoprenoid synthesis and protein prenylation in vitro, only the GGSIs have the potential to directly address disease pathologies characterised by aberrant GGDPS activity and/or dependence on GGPP production.

## 9 | STATINS

Statins are the most widely clinically used IBP inhibitors. These agents inhibit the rate-limiting enzyme HMGCR, thereby impacting both sterol and non-sterol synthesis. Clinically they are used for the management of hypercholesterolemia. Reduction in sterol synthesis induces the cleavage and translocation of SREBPs, subsequently regulating synthesis of cholesterol biosynthesis enzymes as well as increasing expression of LDL receptors.<sup>36,37</sup> In addition to lowering LDL-cholesterol levels, statins have also been shown to inhibit neovascularisation and regulate superoxide levels protecting against a wide variety of cardiovascular diseases, including atherosclerosis,

**TABLE 1** Summary of randomised trials involving statin/chemotherapy combinations in advanced malignancies

Reference	Disease	Trial phase	Treatment	No. of patients	Primary outcome (statin vs. placebo/observation)
Yulian et al. <sup>120</sup>	Locally advanced breast cancer	II	FAC + simvastatin (40 mg)/placebo	66	ORR: 90 vs. 70%, $p = .103$
Han et al. <sup>121</sup>	Relapsed/refractory advanced non-small cell lung cancer	II	Gefitinib ± simvastatin (40 mg)	106	ORR: 38.5 vs. 31.5%, $p = .666$
Konings et al. <sup>125</sup>	Advanced gastric cancer	II	ECC ± pravastatin (40 mg/d)	30	6-mos PFS: 43 vs. 47%
Hong et al. <sup>127</sup>	Advanced pancreatic cancer	II	Gemcitabine + simvastatin (40 mg)/placebo	114	TTP: 2.4 vs. 3.6 mos; $p = .903$
Seckl et al. <sup>123</sup>	Newly diagnosed small cell lung cancer	III	Cisplatin/etoposide + pravastatin (40 mg)/placebo	846	OS: median 10.7 vs. 10.6 mos, $p = .9$
Lim et al. <sup>124</sup>	Relapsed/refractory metastatic colorectal cancer	III	FOLFIRI/XELIRI + simvastatin (40 mg)/placebo	269	PFS: median 5.9 vs. 7.0 mos, $p = .937$
Kim et al. <sup>126</sup>	Advanced gastric cancer	III	XP + simvastatin (40 mg)/placebo	244	PFS: median 5.2 vs. 4.6 mos, $p = .642$

Abbreviations: ECC, epirubicin/cisplatin/capecitabine; FAC, fluorouracil/doxorubicin/cyclophosphamide; FOLFIRI, fluorouracil/leucovorin/irinotecan; ORR, overall response rate; OS, overall survival; PFS, progression free survival; TAE, transarterial embolisation; TTP, time to progression; XELIRI, capecitabine/irinotecan; XP, capecitabine/cisplatin.

ischemia–reperfusion injury, arrhythmia, cardiac fibrosis and pulmonary hypertension.<sup>79–89</sup>

While statins are most commonly used for management of hypercholesteremia, there has been significant interest in their potential as anti-cancer agents.<sup>90</sup> In vitro studies in various malignancies such as breast, ovarian, colon, prostate, lung adenocarcinoma, mesothelioma, acute myeloid leukaemia and multiple myeloma have shown that statins inhibit cell proliferation and invasion and induce apoptosis.<sup>61,91–110</sup> Such statin-induced apoptosis has been confirmed to be the result of GGPP depletion rather than other products downstream of HMGCR such as mevalonate or FPP.<sup>111,112</sup> Further evidence supporting the importance of GGPP depletion in the anti-cancer properties of statins is the finding that disruption of RhoA geranylgeranylation impairs metastasis due to disruption of cell attachment, invasion and migration.<sup>92,111</sup>

However, while these in vitro findings are intriguing, it is noted that the concentrations required to disrupt protein prenylation (typically  $> 1 \mu\text{M}$ ) are much higher than those required to inhibit cholesterol biosynthesis,<sup>26</sup> thus standard doses of clinically utilised statins are unlikely to impact protein prenylation in vivo.<sup>113–117</sup> While several phase I trials showed higher doses of statins could result in serum levels in the micromolar range, these doses were also associated with unwanted side effects with minimal anti-tumour effects.<sup>114,118</sup> In the Thibault et al., phase I study, it was noted that myopathy was the dose-limiting toxicity of high-dose lovastatin treatment and that

treatment with ubiquinone could both reverse and prevent this toxicity.<sup>118</sup> Myopathy was not noted in another phase I study of high-dose lovastatin, perhaps secondary to differences in treatment duration.<sup>119</sup>

A few prospective randomised trials have evaluated whether the addition of statins to standard chemotherapy agents/regimens improve anti-tumour efficacy or survival outcomes (Table 1).<sup>120–127</sup> In aggregate these studies have shown that standard doses of statins failed to significantly improve response rates or survival outcomes in a variety of solid tumour populations. A study in which patients received adjuvant pravastatin following completion of transarterial embolisation and 5-fluorouracil treatment for advanced HCC, reported improvement in median overall survival (18 months for the pravastatin group vs. 9 months for the observation group,  $p = .006$ ).<sup>122</sup> Overall, the generally negative outcomes of these randomised studies are most likely a consequence of standard statin dosing being insufficient to significantly impair protein geranylgeranylation in vivo.

## 10 | NITROGENOUS BIPHOSPHONATES

Biphosphonate-based drugs have been around for over 50 years.<sup>128</sup> Biphosphonates act as non-hydrolysable analogues of inorganic pyrophosphate that share a common phosphorous–carbon–phosphorous backbone which

coordinates the chelation of calcium ions. Originally these drugs were determined useful because of their calcium binding properties, rendering them a treatment for bone disease. More recently developed bisphosphonates, such as zoledronic acid, were discovered to inhibit FDPS and osteoclast resorption. These inhibitors belong to the class of NBPs which are approved for treatment of various bone diseases such as osteoporosis, metastatic bone disease and myeloma bone disease. While these agents specifically inhibit FDPS, it was discovered that the mechanism behind their effects on osteoclasts was due to downstream depletion of GGPP.<sup>129,130,195</sup>

In vitro studies have shown NBPs to have a number of anti-tumour effects, including inhibiting tumour cell proliferation, inducing apoptosis, inhibiting adhesion and invasion, having anti-angiogenic properties as well as synergistic effects with standard anti-neoplastic drugs.<sup>132–143</sup> In several studies, depletion of GGPP was noted to be a key mechanism underlying the observed anti-tumour effects.<sup>144–147</sup> There have also been several studies that demonstrated that NBPs have immunomodulatory activities as a result of activation and proliferation of V $\gamma$ 9V $\delta$ 2 T cells, leading to anti-cancer activity.<sup>136,148–150</sup> The underlying mechanism for this phenomenon is related to NBP-induced increase in intracellular IPP levels, as both IPP and ApppI (an ATP analogue resulting from covalent binding of IPP to AMP) serve as phosphoantigens which stimulate V $\gamma$ 9V $\delta$ 2 T cell expansion.<sup>151–154</sup>

In clinical studies, NBPs have been shown to decrease skeletal morbidity in multiple myeloma, breast cancer, prostate cancer, lung cancer and other tumours that metastasize to the bone.<sup>155–158</sup> There has been less certainty as to whether NBPs impact survival outcomes in a manner independent of the effects on skeletal-related events (SREs). In a phase III clinical trial evaluating the effects of bisphosphonate and thalidomide therapy for newly diagnosed multiple myeloma patients, zoledronic acid was found to improve overall survival and progression-free survival while significantly lowering the SRE risk.<sup>159,160</sup> The effect of zoledronic acid on overall survival was reported to be independent of the reduction in SREs, suggesting more direct anti-myeloma effects,<sup>159,160</sup> although a subsequent analysis of the data suggested this effect did not quite reach statistical significance ( $p = .0515$ ).<sup>161</sup> A meta-analysis of eight randomised studies involving patients with early stage breast cancer suggested that adjuvant bisphosphonate use was associated with a reduction in the rate of breast cancer recurrence in the bone (relative risk 0.83,  $p = .004$ ) and modest reduction in breast cancer mortality (relative risk 0.91,  $p = .04$ ).<sup>162</sup> Several studies have suggested more direct anti-tumour activity of zoledronic acid in the setting of breast cancer where enhanced tumour cell apoptosis or clearance of bone marrow disseminated

tumour cells have been observed.<sup>163–165</sup> However, there are also multiple randomised trials that have failed to demonstrate improvement in non-bone related outcomes.<sup>166–173</sup> Overall, it has not been apparent that NBPs have substantial anti-cancer activities outside of the setting of bone marrow/bone disease, which is likely a consequence of their limited systemic distribution due to their high bone affinity.<sup>174,175</sup>

## 11 | GGDPS INHIBITORS

Given the therapeutic interest in more selectively targeting GGDPS (and protein geranylgeranylation) without impacting processes upstream in the IBP, efforts towards developing specific GGSIs were initiated. Early generations of GGSIs included bisphosphonates containing isoprenoid substituents.<sup>176</sup> One of the first discovered GGSIs, digeranyl bisphosphonate (DGBP), was found to inhibit GGDPS with an IC<sub>50</sub> of around 200 nM.<sup>177</sup> While crystallography studies evaluating DGBP binding to human GGDPS have not been reported, studies in *Saccharomyces cerevisiae* revealed that at least one geranyl chain on the central carbon of the bisphosphonate headgroup is required for specific inhibition of GGDPS. The bisphosphonate head group was found to complex with the magnesium ions with the two prenyl side chains occupying the FPP binding site and the GGPP product-binding site.<sup>178</sup>

Later advancements led to the development of isoprenoid bisphosphonates containing a triazole linker group.<sup>179</sup> Extensive structure-function analysis of a series of triazole bisphosphonates revealed the importance of alkyl chain length and olefin stereochemistry in determining inhibitor potency, and later studies showed the impact of different substituents at the  $\alpha$ -position.<sup>179–184</sup>

