

Crosstalk Between mTOR and p70S6 Kinase

Talat Ilyass¹, Iram Rashid ², Shafat A. Latoo^{1,*}

¹ Department of Biochemistry, Cluster University, Srinagar, Jammu and Kashmir, India; (0009-0008-1401-1962)

² Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Jammu and Kashmir, India; (0009-0000-0258-9013)

Abstract

The mechanistic target of rapamycin (mTOR) sits at the center of the PI3K/mTOR axis, a signaling network frequently dysregulated in human malignancies. mTOR integrates environmental cues, such as nutrient availability, growth factors, and cellular energy to coordinate anabolic processes essential for cell growth and proliferation. Traditionally, mTOR Complex 1 (mTORC1) was seen as unidirectionally activating its primary downstream effector, p70 ribosomal S6 kinase (p70S6K), recent evidence positions p70S6K as a critical determinant of therapeutic outcome. This review challenges the reductionist view of p70S6K as merely a translational regulator, exploring its mutual governance with both mTORC1 and the rapamycin-insensitive mTORC2. p70S6K functions as a feedback node modulating insulin signaling via Insulin Receptor Substrate-1 (IRS-1) and cross-regulates mTORC2 through direct phosphorylation of Rictor. In oncology, these homeostatic loops drive therapeutic resistance: pharmacological mTORC1 inhibition inadvertently relieves these brakes, reactivating survival signaling and undermining treatment durability. Furthermore, we address the functional divergence between the S6K1 and S6K2 isoforms, emphasizing the overlooked nuclear functions of S6K2 and hypothesizing that their phase-separation properties are a mechanism of adaptive stress resistance that may allow malignant cells to sequester survival signaling from pharmacological attack. Through the combined perspectives of structural biology and phosphoproteomics, we highlight recent discoveries, including the controversial physiological role of mTORC2 in phosphorylating the S6K1 hydrophobic motif and the ribosome as a physical scaffold dictating spatiotemporal signaling between mTOR complexes. Finally, we discuss how combinatorial strategies targeting this crosstalk, exemplified by the VIKTORIA-1 trial, offer more favorable therapeutic outcomes than single-node inhibition.

Keywords:

p70S6 kinase; mTOR; signaling; cancer; VIKTORIA-1 clinical trial

1. Introduction

The ability of eukaryotic cells to oversee growth, proliferation, and metabolism with environmental nutrient availability is rudimentary to cellular homeostasis. Few disruptions to this balance carry graver clinical consequences than the dysregulation of mTOR axis in cancer. While the pharmacological inhibition of mTOR has become a mainstay of targeted oncology, first-generation treatments have repeatedly demonstrated limited clinical durability. The reason lies in a therapeutic paradox: inhibiting mTOR severs the critical negative feedback loops that maintain network homeostasis, inadvertently reactivating parallel survival pathways [1,2]. Understanding the molecular basis of this paradox is the central aim of this review.

mTOR functions as the central regulatory hub for anabolic processes within the cell. mTOR manifests in two structurally and functionally distinct complexes: the rapamycin-sensitive mTOR Complex 1 (mTORC1), defined by its Raptor subunit, and the rapamycin-insensitive mTOR Complex 2 (mTORC2), distinguished by Rictor [3,4]. To affect the cellular machinery, mTORC1 relies on a primary downstream effector: the p70 ribosomal S6 kinase (p70S6K), which exists as the twin isoforms S6K1 and S6K2. For years, the canonical doctrine described this relationship as a strictly linear transduction event, whereas mTORC1 activates p70S6K to drive ribosome biogenesis and protein synthesis.

* Corresponding Author:

Shafat A. Latoo, Department of Biochemistry, Cluster University, Srinagar, Jammu and Kashmir, India, e-mail@email.com (0000-0003-0003-3251)



© 2026 Copyright by the Authors.

Licensed as an open access article using a CC BY 4.0 license.

However, recent biochemical and structural observations reveal that this linear model is insufficient to explain clinical drug resistance. The relationship is not a one-directional communication, but a reciprocal dialogue characterized by bidirectional communication and highly integrated, context-dependent feedback loops. This complex network fundamentally dictates both physiological health and disease states. Rather than acting solely as a downstream target, p70S6K functions as a critical feedback node. It reaches upstream to inhibit insulin signaling via Insulin Receptor Substrate-1 (IRS-1), a mechanism that actively aggravates insulin resistance in metabolic conditions like type 2 diabetes [5,6]. Similarly, S6K1 directly phosphorylates Rictor, suppressing mTORC2 and Akt survival signaling.