Other bisphosphonate-based GGSIs include thienopyrimidine bisphosphonate-based (Th-BP) compounds. The extension of the thienopyrimidine moiety was shown to increase potency due to its extension into the IPP binding site. However, this was only seen in compounds with a modification at the C-2 position, while modification at the C-6 position was found to increase potency for inhibition of FDPS instead.<sup>185,186</sup>

Studies are ongoing to understand the systemic effects of GGSIs. In studies involving triazole bisphosphonate GGSIs, hepatotoxicity was determined to be the dose-limiting toxicity, with no effects observed on haematological, cardiac or renal parameters.<sup>187–189</sup> These studies suggested that the hepatotoxicity was a consequence of GGDPS inhibition and not due to the triazole moiety, since the use of a structurally-similar compound (differing by having one less carbon in the isoprenoid side chain)

with ~400-fold less potency as a GGSI (RAM3059) did not induce liver damage at doses >threefold higher than the maximal tolerated dose (MTD) of the lead compound.<sup>189</sup> However, further studies are necessary to confirm that these effects are target based and not specific to the compound structure. It was also confirmed that doses below the MTD could be safely administered and result in inhibition of protein geranylgeranylation *in vivo*.<sup>189</sup> In addition, the combination of low-dose statin with low-dose GGSI resulted in undetectable hepatic GGPP levels and enhanced the hepatotoxic effects, suggesting an association between on-target effects (reduction of GGPP) and hepatotoxicity.<sup>188</sup>

A non-triazole containing GGSI was found to cause significant weight loss in a mouse model of prostate cancer.<sup>190</sup> The weight loss suggested possible toxicity from the GGSI, however this same result was not seen in non-tumour bearing mice when treated with the inhibitor, suggesting that the GGSI alone was not responsible for the toxicity.<sup>190</sup>

In 2018, Lacbay et al. screened over 200 Th-BP analogues and determined one compound (11c) to disrupt protein geranylgeranylation and decrease serum M-protein levels in a mouse model of myeloma.<sup>185</sup> Interestingly, following a 10-day treatment course (3 mg/kg/day via intraperitoneal injection), serological analyses revealed that three out of the seven treated animals had markedly elevated (>10-fold upper limit of normal (ULN)) AST levels and three more with >twofold ULN increases. Elevations in ALT levels were also observed in five animals, ranging from twofold to 12-fold ULN. In 2022, Lee et al. conducted further studies with the same Th-BP GGSI.<sup>186</sup> Here, they reported that seven days after administration of a single dose of GGSI (up to 10 mg/kg via IV administration), no abnormalities in liver function tests were observed. Whether this dose level was sufficient to substantially alter hepatic GGPP levels or disrupt hepatic protein geranylgeranylation was not disclosed. In addition, the pharmacokinetic studies revealed a terminal half-life of 5.34 h, significantly shorter than the previously reported triazole bisphosphonates,<sup>187,189</sup> thus making it difficult to draw any definitive conclusions regarding class effect versus specific agent effect and hepatotoxicity.

Finally, a dual FDPS–GGDPS inhibitor also showed potent activity without signs of toxicity in a murine xenograft model utilising SK-ES-1 sarcoma cells, although the only reported metric of toxicity was animal weight.<sup>191</sup> In aggregate, the available preclinical literature has demonstrated the feasibility of systemically administering GGSI therapy, although further studies are needed to better understand the observed hepatic toxicity and thus maximise the therapeutic window.

## 12 | THERAPEUTIC EFFECTS OF GGDPS INHIBITORS

In contrast to statins and FDPS inhibitors which globally disrupt sterol synthesis and protein prenylation, inhibition of GGDPS depletes GGPP levels, thereby impacting protein geranylgeranylation, without inhibiting farnesylation or sterol synthesis. It is important to note that both *in vitro* and *in vivo* data suggest that the effects of GGDPS inhibition on a myriad of cellular processes are primarily due to disruption of geranylgeranylation.<sup>77,130,131,178,192–195</sup> However, there are also data that suggest that GGSI-induced FPP accumulation can induce apoptosis as a consequence of conversion of FPP to farnesol.<sup>196</sup>

GGDPS inhibition is associated with anti-proliferative effects in a variety of malignancies as a result of disruption of geranylgeranylation of proteins essential for cell growth and survival processes (Figure 5).<sup>78,146,197</sup> While Ras proteins are primarily farnesylated by FTase I, the Rho and Rab families are geranylgeranylated by GGTase I and GGTase II respectively. Rho proteins such as Rho, Rac and Cdc42 are important for cytoskeletal reorganisation and regulate dynamics such as cell polarity, cell motility and membrane protrusion. Rab proteins are important in intracellular membrane trafficking and regulate vesicle formation, transport, docking and fusion. Understanding the impact of GGDPS inhibition on Rho and Rab protein localisation and function is essential for understanding the potential therapeutic benefit of these inhibitors.

*In vitro* studies showed that the GGSI DGBP inhibited cell migration in a human breast cancer cell line MDA-MB-231 and induced autophagy in both MDA-MB-13 and PC3 prostate cancer cells.<sup>197,198</sup> LC3-II accumulation (a marker of autophagic flux) was also evident when multiple myeloma cell lines were treated with DGBP.<sup>199</sup> However, the use of GGTase I and GGTase II specific inhibitors did not recapitulate the effects of DGBP, indicating that DGBP effects are not solely due to disruption of geranylgeranylation. Another interesting study found that GGSI-induced GGPP depletion inhibited micropinocytosis of GGPP leading to amino acid starvation and apoptosis.<sup>77</sup> However, these effects were specific to MCF10A PTEN knockout cells and K-RasG12V expressing cells, suggesting these effects may be specific to certain oncogenic cell types.

One study found DGBP to inhibit proliferation and induce apoptosis in lymphocytic leukaemia cells more potently than the FDPS inhibitor zoledronate.<sup>146</sup> Addition of GGPP following DGBP treatment was shown to abrogate the anti-proliferative effects, indicating GGPP depletion to be the key mechanism underlying DGBP effects. DGBP was also found to induce apoptosis in T-cell acute lymphoblastic leukaemia (T-ALL) cell lines.<sup>200</sup> A potential

mechanism was postulated, connecting disruption of Rab7 localisation due to GGPP depletion with inhibition of Notch1 expression.<sup>201</sup> Notch1 is altered in over 60% of T-ALL cases and has been shown to promote proliferation and differentiation.<sup>201</sup> It was concluded that disruption of Rab7 localisation inhibited Notch1 proliferation, allowing for caspase activation and apoptosis of T-ALL cells.

The GGSIs disodium [(6Z,11E,15E)-9-[bis(sodiooxy)phosphoryl]-17-hydroxy-2,6,12,6-tetramethylheptadeca-2,6,11,15-tetraen-9-yl]phosphonate (GGOHBP) was found to significantly decrease adrenal gland metastasis in a murine model of human prostate cancer.<sup>190</sup> In a separate study it was also reported to significantly reduce whole body tumour burden, slow tumour development and prolong survival in a murine model of human prostate cancer.<sup>202</sup> Lipophilic bisphosphonate BPH-1222 in combination with rapamycin was found to potently suppress tumour growth in a murine model of lung adenocarcinoma.<sup>203</sup> BPH-1222 is an analogue of zoledronate that targets both FDPS and GGDPS. Treatment with BPH-1222 alone was found to block K-Ras prenylation initiating ER stress and autophagy, but it did not ultimately induce apoptosis. Combination treatment of the bisphosphonate along with rapamycin allowed for the induction of autophagy and concomitant inhibition of the mTOR pathway which had previously allowed for tumour cell survival.<sup>203</sup> Similarly, a dual FDPS–GGDPS inhibitor was found to delay tumour growth in a mouse xenograft sarcoma model.<sup>191</sup>

In malignancies characterised by aberrant protein secretion, disruption of Rab geranylgeranylation was found to be the primary mechanism underlying GGSIs-induced cytotoxicity. Treatment of multiple myeloma cells with GGSIs or GGTase II inhibitors results in activation of the unfolded protein response (UPR) pathway and apoptosis.<sup>187,192</sup> Accumulation of monoclonal protein in the ER was shown to cause ER stress and activation of the UPR.<sup>192</sup> Prolonged activation of the UPR, such as with GGSIs-mediated inhibition of Rab activity, leads to activation of the apoptotic pathway. The previously mentioned novel triazole-based GGSIs were shown to potently disrupt monoclonal protein secretion in multiple myeloma cells leading to apoptosis.<sup>179,181</sup> These same triazole bisphosphonates were also found to decrease tumour growth in mice with multiple myeloma flank xenografts.<sup>187</sup> Similar results were reported with the use of a Th-BP inhibitor in both in vitro and in vivo models of multiple myeloma.<sup>193</sup>

GSIs have also shown efficacy in models of pancreatic ductal adenocarcinoma (PDAC). PDAC is characterised by the abnormal production of aberrantly glycosylated mucins.<sup>204</sup> Treatment of PDAC cell lines with a GSI was found to disrupt intracellular trafficking of key mucins like MUC1 leading to activation of the UPR and subse-

quent apoptosis.<sup>194</sup> These results were recapitulated in vivo where triazole bisphosphonate GSI therapy significantly slowed tumour growth in two different mouse models of PDAC.<sup>194</sup>

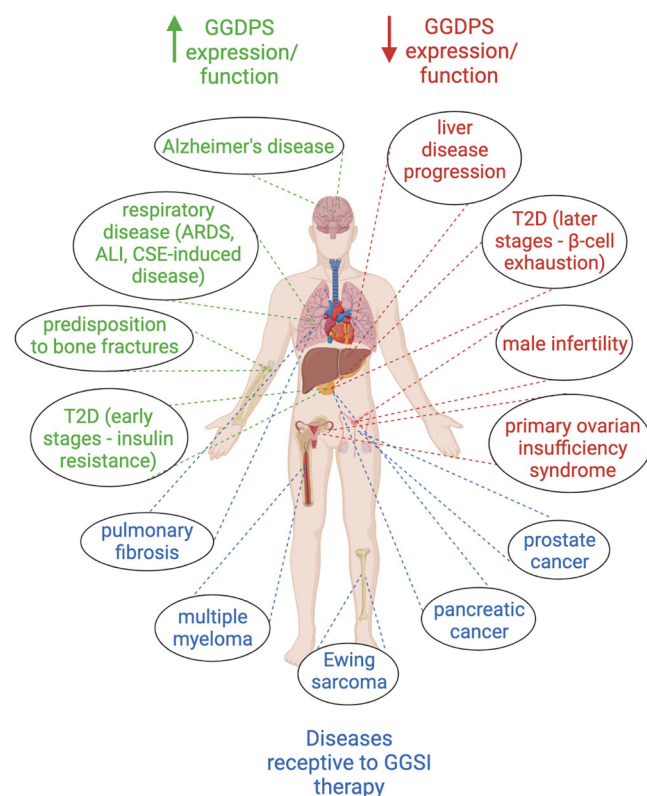
GSIs may also be therapeutically relevant in bone disorders. Bone diseases are commonly treated with NBPs which result in disruption of cytoskeletal arrangement through disruption of the F-actin ring and ruffled border necessary for osteoclast function.<sup>130,131,195</sup> As these effects on osteoclast formation result from depletion of GGPP rather than FPP, GSIs may be an alternative to FDPS inhibitors.<sup>195</sup> Therefore, the use of GSIs, especially in the case of bone pain or fractures resulting from the primary disease, such as in the case of multiple myeloma, may hold promise in treating both the primary disease as well as preventing bone resorption. Further potential benefits include the greater systemic distribution of the triazole bisphosphonate GSIs compared with NBPs.<sup>187</sup>

Finally, there has also been interest in the therapeutic potential of GSIs in pulmonary fibrosis. DGBP was found to abrogate pulmonary fibrosis in a mouse model utilising bleomycin-induced lung injury.<sup>74</sup> DGBP disrupted localisation of Rac1 to the mitochondria of alveolar macrophages, which in turn attenuated mitochondrial oxidative stress levels and limited the fibrotic lung response.<sup>74</sup>

## 13 | CONCLUSIONS

In this review, we provide a comprehensive overview of the role of GGDPS in normal physiological processes. While the factors that impact the expression and regulation of the enzyme in pathophysiological conditions remain incompletely understood, it is evident that the EGR1/GGDPS/Ras/ERK1/2 pathway plays an important role. However, whether there are other modulators or pathways that impact GGDPS activity remain to be determined.

It is evident that GGDPS is a contributor to a wide range of human pathologies, including liver disease, T2D, pulmonary disease and malignancy, and therefore is an enzyme of significant clinical interest (Figure 8). Available preclinical studies involving GSIs have demonstrated efficacy in several malignancy types that are currently considered incurable (e.g., multiple myeloma and PDAC) (Figure 8). In addition, preclinical studies with a GSI have revealed efficacy in pulmonary fibrosis, a disease with limited therapeutic options. Overall, there is substantial rationale for the further development of GSIs and to expand the scope of GSI therapeutic intervention research to not only include other malignancies, but also other disease states such as liver disease and T2D.



**FIGURE 8** Overview of the pathological processes impacted by altered GGDPS expression or activity

Ultimately, successful translation to the clinic will depend not only on understanding the GGSIs' disease-modulating effects but also the impact of targeting GGDPS in normal cells. Thus, continued exploration of this enzyme's role in normal human physiology and pathophysiology is of critical importance.


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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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## REFERENCES

- Kawamukai M. Biosynthesis of coenzyme Q in eukaryotes. *Biosci Biotechnol Biochem*. 2016;80(1):23-33. doi: [10.1080/09168451.2015.1065172](https://doi.org/10.1080/09168451.2015.1065172)
- Elsabrouty R, Jo Y, Hwang S, Jun DJ, DeBose-Boyd RA. Type 1 polyisoprenoid diphosphate phosphatase modulates geranylgeranyl-mediated control of HMG CoA reductase and UBIAD1. *Elife*. 2021. doi: [10.7554/eLife.64688](https://doi.org/10.7554/eLife.64688)
- Taylor JS, Reid TS, Terry KL, Casey PJ, Beese LS. Structure of mammalian protein geranylgeranyltransferase type-I. *EMBO J*. 2003;22(22):5963-5974. doi: [10.1093/emboj/cdg571](https://doi.org/10.1093/emboj/cdg571)
- Thomä NH, Iakovenko A, Owen D, et al. Phosphoisoprenoid binding specificity of geranylgeranyltransferase type II. *Biochemistry*. 2000;39(39):12043-12052. doi: [10.1021/bi000835m](https://doi.org/10.1021/bi000835m)
- Kuchay S, Wang H, Marzio A, et al. GGTase3 is a newly identified geranylgeranyltransferase targeting a ubiquitin ligase. *Nat Struct Mol Biol*. 2019;26(7):628-636. doi: [10.1038/s41594-019-0249-3](https://doi.org/10.1038/s41594-019-0249-3)
- Losada de la Lastra A, Hassan S, Tate EW. Deconvoluting the biology and druggability of protein lipidation using chemical proteomics. *Curr Opin Chem Biol*. 2021;60:97-112. doi: [10.1016/j.cbpa.2020.10.002](https://doi.org/10.1016/j.cbpa.2020.10.002)
- Reid TS, Terry KL, Casey PJ, Beese LS. Crystallographic analysis of CaaX prenyltransferases complexed with substrates defines rules of protein substrate selectivity. *J Mol Biol*. 2004;343(2):417-433. doi: [10.1016/j.jmb.2004.08.056](https://doi.org/10.1016/j.jmb.2004.08.056)
- Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. *Nat Rev Cancer*. 2011;11(11):775-791. doi: [10.1038/nrc3151](https://doi.org/10.1038/nrc3151)
- Sun J, Qian Y, Hamilton AD, Sebti SM. Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. *Oncogene*. 1998;16(11):1467-1473. doi: [10.1038/sj.onc.1201656](https://doi.org/10.1038/sj.onc.1201656)
- Seabra MC, Reiss Y, Casey PJ, Brown MS, & Goldstein JL (1991). Protein farnesyltransferase and geranylgeranyltransferase share a common  $\alpha$  subunit. *Cell*, 65(3), 429–434. [https://doi.org/10.1016/0092-8674\(91\)90460-g](https://doi.org/10.1016/0092-8674(91)90460-g)
- Lackner MR, Kindt RM, Carroll PM, et al. Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors. *Cancer Cell*. 2005;7(4):325-336. <https://doi.org/10.1016/j.ccr.2005.03.024>
- Desnoyers L, Anant JS, Seabra MC. Geranylgeranylation of Rab proteins. *Biochem Soc Trans*. 1996;24(3):699-703. <https://doi.org/10.1042/bst0240699>
- Palsuledesai CC, Distefano MD. Protein prenylation: enzymes, therapeutics, and biotechnology applications. *ACS Chem Biol*. 2015;10(1):51-62. <https://doi.org/10.1021/cb500791f>
- Roth AF, & Davis NG (2020). Geranylgeranyl generosity: a new prenyl-transferase gives a fat to a SNARE protein. *The EMBO Journal*, 39(8). Portico. <https://doi.org/10.15252/embj.2020104744>
- Shirakawa R, Goto-Ito S, Goto K, et al. A SNARE geranylgeranyltransferase essential for the organization of the Golgi apparatus. *EMBO J*. 2020;39(8):e104120. doi:[10.15252/embj.2019104120](https://doi.org/10.15252/embj.2019104120)
- Storck EM, Morales-Sanfrutos J, Serwa RA, et al. Dual chemical probes enable quantitative system-wide analysis of protein prenylation and prenylation dynamics. *Nat Chem*. 2019;11(6):552-561. <https://doi.org/10.1038/s41557-019-0237-6>
- Kavanagh KL, Dunford JE, Bunkoczi G, Russell RG, Oppermann U The crystal structure of human geranylgeranyl pyrophosphate synthase reveals a novel hexameric arrangement and inhibitory product binding. *The Journal of biological chemistry*. 2006;281(31):22004-22012. <https://doi.org/10.1074/jbc.M602603200>