In oncology, the pharmacological inhibition of mTOR unintentionally releases these exact loops, reactivating compensatory survival signaling and limiting clinical efficacy. Therefore, this review seeks to move beyond reductionist textbook models of unidirectional flow to synthesize the mutual regulatory interactions defining this signaling axis.

We aim to break down both canonical and non-canonical activation mechanisms of p70S6K, analyze the functional divergence of S6K1 and S6K2 (including frequently overlooked nuclear and spatial functions), and investigate how the dysregulation of this crosstalk drives adaptive drug resistance.

Finally, we will identify unresolved controversies, such as the true identity of the physiological hydrophobic motif kinase, and formulate a new hypothesis regarding the phase-separation properties of S6K isoforms during cellular stress. Ultimately, making these intricate feedback loops come to light, it is also essential to developing better therapeutics, ensuring that our mechanistic understanding of this cellular dialogue effectively translates to the clinic.

1.1. The Molecular Architecture of mTOR Complexes

To truly grasp the delicate crosstalk between mTOR and p70S6 Kinase (p70S6K), we must first define the structural framework that dictates their interactions. The mechanistic target of rapamycin (mTOR) is a massive, highly conserved serine/threonine kinase (approximately 289 kDa) belonging to the phosphoinositide 3-kinase-related kinase (PIKK) family. It serves as the catalytic core of two structurally and functionally distinct multiprotein complexes: “mTORC1 and mTORC2.” Where specialized accessory proteins control the respective substrates, subcellular localizations, and sensitivities to pharmacological inhibition.

1.1.1. mTOR Complex 1 (mTORC1): The Anabolic Engine

mTORC1 has historically had the image of being the rapamycin-sensitive mTOR signaling complex that functions to sense nutrient (amino acids), energy (ATP), and growth factor status [3].

The primary functions of mTORC1 serve as the primary anabolic engine for protein synthesis, ribosome biogenesis, and lipogenesis while actively inhibiting autophagy [3].

mTORC1 consists of several different subunits, which define its composition:

- **Raptor (*rapamycin-sensitive mTOR regulatory associated protein*)** is the primary core component of mTORC1, as it is the major allosteric regulator. Raptor has a crucial function as it recruits downstream substrates such as p70S6K via binding to TOS motifs, bringing them into close proximity to the mTOR kinase domain to mediate phosphorylation.
- **mLST8 (*mammalian lethal with SEC13 protein 8*)**: Core component that binds adjacent to the catalytic cleft of mTOR kinase domain, stabilizing an active conformation of the kinase domain.
- **Endogenous Inhibitors (*PRAS40 and Deptor*)**: These proteins bind to mTORC1 and prevent its activity in the absence of upstream signals that will signal the dissociation of PRAS40 from the mTORC1 complex.

1.1.2. mTOR Complex 2 (mTORC2): The Survival and Cytoskeletal Regulator

The organization of the mTORC2 structure differs substantially from that of mTORC1, even though they share the mTOR catalytic core, as well as the mLST8 component of mTORC1; the main function of mTORC2 is to control cellular survival and cytoskeletal organization through the phosphorylation of Akt at Ser473 [3].

Rictor is the defining mTORC2 component, which replaces Raptor. Rictor also lacks binding to TOS motifs but instead points to the AGC family of kinases (such as Akt, SGK1, and PKC), which are phosphorylated by the mTORC2 kinase.

The integrity of the mTORC2 structure depends on mSin1 (the mammalian SAPK-interacting protein 1). mSin1 has a PH domain that helps tether mTORC2 to the plasma membrane and serves as a central hub for feedback inhibition by p70S6K.

In addition, while it was originally believed to be rapamycin-insensitive due to the physical obstruction of the rapamycin target (FKBP12/FRB) by Rictor, rapamycin treatment will ultimately inhibit mTORC2 if given over an extended duration, as this will sequester the new pool of mTOR kinases produced during the rapamycin treatment [3].