18. Lisniansky M, Yariv E, Segal O, et al. Metal coordination is crucial for geranylgeranyl diphosphate synthase-bisphosphonate interactions: a crystallographic and computational analysis. *Mol Pharmacol*. 2019;96(5):580-588. <https://doi.org/10.1124/mol.119.117499>
19. Zheng H, Chruszcz M, Lasota P, Lebiada L, Minor W Data mining of metal ion environments present in protein structures. *J Inorg Biochem*. 2008;102(9):1765-1776. <https://doi.org/10.1016/j.jinorgbio.2008.05.006>
20. Miyagi Y, Matsumura Y, Sagami H Human geranylgeranyl diphosphate synthase is an octamer in solution. *J Biochem*. 2007;142(3):377-381. <https://doi.org/10.1093/jb/mvm144>
21. Zhang H, & Li Z.-X. (2014). A Type-III insect geranylgeranyl diphosphate synthase with a novel catalytic property. *Protein & Peptide Letters*, 21(7), 615–623. <https://doi.org/10.2174/0929866521666140214123942>
22. Ding BY, Niu J, Shang F, Yang L, Chang TY, Wang JJ. Characterization of the geranylgeranyl diphosphate synthase gene in *Acyrtosiphon pisum* (Hemiptera: Aphididae) and its association with carotenoid biosynthesis. *Front Physiol*. 2019;10:1398. <https://doi.org/10.3389/fphys.2019.01398>
23. Kuzuguchi T, Morita Y, Sagami I, Sagami H, Ogura K Human geranylgeranyl diphosphate synthase. cDNA cloning and expression. *J Biol Chem*. 1999;274(9):5888-5894. <https://doi.org/10.1074/jbc.274.9.5888>
24. Poulter CD, Argyle JC, Mash EA. Farnesyl pyrophosphate synthetase. Mechanistic studies of the 1'-4 coupling reaction with 2-fluorogeranyl pyrophosphate. *J Biol Chem*. 1978;253(20):7227-7233.
25. Szabo CM, Matsumura Y, Fukura S, Martin MB, Sanders JM, Sengupta S, Cieslak JA, Loftus TC, Lea CR, Lee H-J, Koohang A, Coates RM, Sagami H, & Oldfield E (2002). Inhibition of geranylgeranyl diphosphate synthase by bisphosphonates and diphosphates: A potential route to new bone antiresorption and antiparasitic agents. *Journal of Medicinal Chemistry*, 45(11), 2185–2196. <https://doi.org/10.1021/jm010412y>
26. Lutz RJ, McLain TM, Sinensky M Feedback inhibition of polyisoprenyl pyrophosphate synthesis from mevalonate in vitro. Implications for protein prenylation. *J Biol Chem*. 1992;267(12):7983-7986.
27. Sagami H, Morita Y, Ogura K Purification and properties of geranylgeranyl-diphosphate synthase from bovine brain. *J Biol Chem*. 1994;269(32):20561-20566.
28. Guo RT, Cao R, Liang PH, et al. Bisphosphonates target multiple sites in both cis- and trans-prenyltransferases. *Proc Natl Acad Sci U S A*. 2007;104(24):10022-10027. <https://doi.org/10.1073/pnas.0702254104>
29. Gokey NG, Lopez-Anido C, Gillian-Daniel AL, Svaren J. Early growth response 1 (Egr1) regulates cholesterol biosynthetic gene expression. *J Biol Chem*. 2011;286(34):29501-29510. <https://doi.org/10.1074/jbc.M111.263509>
30. Shen N, Shao Y, Lai SS, et al. GGPPS, a new EGR-1 target gene, reactivates ERK 1/2 signaling through increasing Ras prenylation. *Am J Pathol*. 2011;179(6):2740-2750. <https://doi.org/10.1016/j.ajpath.2011.08.011>
31. Shen N, Yu X, Pan FY, Gao X, Xue B, Li CJ. An early response transcription factor, Egr-1, enhances insulin resistance in type 2 diabetes with chronic hyperinsulinism. *J Biol Chem*. 2011;286(16):14508-14515. <https://doi.org/10.1074/jbc.M110.190165>
32. Yu X, Shen N, Zhang ML, et al. Egr-1 decreases adipocyte insulin sensitivity by tilting PI3K/Akt and MAPK signal balance in mice. *Embo j*. 2011;30(18):3754-3765. <https://doi.org/10.1038/emboj.2011.277>
33. Tao W, Shi JF, Zhang Q, Xue B, Sun YJ, Li CJ. Egr-1 enhances drug resistance of breast cancer by modulating MDR1 expression in a GGPPS-independent manner. *Biomed Pharmacother*. 2013;67(3):197-202. <https://doi.org/10.1016/j.biopha.2013.01.001>
34. Buchou C, Laud-Duval K, van der Ent W, et al. Upregulation of the Mevalonate Pathway through EWSR1-FLI1/EGR2 Regulatory Axis Confers Ewing Cells Exquisite Sensitivity to Statins. *Cancers (Basel)*. 2022;14(9)<https://doi.org/10.3390/cancers14092327>
35. Ericsson J, Greene JM, Carter KC, et al. Human geranylgeranyl diphosphate synthase: isolation of the cDNA, chromosomal mapping and tissue expression. *J Lipid Res*. 1998;39(9):1731-1739.
36. Waku T, Hagiwara T, Tamura N, et al. NRF3 upregulates gene expression in SREBP2-dependent mevalonate pathway with cholesterol uptake and lipogenesis inhibition. *iScience*. 2021;24(10):103180. <https://doi.org/10.1016/j.isci.2021.103180>
37. Clendening JW, Penn LZ. Targeting tumor cell metabolism with statins. *Oncogene*. 2012;31(48):4967-4978. <https://doi.org/10.1038/onc.2012.6>
38. Zhao Y, Wu TY, Zhao MF, Li CJ. The balance of protein farnesylation and geranylgeranylation during the progression of nonalcoholic fatty liver disease. *J Biol Chem*. 2020;295(15):5152-5162. <https://doi.org/10.1074/jbc.REV119.008897>
39. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J Lipid Res*. 2009;50 Suppl(Suppl):S15-27. <https://doi.org/10.1194/jlr.R800054-JLR200>
40. Rawson RB. The SREBP pathway—insights from Insigns and insects. *Nat Rev Mol Cell Biol*. 2003;4(8):631-640. <https://doi.org/10.1038/nrm1174>
41. Diao F, Jiang C, Wang XX, et al. Alteration of protein prenylation promotes spermatogonial differentiation and exhausts spermatogonial stem cells in newborn mice. *Sci Rep*. 2016;6:28917. <https://doi.org/10.1038/srep28917>
42. Zhu R, Wang J, Feng T, et al. The alteration of RhoA geranylgeranylation and Ras farnesylation breaks the integrity of the blood-testis barrier and results in hypospermatogenesis. *Cell Death Dis*. 2019;10(6):450. <https://doi.org/10.1038/s41419-019-1688-9>
43. Foley AR, Zou Y, Dunford JE, et al. GGPS1 mutations cause muscular dystrophy/hearing loss/ovarian insufficiency syndrome. *Ann Neurol*. 2020;88(2):332-347. <https://doi.org/10.1002/ana.25772>
44. Tucker EJ, Rius R, Jaillard S, et al. Genomic sequencing highlights the diverse molecular causes of Perrault syndrome: a peroxisomal disorder (PEX6), metabolic disorders (CLPP, GGPS1), and mtDNA maintenance/translation disorders (LARS2, TFAM). *Hum Genet*. 2020;139(10):1325-1343. <https://doi.org/10.1007/s00439-020-02176-w>
45. Jiang C, Diao F, Sang YJ, et al. GGPP-mediated protein geranylgeranylation in oocyte is essential for the establishment of oocyte-granulosa cell communication and primary-secondary follicle transition in mouse ovary. *PLoS*

- Genet.* 2017;13(1):e1006535. <https://doi.org/10.1371/journal.pgen.1006535>
46. Sang YJ, Wang Q, Zheng F, et al. Ggps1 deficiency in the uterus results in dystocia by disrupting uterine contraction. *J Mol Cell Biol.* 2021;13(2):116-127. <https://doi.org/10.1093/jmcb/mjaa066>
  47. Wang XX, Ying P, Diao F, et al. Altered protein prenylation in Sertoli cells is associated with adult infertility resulting from childhood mumps infection. *J Exp Med.* 2013;210(8):1559-1574. <https://doi.org/10.1084/jem.20121806>
  48. Bae JW, Kim SH, Kim DH, et al. Ras-related proteins (Rab) are key proteins related to male fertility following a unique activation mechanism. *Reprod Biol.* 2019;19(4):356-362. <https://doi.org/10.1016/j.repbio.2019.10.001>
  49. Choi HJ, Choi JY, Cho SW, et al. Genetic polymorphism of geranylgeranyl diphosphate synthase (GGSP1) predicts bone density response to bisphosphonate therapy in Korean women. *Yonsei Med J.* 2010;51(2):231-238. <https://doi.org/10.3349/ymj.2010.51.2.231>
  50. Roca-Ayats N, Balcels S, Garcia-Giralt N, et al. GGPS1 mutation and atypical femoral fractures with bisphosphonates. *N Engl J Med.* 2017;376(18):1794-1795. <https://doi.org/10.1056/NEJMc1612804>
  51. Roca-Ayats N, Ng PY, Garcia-Giralt N, et al. Functional characterization of a GGPPS variant identified in atypical femoral fracture patients and delineation of the role of GGPPS in bone-relevant cell types. *J Bone Miner Res.* 2018;33(12):2091-2098. <https://doi.org/10.1002/jbmr.3580>
  52. Marozik P, Alekna V, Rudenko E, et al. Bone metabolism genes variation and response to bisphosphonate treatment in women with postmenopausal osteoporosis. *PLoS One.* 2019;14(8):e0221511. <https://doi.org/10.1371/journal.pone.0221511>
  53. Chen Z, Xu N, Chong D, et al. Geranylgeranyl pyrophosphate synthase facilitates the organization of cardiomyocytes during mid-gestation through modulating protein geranylgeranylation in mouse heart. *Cardiovasc Res.* 2018;114(7):965-978. <https://doi.org/10.1093/cvr/cvy042>
  54. Chong D, Chen Z, Guan S, et al. Geranylgeranyl pyrophosphate-mediated protein geranylgeranylation regulates endothelial cell proliferation and apoptosis during vasculogenesis in mouse embryo. *J Genet Genomics.* 2021;48(4):300-311. <https://doi.org/10.1016/j.jgg.2021.03.009>
  55. Liu J, Jiang S, Zhao Y, et al. Geranylgeranyl diphosphate synthase (GGPPS) regulates non-alcoholic fatty liver disease (NAFLD)-fibrosis progression by determining hepatic glucose/fatty acid preference under high-fat diet conditions. *J Pathol.* 2018;246(3):277-288. <https://doi.org/10.1002/path.5131>
  56. Jiang S, Shen D, Jia WJ, et al. GGPPS-mediated Rab27A geranylgeranylation regulates  $\beta$  cell dysfunction during type 2 diabetes development by affecting insulin granule docked pool formation. *J Pathol.* 2016;238(1):109-119. <https://doi.org/10.1002/path.4652>
  57. Tao W, Wu J, Xie BX, et al. Lipid-induced muscle insulin resistance is mediated by GGPPS via modulation of the RhoA/Rho kinase signaling pathway. *J Biol Chem.* 2015;290(33):20086-20097. <https://doi.org/10.1074/jbc.M115.657742>
  58. Xu WJ, Wang XX, Jin JJ, et al. Inhibition of GGPPS1 attenuated LPS-induced acute lung injury and was associated with NLRP3 inflammasome suppression. *Am J Physiol Lung Cell Mol Physiol.* 2019;316(3):L567-L577. <https://doi.org/10.1152/ajplung.00190.2018>
  59. Li D, Li C, Wang T, et al. Geranylgeranyl diphosphate synthase 1 knockdown suppresses NLRP3 inflammasome activity via promoting autophagy in sepsis-induced acute lung injury. *Int Immunopharmacol.* 2021;100:108106. <https://doi.org/10.1016/j.intimp.2021.108106>
  60. Wan B, Xu W, Chen M, Sun S, Jin J, Lv Y, Zhan P, Zhu S, Wang X, Lv T-F., & Song Y (2020). Geranylgeranyl diphosphate synthase 1 knockout ameliorates ventilator-induced lung injury via regulation of TLR2/4-AP-1 signaling. *Free Radical Biology and Medicine*, 147, 159-166. <https://doi.org/10.1016/j.freeradbiomed.2019.12.024>
  61. Wang X, Xu W, Zhan P, et al. Overexpression of geranylgeranyl diphosphate synthase contributes to tumour metastasis and correlates with poor prognosis of lung adenocarcinoma. *J Cell Mol Med.* 2018;22(4):2177-2189. <https://doi.org/10.1111/jcmm.13493>
  62. Dai B, Li Q, Song X, et al. Knockdown of Ggps1 in chondrocyte expedites fracture healing by accelerating the progression of endochondral ossification in mice. *J Bone Miner Metab.* 2018;36(2):133-147. <https://doi.org/10.1007/s00774-017-0824-9>
  63. Pelleieux S, Picard C, Lamarre-Th eroux L, et al. Isoprenoids and tau pathology in sporadic Alzheimer's disease. *Neurobiol Aging.* 2018;65:132-139. <https://doi.org/10.1016/j.neurobiolaging.2018.01.012>
  64. Hooff GP, Wood WG, Muller WE, Eckert GP. Isoprenoids, small GTPases and Alzheimer's disease. *Biochim Biophys Acta.* 2010;1801(8):896-905. <https://doi.org/10.1016/j.bbali.2010.03.014>
  65. Eckert GP, Hooff GP, Strandjord DM, et al. Regulation of the brain isoprenoids farnesyl- and geranylgeranylpyrophosphate is altered in male Alzheimer patients. *Neurobiol Dis.* 2009;35(2):251-257. <https://doi.org/10.1016/j.nbd.2009.05.005>
  66. Zhou Y, Suram A, Venugopal C, et al. Geranylgeranyl pyrophosphate stimulates gamma-secretase to increase the generation of Abeta and APP-CTFgamma. *FASEB J.* 2008;22(1):47-54. <https://doi.org/10.1096/fj.07-8175com>
  67. Gendaszewska-Darmach E, Garstka MA, Blazewska KM. Targeting Small GTPases and Their Prenylation in Diabetes Mellitus. *J Med Chem.* 2021;64(14):9677-9710. <https://doi.org/10.1021/acs.jmedchem.1c00410>
  68. Vicent D, Maratos-Flier E, Kahn CR. The branch point enzyme of the mevalonate pathway for protein prenylation is overexpressed in the ob/ob mouse and induced by adipogenesis. *Mol Cell Biol.* 2000;20(6):2158-2166. <https://doi.org/10.1128/MCB.20.6.2158-2166.2000>
  69. Campos C Chronic hyperglycemia and glucose toxicity: pathology and clinical sequelae. *Postgrad Med.* 2012;124(6):90-97. <https://doi.org/10.3810/pgm.2012.11.2615>
  70. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest.* 2006;116(7):1802-1812. <https://doi.org/10.1172/jci29103>
  71. Sylow L, Jensen TE, Kleinert M, et al. Rac1 signaling is required for insulin-stimulated glucose uptake and is dysregulated in insulin-resistant murine and human skeletal muscle. *Diabetes.* 2013;62(6):1865-1875. <https://doi.org/10.2337/db12-1148>
  72. Yu DC, Liu J, Chen J, et al. GGPPS1 predicts the biological character of hepatocellular carcinoma in patients with cirrhosis.

- BMC Cancer*. 2014;14:248. <https://doi.org/10.1186/1471-2407-14-248>
73. Jin J, Qian H, Wan B, et al. Geranylgeranyl diphosphate synthase deficiency hyperactivates macrophages and aggravates lipopolysaccharide-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2021;320(6):L1011-L1024. <https://doi.org/10.1152/ajplung.00281.2020>
  74. Osborn-Heaford HL, Murthy S, Gu L, et al. Targeting the isoprenoid pathway to abrogate progression of pulmonary fibrosis. *Free Radic Biol Med*. 2015;86:47-56. <https://doi.org/10.1016/j.freeradbiomed.2015.04.031>
  75. Chen M, Wan B, Zhu S, et al. Geranylgeranyl diphosphate synthase deficiency aggravates lung fibrosis in mice by modulating TGF- $\beta$ 1/BMP-4 signaling. *Biol Chem*. 2019;400(12):1617-1627. <https://doi.org/10.1515/hsz-2019-0168>
  76. Guo C, Wan R, He Y, et al. Therapeutic targeting of the mevalonate-geranylgeranyl diphosphate pathway with statins overcomes chemotherapy resistance in small cell lung cancer. *Nat Cancer*. 2022;3(5):614-628. <https://doi.org/10.1038/s43018-022-00358-1>
  77. Jiao Z, Cai H, Long Y, et al. Statin-induced GGPP depletion blocks macropinocytosis and starves cells with oncogenic defects. *Proc Natl Acad Sci U S A*. 2020;117(8):4158-4168. <https://doi.org/10.1073/pnas.1917938117>
  78. Agabiti SS, Liang Y, Wiemer AJ. Molecular mechanisms linking geranylgeranyl diphosphate synthase to cell survival and proliferation. *Mol Membr Biol*. 2016;33(1-2):1-11. <https://doi.org/10.1080/09687688.2016.1213432>
  79. Park HJ, Zhang Y, Georgescu SP, Johnson KL, Kong D, Galper JB. Human umbilical vein endothelial cells and human dermal microvascular endothelial cells offer new insights into the relationship between lipid metabolism and angiogenesis. *Stem Cell Rev*. 2006;2(2):93-102. <https://doi.org/10.1007/s12015-006-0015-x>
  80. Tang D, Park HJ, Georgescu SP, Sebt SM, Hamilton AD, Galper JB. Simvastatin potentiates tumor necrosis factor alpha-mediated apoptosis of human vascular endothelial cells via the inhibition of the geranylgeranylation of RhoA. *Life Sci*. 2006;79(15):1484-1492. <https://doi.org/10.1016/j.lfs.2006.04.019>
  81. Lee TK, Poon RT, Yuen AP, et al. Rac activation is associated with hepatocellular carcinoma metastasis by up-regulation of vascular endothelial growth factor expression. *Clin Cancer Res*. 2006;12(17):5082-5089. <https://doi.org/10.1158/1078-0432.Ccr-05-2794>
  82. Massaro M, Zampolli A, Scoditti E, et al. Statins inhibit cyclooxygenase-2 and matrix metalloproteinase-9 in human endothelial cells: anti-angiogenic actions possibly contributing to plaque stability. *Cardiovasc Res*. 2010;86(2):311-320. <https://doi.org/10.1093/cvr/cvp375>
  83. Elnakish MT, Hassanain HH, Janssen PM, Angelos MG, Khan M. Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. *J Pathol*. 2013;231(3):290-300. <https://doi.org/10.1002/path.4255>
  84. Emelyanova L, Sra A, Schmuck EG, et al. Impact of statins on cellular respiration and de-differentiation of myofibroblasts in human failing hearts. *ESC Heart Fail*. 2019;6(5):1027-1040. <https://doi.org/10.1002/ehf2.12509>
  85. Wong MJ, Kantores C, Ivanovska J, Jain A, Jankov RP. Simvastatin prevents and reverses chronic pulmonary hypertension in newborn rats via pleiotropic inhibition of RhoA signaling. *Am J Physiol Lung Cell Mol Physiol*. 2016;311(5):L985-L999. <https://doi.org/10.1152/ajplung.00345.2016>
  86. Girgis RE, Li D, Zhan X, et al. Attenuation of chronic hypoxic pulmonary hypertension by simvastatin. *Am J Physiol Heart Circ Physiol*. 2003;285(3):H938-945. <https://doi.org/10.1152/ajpheart.01097.2002>
  87. Murata T, Kinoshita K, Hori M, et al. Statin protects endothelial nitric oxide synthase activity in hypoxia-induced pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2005;25(11):2335-2342. <https://doi.org/10.1161/01.Atv.0000186184.33537.48>
  88. Zhang Z, Wang M, Xue SJ, Liu DH, Tang YB. Simvastatin ameliorates angiotensin II-induced endothelial dysfunction through restoration of Rho-BH4-eNOS-NO pathway. *Cardiovasc Drugs Ther*. 2012;26(1):31-40. <https://doi.org/10.1007/s10557-011-6351-3>
  89. Wang Q, Guo YZ, Zhang YT, et al. The effects and mechanism of atorvastatin on pulmonary hypertension due to left heart disease. *PLoS One*. 2016;11(7):e0157171. <https://doi.org/10.1371/journal.pone.0157171>
  90. Hindler K, Cleeland CS, Rivera E, Collard CD. The role of statins in cancer therapy. *Oncologist*. 2006;11(3):306-315. <https://doi.org/10.1634/theoncologist.11-3-306>
  91. Wilke M, Göbel A, Rauner M, et al. Zoledronic acid and atorvastatin inhibit  $\alpha$ v $\beta$ 3-mediated adhesion of breast cancer cells. *J Bone Oncol*. 2014;3(1):10-17. <https://doi.org/10.1016/j.jbo.2014.02.001>
  92. Denoyelle C, Vasse M, Körner M, et al. Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: an in vitro study. *Carcinogenesis*. 2001;22(8):1139-1148. <https://doi.org/10.1093/carcin/22.8.1139>
  93. Wang T, Seah S, Loh X, et al. Simvastatin-induced breast cancer cell death and deactivation of PI3K/Akt and MAPK/ERK signalling are reversed by metabolic products of the mevalonate pathway. *Oncotarget*. 2016;7(3):2532-2544. doi:10.18632/oncotarget.6304
  94. Addeo R, Altucci L, Battista T, et al. Stimulation of human breast cancer MCF-7 cells with estrogen prevents cell cycle arrest by HMG-CoA reductase inhibitors. *Biochem Biophys Res Commun*. 1996;220(3):864-870. <https://doi.org/10.1006/bbrc.1996.0494>
  95. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med*. 2008;14(12):1351-1356. <https://doi.org/10.1038/nm.1890>
  96. Kobayashi Y, Kashima H, Rahmanto YS, et al. Drug repositioning of mevalonate pathway inhibitors as antitumor agents for ovarian cancer. *Oncotarget*. 2017;8(42):72147-72156. doi:10.18632/oncotarget.20046
  97. Feng JL, Dixon-Suen SC, Jordan SJ, Webb PM. Statin use and survival among women with ovarian cancer: an Australian national data-linkage study. *Br J Cancer*. 2021;125(5):766-771. <https://doi.org/10.1038/s41416-021-01460-4>
  98. Majidi A, Na R, Jordan SJ, De Fazio A, Webb PM. Statin use and survival following a diagnosis of ovarian cancer: A prospective