This structural distinction carries direct pharmacological weight, because rapalogs act through FKBP12/FRB and only partially engage mTORC2, tumors frequently escape mTORC1-selective inhibition by falling back on mTORC2-driven Akt survival signaling, which is the rationale behind developing ATP-competitive inhibitors that block both complexes simultaneously [7].

I.1.3. Stoichiometric Crosstalk: The Shared Core

Apprehending this architectural dichotomy is essential. While textbooks distinguish between these complexes, structural biology indicates that the catalytic mTOR subunit and mLST8 in both complexes can compete with one another due to the fact that they share the same components [3].

The sharing of these elements suggests a mechanism for what can be revealed as “stoichiometric crosstalk,” whereby the sequestration of mTOR into one of the two complexes may have a negative effect on the formation of the second complex [3].

As an example, if a cell produces excess Raptor, it could potentially sequester mTOR into mTORC1 and thereby leave a reduced amount of mTOR available for use by mTORC2.

As a result, the first level of crosstalk will occur not through phosphorylation but rather through the precise stoichiometric balance of each of the two complexes being formed.

I.2. p70S6 Kinase: Overview

I.2.1. The Domain Structure of p70S6K

The structural basis for the regulation of the p70S6K protein Kinase has a modular functional domain structure. When p70S6K exists in its basal resting state, it is in a closed inactive conformation.

The major regions of the p70S6K Kinase include:

- **The N-terminal Domain:** Contains the TOR Signaling (TOS) Motif, an acidic motif that mediates p70S6K's direct association with the Raptor subunit of mTORC1.
- **Catalyst Domain:** The conserved AGC Kinase Core that contains the ATP binding pocket and the T-loop Activation Loop. Phosphorylation of a specific threonine residue (Thr229) within the T-loop by PDK1 is a required condition for p70S6K's catalytic function.
- **Linker Domain:** Serves as a critical structural connector between the Catalyst Domain and the C-Terminus of p70S6K, that contains the Hydrophobic Motif (HM or Thr389), which is the rate-limiting step for activation once phosphorylated.
- **Autoinhibitory Domain (AID) at C-Terminus:** The basic region behaves like a pseudosubstrate. In the inactive form, it folds onto itself and overlaps with both the N-terminal and C-terminal catalytic domains, physically covering the active site, preventing phosphorylation of both the T-loop and HM.

I.2.2. Structural Divergence of S6K Isoforms: S6K1 vs. S6K2

Although commonly known as one molecule, mammalian genomes encode two forms (homologs) of p70S6K protein: S6K1 encoded on chromosome 17q23 by the gene *RPS6KB1*, and *S6K2* located at 11q13 by the gene *RPS6KB2*. Notably, 17q23 is a recurrently amplified locus in human breast cancer, directly implicating this isoform and its structural framework in oncogenesis [8].

Originally, it was believed that these two proteins were redundant because of their >80% sequence similarity (identity) in the active site of their respective catalytic kinase domains. However, there are substantial differences

between S6K1 and S6K2 with respect to both the amino-terminal and carboxyl-terminal regions of the proteins that regulate both their individual intracellular locations, partner proteins, and cellular functions.

S6 Kinase 1 (S6K1): The prototypical isoform and major reagent for somatic cell growth and metabolic regulation, S6K1 is predominantly present in the cytoplasm as its shorter isoform, p70. The PDZ binding region located at the carboxy-terminal end of S6K1 allows this kinase to interact with the actin cytoskeleton and other proteins, such as neurabin. S6K1 is the major enzyme for phosphorylation of ribosomal protein S6 and initiation of the negative feedback loop via Insulin Receptor Substrate-1 (IRS-1).

S6 Kinase 2 (S6K2): The presence of a putative Nuclear Localization Signal (NLS) in the carboxy terminus of S6K2 directs it primarily to the cell nucleus. In addition, the presence of a proline-rich sequence at the carboxy terminus of S6K2 provides functional interaction with scaffold proteins containing SH3 or WW domains. Because of the nuclear localization of S6K2, it can mediate events that are not simply due to general translation, but can also affect cellular processes such as chromatin remodeling and RNA processing. S6K2 is the major enzyme for phosphorylating Histone H3 at threonine 45 and is heavily involved in the continued persistence of stress granules during periods of cellular stress.