- observational study. *Int J Cancer*. 2021;148(7):1608-1615. <https://doi.org/10.1002/ijc.33333>
99. Irvin S, Clarke MA, Trabert B, Wentzensen N Systematic review and meta-analysis of studies assessing the relationship between statin use and risk of ovarian cancer. *Cancer Causes Control*. 2020;31(10):869-879. <https://doi.org/10.1007/s10552-020-01327-8>
  100. Wang Y, Ren F, Song Z, Chen P, Liu S, Ouyang L Statin use and the risk of ovarian and endometrial cancers: a meta-analysis. *BMC Cancer*. 2019;19(1):730. <https://doi.org/10.1186/s12885-019-5954-0>
  101. Luo N, Guo J, Chen L, Yang W, Qu X, Cheng Z ARHGAP10, downregulated in ovarian cancer, suppresses tumorigenicity of ovarian cancer cells. *Cell Death Dis*. 2016;7(3):e2157. <https://doi.org/10.1038/cddis.2015.401>
  102. Jang HJ, Hong EM, Park SW, et al. Statin induces apoptosis of human colon cancer cells and downregulation of insulin-like growth factor 1 receptor via proapoptotic ERK activation. *Oncol Lett*. 2016;12(1):250-256. <https://doi.org/10.3892/ol.2016.4569>
  103. Hoque A, Chen H, Xu XC. Statin induces apoptosis and cell growth arrest in prostate cancer cells. *Cancer Epidemiol Biomarkers Prev*. 2008;17(1):88-94. <https://doi.org/10.1158/1055-9965.Epi-07-0531>
  104. Miyazawa Y, Sekine Y, Kato H, Furuya Y, Koike H, Suzuki K Simvastatin up-regulates annexin A10 that can inhibit the proliferation, migration, and invasion in androgen-independent human prostate cancer cells. *Prostate*. 2017;77(4):337-349. <https://doi.org/10.1002/pros.23273>
  105. Yu X, Pan Y, Ma H, Li W Simvastatin inhibits proliferation and induces apoptosis in human lung cancer cells. *Oncol Res*. 2013;20(8):351-357. <https://doi.org/10.3727/096504013x13657689382897>
  106. Okamoto S, Jiang Y, Kawamura K, et al. Zoledronic acid induces apoptosis and S-phase arrest in mesothelioma through inhibiting Rab family proteins and topoisomerase II actions. *Cell Death Dis*. 2014;5(11):e1517. <https://doi.org/10.1038/cddis.2014.475>
  107. Longo J, Smirnov P, Li Z, et al. The mevalonate pathway is an actionable vulnerability of t(4;14)-positive multiple myeloma. *Leukemia*. 2021;35(3):796-808. <https://doi.org/10.1038/s41375-020-0962-2>
  108. Wong WW, Dimitroulakos J, Minden MD, Penn LZ. HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia*. 2002;16(4):508-519. <https://doi.org/10.1038/sj.leu.2402476>
  109. Gong L, Xiao Y, Xia F, et al. The mevalonate coordinates energy input and cell proliferation. *Cell Death Dis*. 2019;10(4):327. <https://doi.org/10.1038/s41419-019-1544-y>
  110. Marcelli M, Cunningham GR, Haidacher SJ, et al. Caspase-7 is activated during lovastatin-induced apoptosis of the prostate cancer cell line LNCaP. *Cancer Res*. 1998;58(1):76-83.
  111. Agarwal B, Rao CV, Bhendwal S, et al. Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. *Gastroenterology*. 1999;117(4):838-847. [https://doi.org/10.1016/s0016-5085\(99\)70342-2](https://doi.org/10.1016/s0016-5085(99)70342-2)
  112. Xia Z, Tan MM, Wong WW, Dimitroulakos J, Minden MD, Penn LZ. Blocking protein geranylgeranylation is essential for lovastatin-induced apoptosis of human acute myeloid leukemia cells. *Leukemia*. 2001;15(9):1398-1407. <https://doi.org/10.1038/sj.leu.2402196>
  113. Joentausta RM, Rannikko A, Murtola TJ. Prostate cancer survival among statin users after prostatectomy in a Finnish nationwide cohort. *Prostate*. 2019;79(6):583-591. <https://doi.org/10.1002/pros.23768>
  114. Kornblau SM, Banker DE, Stirewalt D, et al. Blockade of adaptive defensive changes in cholesterol uptake and synthesis in AML by the addition of pravastatin to idarubicin + high-dose Ara-C: a phase 1 study. *Blood*. 2007;109(7):2999-3006. <https://doi.org/10.1182/blood-2006-08-044446>
  115. Fatehi Hassanabad A. Current perspectives on statins as potential anti-cancer therapeutics: clinical outcomes and underlying molecular mechanisms. *Transl Lung Cancer Res*. 2019;8(5):692-699. doi:10.21037/tlcr.2019.09.08
  116. Bjorkhem-Bergman L, Lindh JD, Bergman P What is a relevant statin concentration in cell experiments claiming pleiotropic effects? *Br J Clin Pharmacol*. 2011;72(1):164-165. <https://doi.org/10.1111/j.1365-2125.2011.03907.x>
  117. Gyawali B, Pantziarka P, Crispino S, Bouche G Does the oncology community have a rejection bias when it comes to repurposed drugs? *Ecancermedicalscience*. 2018;12:ed76. <https://doi.org/10.3332/ecancer.2018.ed76>
  118. Thibault A, Samid D, Tompkins AC, et al. Phase I study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. *Clin Cancer Res*. 1996;2(3):483-491.
  119. Holstein SA, Knapp HR, Clamon GH, Murry DJ, Hohl RJ. Pharmacodynamic effects of high dose lovastatin in subjects with advanced malignancies. *Cancer Chemother Pharmacol*. 2006;57(2):155-164. <https://doi.org/10.1007/s00280-005-0013-8>
  120. Yulian ED, Siregar NC, Bajuardji. Combination of simvastatin and FAC improves response to neoadjuvant chemotherapy in locally advanced breast cancer. *Cancer Res Treat*. 2021;53(4):1072-1083. <https://doi.org/10.4143/crt.2020.1024>
  121. Han JY, Lee SH, Yoo NJ, et al. A randomized phase II study of gefitinib plus simvastatin versus gefitinib alone in previously treated patients with advanced non-small cell lung cancer. *Clin Cancer Res*. 2011;17(6):1553-1560. <https://doi.org/10.1158/1078-0432.Ccr-10-2525>
  122. Kawata S, Yamasaki E, Nagase T, et al. Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. *Br J Cancer*. 2001;84(7):886-891. <https://doi.org/10.1054/bjoc.2000.1716>
  123. Seckl MJ, Ottensmeier CH, Cullen M, et al. Multicenter, Phase III, randomized, double-blind, placebo-controlled trial of pravastatin added to first-line standard chemotherapy in small-cell lung cancer (LUNGSTAR). *J Clin Oncol*. 2017;35(14):1506-1514. <https://doi.org/10.1200/JCO.2016.69.7391>
  124. Lim SH, Kim TW, Hong YS, et al. A randomised, double-blind, placebo-controlled multi-centre phase III trial of XELIRI/FOLFIRI plus simvastatin for patients with metastatic colorectal cancer. *Br J Cancer*. 2015;113(10):1421-1426. <https://doi.org/10.1038/bjc.2015.371>
  125. Konings IR, van der Gaast A, van der Wijk LJ, de Jongh FE, Eskens FA, Sleijfer S The addition of pravastatin to chemotherapy in advanced gastric carcinoma: a randomised phase II trial. *Eur J Cancer*. 2010;46(18):3200-3204. <https://doi.org/10.1016/j.ejca.2010.07.036>

126. Kim ST, Kang JH, Lee J, et al. Simvastatin plus capecitabine-cisplatin versus placebo plus capecitabine-cisplatin in patients with previously untreated advanced gastric cancer: a double-blind randomised phase 3 study. *Eur J Cancer*. 2014;50(16):2822-2830. <https://doi.org/10.1016/j.ejca.2014.08.005>
127. Hong JY, Nam EM, Lee J, et al. Randomized double-blinded, placebo-controlled phase II trial of simvastatin and gemcitabine in advanced pancreatic cancer patients. *Cancer Chemother Pharmacol*. 2014;73(1):125-130. <https://doi.org/10.1007/s00280-013-2328-1>
128. Ebetino FH, Hogan AM, Sun S, et al. The relationship between the chemistry and biological activity of the bisphosphonates. *Bone*. 2011;49(1):20-33. <https://doi.org/10.1016/j.bone.2011.03.774>
129. van Beek E, Pieterman E, Cohen L, Löwik C, Papapoulos S Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochem Biophys Res Commun*. 1999;264(1):108-111. <https://doi.org/10.1006/bbrc.1999.1499>
130. Fisher JE, Rogers MJ, Halasy JM, et al. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. *Proc Natl Acad Sci U S A*. 1999;96(1):133-138. <https://doi.org/10.1073/pnas.96.1.133>
131. Maalouf MA, Wiemer AJ, Kuder CH, Hohl RJ, Wiemer DF. Synthesis of fluorescently tagged isoprenoid bisphosphonates that inhibit protein geranylgeranylation. *Bioorg Med Chem*. 2007;15(5):1959-1966. <https://doi.org/10.1016/j.bmc.2007.01.002>
132. Fromiguet O, Lagneaux L, Body JJ. Bisphosphonates induce breast cancer cell death in vitro. *J Bone Miner Res*. 2000;15(11):2211-2221. <https://doi.org/10.1359/jbmr.2000.15.11.2211>
133. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs Ras membrane [correction of impairs membrane] localisation and induces cytochrome c release in breast cancer cells. *Br J Cancer*. 2002;86(9):1479-1486. <https://doi.org/10.1038/sj.bjc.6600297>
134. Boissier S, Ferreras M, Peyruchaud O, et al. Bisphosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. *Cancer Res*. 2000;60(11):2949-2954.
135. van der Pluijm G, Vloedgraven H, van Beek E, van der Wee-Pals L, Löwik C, Papapoulos S Bisphosphonates inhibit the adhesion of breast cancer cells to bone matrices in vitro. *J Clin Invest*. 1996;98(3):698-705. <https://doi.org/10.1172/jci118841>
136. Dieli F, Gebbia N, Poccia F, et al. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. *Blood*. 2003;102(6):2310-2311. <https://doi.org/10.1182/blood-2003-05-1655>
137. Benford HL, McGowan NW, Helfrich MH, Nuttall ME, Rogers MJ. Visualization of bisphosphonate-induced caspase-3 activity in apoptotic osteoclasts in vitro. *Bone*. 2001;28(5):465-473. [https://doi.org/10.1016/s8756-3282\(01\)00412-4](https://doi.org/10.1016/s8756-3282(01)00412-4)
138. Boissier S, Magnetto S, Frappart L, et al. Bisphosphonates inhibit prostate and breast carcinoma cell adhesion to unmineralized and mineralized bone extracellular matrices. *Cancer Res*. 1997;57(18):3890-3894.
139. Jagdev SP, Coleman RE, Shipman CM, Rostami HA, Croucher PI. The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence for synergy with paclitaxel. *Br J Cancer*. 2001;84(8):1126-1134. <https://doi.org/10.1054/bjoc.2001.1727>
140. Neville-Webbe HL, Evans CA, Coleman RE, Holen I Mechanisms of the synergistic interaction between the bisphosphonate zoledronic acid and the chemotherapy agent paclitaxel in breast cancer cells in vitro. *Tumour Biol*. 2006;27(2):92-103. <https://doi.org/10.1159/000092489>
141. Derenne S, Amiot M, Barille S, et al. Zoledronate is a potent inhibitor of myeloma cell growth and secretion of IL-6 and MMP-1 by the tumoral environment. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1999;14(12):2048-2056. <https://doi.org/10.1359/jbmr.1999.14.12.2048>
142. Wood J, Bonjean K, Ruetz S, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. *The Journal of pharmacology and experimental therapeutics*. 2002;302(3):1055-1061. <https://doi.org/10.1124/jpet.102.035295>
143. Tassone P, Tagliaferri P, Viscomi C, et al. Zoledronic acid induces antiproliferative and apoptotic effects in human pancreatic cancer cells in vitro. *British journal of cancer*. 2003;88(12):1971-1978. <https://doi.org/10.1038/sj.bjc.6600986>
144. Tenta R, Tiblalex D, Sotiriou E, Lembessis P, Manoussakis M, Koutsilieris M Bone microenvironment-related growth factors modulate differentially the anticancer actions of zoledronic acid and doxorubicin on PC-3 prostate cancer cells. *Prostate*. 2004;59(2):120-131. <https://doi.org/10.1002/pros.10363>
145. Tenta R, Pitulis N, Tiblalex D, et al. Mechanisms of the action of zoledronic acid on human MG-63 osteosarcoma cells. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. 2008;40(11):737-745. <https://doi.org/10.1055/s-2008-1078753>
146. Agabiti SS, Li J, Wiemer AJ. Geranylgeranyl diphosphate synthase inhibition induces apoptosis that is dependent upon GGPP depletion, ERK phosphorylation and caspase activation. *Cell Death Dis*. 2017;8(3):e2678. <https://doi.org/10.1038/cddis.2017.101>
147. Jiang P, Zhang P, Mukthavaram R, et al. Anti-cancer effects of nitrogen-containing bisphosphonates on human cancer cells. *Oncotarget*. 2016;7(36):57932-57942. doi:10.18632/oncotarget.10773
148. Kunzmann V, Bauer E, Wilhelm M Gamma/delta T-cell stimulation by pamidronate. *The New England journal of medicine*. 1999;340(9):737-738. <https://doi.org/10.1056/nejm199903043400914>
149. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M Stimulation of gammadelta T cells by amino-bisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood*. 2000;96(2):384-392.
150. Benzaid I, Monkkonen H, Stresing V, et al. High phospho-antigen levels in bisphosphonate-treated human breast tumors promote Vgamma9Vdelta2 T-cell chemotaxis and cytotoxicity in vivo. *Cancer research*. 2011;71(13):4562-4572. <https://doi.org/10.1158/0008-5472.CAN-10-3862>
151. Roelofs AJ, Jauhainen M, Monkkonen H, Rogers MJ, Monkkonen J, Thompson K Peripheral blood monocytes are responsible for gammadelta T cell activation induced by

- zoledronic acid through accumulation of IPP/DMAPP. *British journal of haematology*. 2009;144(2):245-250. <https://doi.org/10.1111/j.1365-2141.2008.07435.x>
152. Vantourout P, Mookerjee-Basu J, Rolland C, et al. Specific requirements for Vgamma9Vdelta2 T cell stimulation by a natural adenylated phosphoantigen. *Journal of immunology*. 2009;183(6):3848-3857. <https://doi.org/10.4049/jimmunol.0901085>
  153. Monkkonen H, Auriola S, Lehenkari P, et al. A new endogenous ATP analog (AppI) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates. *Br J Pharmacol*. 2006;147(4):437-445. <https://doi.org/10.1038/sj.bjp.0706628>
  154. Mitrofan LM, Pelkonen J, Monkkonen J The level of ATP analog and isopentenyl pyrophosphate correlates with zoledronic acid-induced apoptosis in cancer cells in vitro. *Bone*. 2009;45(6):1153-1160. <https://doi.org/10.1016/j.bone.2009.08.010>
  155. Berenson JR, Rosen LS, Howell A, et al. Zoledronic acid reduces skeletal-related events in patients with osteolytic metastases. *Cancer*. 2001;91(7):1191-1200. [https://doi.org/10.1002/1097-0142\(20010401\)91:7<1191::aid-cnrcr1119>3.0.co;2-0](https://doi.org/10.1002/1097-0142(20010401)91:7<1191::aid-cnrcr1119>3.0.co;2-0)
  156. Saad F, Gleason DM, Murray R, et al. A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. *Journal of the National Cancer Institute*. 2002;94(19):1458-1468. <https://doi.org/10.1093/jnci/94.19.1458>
  157. Rosen LS, Gordon D, Kaminski M, et al. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind, comparative trial. *Cancer journal*. 2001;7(5):377-387.
  158. Rosen LS, Gordon D, Tchekmedyian S, et al. Zoledronic acid versus placebo in the treatment of skeletal metastases in patients with lung cancer and other solid tumors: a phase III, double-blind, randomized trial—the Zoledronic Acid Lung Cancer and Other Solid Tumors Study Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2003;21(16):3150-3157. <https://doi.org/10.1200/JCO.2003.04.105>
  159. Morgan GJ, Davies FE, Gregory WM, et al. First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. *Lancet*. 2010;376(9757):1989-1999. [https://doi.org/10.1016/S0140-6736\(10\)62051-X](https://doi.org/10.1016/S0140-6736(10)62051-X)
  160. Morgan GJ, Davies FE, Gregory WM, et al. Long-term follow-up of MRC Myeloma IX trial: survival outcomes with bisphosphonate and thalidomide treatment. *Clin Cancer Res*. 2013;19(21):6030-6038. <https://doi.org/10.1158/1078-0432.CCR-12-3211>
  161. Morgan GJ. Further analyses of the Myeloma IX Study. *Lancet*. 2011;378(9793):768-769. [https://doi.org/10.1016/S0140-6736\(11\)61374-3](https://doi.org/10.1016/S0140-6736(11)61374-3)
  162. Early Breast Cancer Trialists' Collaborative G. Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. *Lancet*. 2015;386(10001):1353-1361. [https://doi.org/10.1016/S0140-6736\(15\)60908-4](https://doi.org/10.1016/S0140-6736(15)60908-4)
  163. Foroni C, Milan M, Strina C, et al. Pure anti-tumor effect of zoledronic acid in naive bone-only metastatic and locally advanced breast cancer: proof from the “biological window therapy”. *Breast Cancer Res Treat*. 2014;144(1):113-121. <https://doi.org/10.1007/s10549-014-2840-y>
  164. Aft R, Naughton M, Trinkaus K, et al. Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial. *Lancet Oncol*. 2010;11(5):421-428. [https://doi.org/10.1016/S1470-2045\(10\)70054-1](https://doi.org/10.1016/S1470-2045(10)70054-1)
  165. Banyas M, Solomayer EF, Gebauer G, et al. Influence of zoledronic acid on disseminated tumor cells in bone marrow and survival: results of a prospective clinical trial. *BMC Cancer*. 2013;13:480. <https://doi.org/10.1186/1471-2407-13-480>
  166. Coleman RE, Marshall H, Cameron D, et al. Breast-cancer adjuvant therapy with zoledronic acid. *The New England journal of medicine*. 2011;365(15):1396-1405. <https://doi.org/10.1056/NEJMoa1105195>
  167. James ND, Pirrie SJ, Pope AM, et al. Clinical outcomes and survival following treatment of metastatic castrate-refractory prostate cancer with docetaxel alone or with strontium-89, zoledronic acid, or both: the TRAPEZE randomized clinical trial. *JAMA oncology*. 2016;2(4):493-499. <https://doi.org/10.1001/jamaoncol.2015.5570>
  168. Jallouk AP, Paravastu S, Weilbaecher K, Aft RL. Long-term outcome of (neo)adjuvant zoledronic acid therapy in locally advanced breast cancer. *Breast Cancer Res Treat*. 2021;187(1):135-144. <https://doi.org/10.1007/s10549-021-06100-2>
  169. Li S, Chen P, Pei Y, et al. Addition of zoledronate to chemotherapy in patients with osteosarcoma treated with limb-sparing surgery: a phase III clinical trial. *Med Sci Monit*. 2019;25:1429-1438. doi:10.12659/MSM.913236
  170. Ishikawa T, Akazawa K, Hasegawa Y, et al. Survival outcomes of neoadjuvant chemotherapy with zoledronic acid for HER2-negative breast cancer. *J Surg Res*. 2017;220:46-51. <https://doi.org/10.1016/j.jss.2017.05.066>
  171. Kamba T, Kamoto T, Maruo S, et al. A phase III multicenter, randomized, controlled study of combined androgen blockade with versus without zoledronic acid in prostate cancer patients with metastatic bone disease: results of the ZAPCA trial. *Int J Clin Oncol*. 2017;22(1):166-173. <https://doi.org/10.1007/s10147-016-1037-2>
  172. Piperno-Neumann S, Le Deley MC, Redini F, et al. Zoledronate in combination with chemotherapy and surgery to treat osteosarcoma (OS2006): a randomised, multicentre, open-label, phase 3 trial. *Lancet Oncol*. 2016;17(8):1070-1080. [https://doi.org/10.1016/S1470-2045\(16\)30096-1](https://doi.org/10.1016/S1470-2045(16)30096-1)
  173. Clive AO, Hooper CE, Edey AJ, et al. A randomised controlled trial of intravenous zoledronic acid in malignant pleural disease: a proof of principle pilot study. *PLoS One*. 2015;10(3):e0118569. <https://doi.org/10.1371/journal.pone.0118569>
  174. Lyseng-Williamson KA. Zoledronic acid: a review of its use in breast cancer. *Drugs*. 2008;68(18):2661-2682. <https://doi.org/10.2165/0003495-200868180-00010>
  175. Chen T, Berenson J, Vescio R, et al. Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. *Journal of clinical pharmacology*.