These structural differences confirm that while S6K1 governs cytosolic translation and systemic metabolic feedback, S6K2 acts as a distinct mediator linking mTOR signaling directly to nuclear transcription and survival pathways (Table 1).

Among the additional substrates in the Table 1, S6K1 also phosphorylates the tumor suppressor Programmed Cell Death 4 (PDCD4) at Ser67, targeting it for SCF β TrCP-mediated ubiquitination and proteasomal degradation; because PDCD4 normally restrains the RNA helicase eIF4A, its removal relieves this brake on cap-dependent translation initiation [9].

Table 1. Structural and Functional Comparison of S6K1 and S6K2.

Feature	p70S6K1 (S6K1)	p70S6K2 (S6K2)
Gene	<i>RPS6KB1</i>	<i>RPS6KB2</i>
Primary Isoforms	p70 (cytosolic), p85 (nuclear)	p54 (nuclear), p56
Localization	Predominantly Cytosolic	Predominantly Nuclear
C-Terminal Domain	PDZ-binding domain (interacts with actin cytoskeleton/neurabin)	Proline-rich domain (interacts with SH3 domains)
NLS/NES	p85 has N-terminal NLS; p70 has C-terminal NES and undergoes stimulus-dependent nuclear-cytoplasmic shuttling	Functional C-terminal NLS
Key Substrates	Ribosomal protein S6, eIF4B, IRS-1, Rictor, PDCD4	Histone H3, hnRNPA1, BCL-XL mRNA
Knockout Phenotype	Small body size (mice), hypoinsulinemia	Normal body size, compensatory S6K1 upregulation
Stress Response	SG Assembly promoter	SG Maintenance/Persistence

Note: PRAS40, discussed above as endogenous TOS-motif-containing inhibitor of mTORC1, is not itself an S6K1/S6K2 substrate and is therefore omitted from the Key substrates row; it instead competes with S6K1 for Raptor docking upstream of S6K1 activation.

1.2.3. p70S6K in Cancer

p70S6K, which was first identified as an essential regulator of mitogen-stimulated protein synthesis, is specifically involved in the translation of a certain class of mRNAs with a 5' terminal oligopyrimidine (TOP) sequence [10]. Experimental disruption of p70S6K activity has been shown to significantly inhibit organismal growth in model animals such as *Drosophila* and mice [11,12]. Subsequent research has demonstrated that p70S6K plays a critical role in controlling the proliferation and survival of cancer cells by influencing multiple molecular signaling pathways [13]. Additionally, a number of recently identified functional pathways suggest that p70S6K signaling plays a role in the genesis of cancer.

An important biological process involved in both adult tissue healing and embryonic development is the epithelial–mesenchymal transition (EMT), which enables epithelial cells to adopt mesenchymal characteristics [14]. An increasing amount of evidence suggests that EMT and cancer stem-like traits, particularly during ovarian cancer invasion and metastasis, depend on p70S6K activation. Activation of the HGF/c-Met pathway stimulates p70S6K signaling, which increases the expression and proteolytic activity of matrix metalloproteinase-9 (MMP9), hence

driving EMT-associated phenotypic alterations and tumor cell invasiveness [15]. Additionally, by upregulating Snail, a transcriptional inhibitor of E-cadherin, p70S6K facilitates the mesenchymal transition [16].

p70S6K also regulates cytoskeletal architecture by stabilizing actin filaments and activating Rho-family GTPases, like Rac1 and Cdc42, which promote cancer cell motility [17–19]. Additionally, p70S6K controls microRNA synthesis and encourages the maturity of miR-145 by disrupting the connection between Dicer and tristetraprolin. Two results of this process are the formation of three-dimensional tumor spheroids and increased N-cadherin expression [20]. Furthermore, p70S6K facilitates spheroid attachment and colonization through the P-cadherin/integrin β 1 signaling pathway, supporting spheroid–peritoneal interactions during metastatic progression [21]. These findings are supported by research demonstrating p70S6K’s function in leukemia formation and hematopoietic stem cell self-renewal, which suggests a broader role for p70S6K in regulating cancer stemness [22]. Human cancers typically activate the mTOR signaling pathway and its downstream serine/threonine kinase p70S6K, and dysregulation of the mTOR pathway is linked to a number of human disease processes, particularly different forms of cancer [23–27].