- 2002;42(11):1228-1236. <https://doi.org/10.1177/009127002762491316>
176. Wiemer AJ, Yu JS, Lamb KM, Hohl RJ, Wiemer DF. Mono- and dialkyl isoprenoid bisphosphonates as geranylgeranyl diphosphate synthase inhibitors. *Bioorg Med Chem.* 2008;16(1):390-399. <https://doi.org/10.1016/j.bmc.2007.09.029>
  177. Wiemer AJ, Tong H, Swanson KM, & Hohl RJ (2007). Dig-eranyl bisphosphonate inhibits geranylgeranyl pyrophosphate synthase. *Biochemical and Biophysical Research Communications*, 353(4), 921–925. <https://doi.org/10.1016/j.bbrc.2006.12.094>
  178. Chen CKM, Hudock MP, Zhang Y, et al. Inhibition of geranylgeranyl diphosphate synthase by bisphosphonates: a crystallographic and computational investigation. *Journal of medicinal chemistry.* 2008;51(18):5594-5607. <https://doi.org/10.1021/jm800325y>
  179. Wills VS, Allen C, Holstein SA, Wiemer DF. Potent triazole bisphosphonate inhibitor of geranylgeranyl diphosphate synthase. *ACS Med Chem Lett.* 2015;6(12):1195-1198. <https://doi.org/10.1021/acsmchemlett.5b00334>
  180. Zhou X, Ferree SD, Wills VS, et al. Geranyl and neryl triazole bisphosphonates as inhibitors of geranylgeranyl diphosphate synthase. *Bioorg Med Chem.* 2014;22(9):2791-2798. <https://doi.org/10.1016/j.bmc.2014.03.014>
  181. Allen C, Kortagere S, Tong H, et al. Olefin isomers of a triazole bisphosphonate synergistically inhibit geranylgeranyl diphosphate synthase. *Mol Pharmacol.* 2017;91(3):229-236. <https://doi.org/10.1124/mol.116.107326>
  182. Wills VS, Metzger JI, Allen C, Varney ML, Wiemer DF, Holstein SA. Bishomoisoprenoid triazole bisphosphonates as inhibitors of geranylgeranyl diphosphate synthase. *Bioorg Med Chem.* 2017;25(8):2437-2444. <https://doi.org/10.1016/j.bmc.2017.02.066>
  183. Matthiesen RA, Varney ML, Xu PC, Rier AS, Wiemer DF, Holstein SA.  $\alpha$ -Methylation enhances the potency of isoprenoid triazole bisphosphonates as geranylgeranyl diphosphate synthase inhibitors. *Bioorg Med Chem.* 2018;26(2):376-385. <https://doi.org/10.1016/j.bmc.2017.10.023>
  184. Fairweather AER, Goetz DB, Schroeder CM, et al. Impact of alpha-modifications on the activity of triazole bisphosphonates as geranylgeranyl diphosphate synthase inhibitors. *Bioorg Med Chem.* 2021;44:116307. <https://doi.org/10.1016/j.bmc.2021.116307>
  185. Lacbay CM, Waller DD, Park J, et al. Unraveling the prenylation-cancer paradox in multiple myeloma with novel geranylgeranyl pyrophosphate synthase (GGPPS) inhibitors. *J Med Chem.* 2018;61(15):6904-6917. <https://doi.org/10.1021/acs.jmedchem.8b00886>
  186. Lee HF, Lacbay CM, Boutin R, et al. Synthesis and evaluation of structurally diverse C-2-substituted thienopyrimidine-based inhibitors of the human geranylgeranyl pyrophosphate synthase. *J Med Chem.* 2022;65(3):2471-2496. <https://doi.org/10.1021/acs.jmedchem.1c01913>
  187. Haney SL, Chhonker YS, Varney ML, et al. In vivo evaluation of isoprenoid triazole bisphosphonate inhibitors of geranylgeranyl diphosphate synthase: impact of olefin stereochemistry on toxicity and biodistribution. *J Pharmacol Exp Ther.* 2019;371(2):327-338. <https://doi.org/10.1124/jpet.119.258624>
  188. Haney SL, Varney ML, Chhonker Y, et al. In vivo evaluation of combination therapy targeting the isoprenoid biosynthetic pathway. *Pharmacol Res.* 2021;167:105528. <https://doi.org/10.1016/j.phrs.2021.105528>
  189. Haney SL, Chhonker YS, Varney ML, Talmon G, Murry DJ, Holstein SA. Preclinical investigation of a potent geranylgeranyl diphosphate synthase inhibitor. *Invest New Drugs.* 2018;36(5):810-818. <https://doi.org/10.1007/s10637-018-0571-3>
  190. Reilly JE, Neighbors JD, Tong H, Henry MD, Hohl RJ. Targeting geranylgeranylation reduces adrenal gland tumor burden in a murine model of prostate cancer metastasis. *Clin Exp Metastasis.* 2015;32(6):555-566. <https://doi.org/10.1007/s10585-015-9727-0>
  191. Zhang Y, Cao R, Yin F, et al. Lipophilic bisphosphonates as dual farnesyl/geranylgeranyl diphosphate synthase inhibitors: an X-ray and NMR investigation. *J Am Chem Soc.* 2009;131(14):5153-5162. <https://doi.org/10.1021/ja808285e>
  192. Holstein SA, Hohl RJ. Isoprenoid biosynthetic pathway inhibition disrupts monoclonal protein secretion and induces the unfolded protein response pathway in multiple myeloma cells. *Leuk Res.* 2011;35(4):551-559. <https://doi.org/10.1016/j.leukres.2010.08.008>
  193. Lacbay CM, Waller DD, Park J, et al. Unraveling the prenylation-cancer paradox in multiple myeloma with novel geranylgeranyl pyrophosphate synthase (GGPPS) inhibitors. *Journal of medicinal chemistry.* 2018;61(15):6904-6917. <https://doi.org/10.1021/acs.jmedchem.8b00886>
  194. Haney SL, Varney ML, Chhonker YS, et al. Inhibition of geranylgeranyl diphosphate synthase is a novel therapeutic strategy for pancreatic ductal adenocarcinoma. *Oncogene.* 2019;38(26):5308-5320. <https://doi.org/10.1038/s41388-019-0794-6>
  195. Coxon FP, Helfrich MH, Van't Hof R, et al. Protein geranylgeranylation is required for osteoclast formation, function, and survival: inhibition by bisphosphonates and GGTI-298. *J Bone Miner Res.* 2000;15(8):1467-1476. <https://doi.org/10.1359/jbmr.2000.15.8.1467>
  196. Wiemer AJ, Wiemer DF, Hohl RJ. Geranylgeranyl diphosphate synthase: an emerging therapeutic target. *Clin Pharmacol Ther.* 2011;90(6):804-812. <https://doi.org/10.1038/clpt.2011.215>
  197. Dudakovic A, Tong H, Hohl RJ. Geranylgeranyl diphosphate depletion inhibits breast cancer cell migration. *Invest New Drugs.* 2011;29(5):912-920. <https://doi.org/10.1007/s10637-010-9446-y>
  198. Wasko BM, Dudakovic A, Hohl RJ. Bisphosphonates induce autophagy by depleting geranylgeranyl diphosphate. *J Pharmacol Exp Ther.* 2011;337(2):540-546. <https://doi.org/10.1124/jpet.110.175521>
  199. Dykstra KM, Allen C, Born EJ, Tong H, Holstein SA. Mechanisms for autophagy modulation by isoprenoid biosynthetic pathway inhibitors in multiple myeloma cells. *Oncotarget.* 2015;6(39):41535-41549. doi:10.18632/oncotarget.6365
  200. Agabiti SS, Li J, Dong W, Poe MM, Wiemer AJ. Regulation of the Notch-ATM-abl axis by geranylgeranyl diphosphate synthase inhibition. *Cell Death Dis.* 2019;10(10):733. <https://doi.org/10.1038/s41419-019-1973-7>

201. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004;306(5694):269-271. <https://doi.org/10.1126/science.1102160>
202. Reilly JE, Neighbors JD, Hohl RJ. Targeting protein geranylgeranylation slows tumor development in a murine model of prostate cancer metastasis. *Cancer Biol Ther*. 2017;18(11):872-882. <https://doi.org/10.1080/15384047.2016.1219817>
203. Xia Y, Liu YL, Xie Y, et al. A combination therapy for KRAS-driven lung adenocarcinomas using lipophilic bisphosphonates and rapamycin. *Sci Transl Med*. 2014;6(263):263ra161. <https://doi.org/10.1126/scitranslmed.3010382>
204. Suh H, Pillai K, Morris DL. Mucins in pancreatic cancer: biological role, implications in carcinogenesis and applications in diagnosis and therapy. *Am J Cancer Res*. 2017;7(6):1372-1383.

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