The cancer cell lines that show resistance to a variety of chemotherapeutic drugs, such as paclitaxel, erlotinib, pemetrexed, cisplatin, and doxorubicin, have often been found to demonstrate upregulation of the Akt/mTOR/p70S6K signaling axis [28,29]. Several inhibitors targeting the PI3K/Akt/mTOR signaling cascade have been explored for suppressing p70S6K activity. XL418, a dual inhibitor of Akt and p70S6K developed by Exelixis, demonstrated strong inhibition of S6 phosphorylation, minimal off-target kinase activity, and acceptable ADME characteristics in preclinical evaluations [30]. LY2780301, developed by Eli Lilly, entered early clinical testing and showed good tolerability, although its anticancer efficacy was limited and drug plasma levels did not correlate well with p70S6K expression [31]. A subsequent Phase IB dose-escalation study reported manageable toxicity and moderate therapeutic benefit when combined with gemcitabine in patients with PI3K/Akt/mTOR pathway alterations [32]. Rapamycin and its analogs (rapalogs) have also been widely studied. Although rapamycin exhibits strong immunosuppressive and anticancer properties, its poor solubility and pharmacokinetic limitations encouraged the development of improved derivatives [33]. Everolimus and temsirolimus have gained FDA approval for treating multiple cancer types, including breast, renal, and neuroendocrine tumors, and have demonstrated promising clinical outcomes [34,35]. Additionally, the dual p70S6K/Akt inhibitor M2698 showed favorable tolerability and partial therapeutic responses when combined with standard breast cancer treatments [36].

I.3. The Mechanisms of p70S6K Activation: Canonical Models and New Paradigms

The classical understanding of S6K1 activation describes an elegant, sequential relay of phosphorylation events. In quiescent cells, S6K1 remains dormant, locked in a “closed,” autoinhibited conformation mediated by an interaction between its N-terminal and C-terminal regulatory domains.

I.3.1. The Canonical Activation Model vs. New Paradigms

Full activation of the S6K1 kinase involves three separate but related phases of sequential activation, which creates a logical “AND” gate that requires multiple simultaneous positive conditions prior to initiating energy-consuming processes associated with protein synthesis.

Step 1: Primal Activation (MAPK Convergence)

The process begins at the C-terminal Autoinhibitory Domain (AID). In this process, mitogenic signals act through proline-directed kinases (such as MAPKs, e.g., ERK or CDKs) and phosphorylate specific Serine and Threonine residues in the AID (Ser411, Ser418, Thr421 & Ser424). The priming effects of this phosphorylation create a conformational change in the AID that allows the pseudosubstrate to be displaced from the active site of the p70S6K catalytic domain (the central catalytic domain and linker region) [37]. At this point, mitogenic signals have activated p70S6K for subsequent activation by nutrient signals (first critical node of crosstalk).

Step 2: Hydrophobic Motif Switch (Thr389)

After the AID has swung open to allow for the displacement of the pseudosubstrate, the hydrophobic motif (HM) amino acid Thr389 is now exposed. The TM threonine is recognized as the primary (and only site that can be phosphorylated) by the mTORC1 System in the canonical sequence. The phosphorylation of Thr389 by the mTORC1 system represents the rate-limiting step for the activation of p70S6K, a universal indicator of mTORC1 activation, because this step is typically inhibited by rapamycin [38].

Step 3: Phosphorylation of Thr229 in the Activation Loop

A high-affinity binding site is formed when PDK1 binds to Thr389. PDK1 can reach Thr229 in the activation loop and phosphorylate it. This is where p70S6K becomes completely active because the catalytic residues are perfectly lined up for the transfer of ATP.

Paradigms Shifting: eIF4E and TOS motif Interaction.

The original assumption about the nature of the S6K1-TOS interaction in relation to mTORC1 signaling has been challenged by recent literature. The original assumption was that the only method by which Raptor (mTORC1's defining subunit) recruited S6K1 was through its binding to the S6K1 TOS motif. Recent biochemistry has identified eIF4E—a eukaryotic initiation factor—as a significant mediator/receptor in the mTORC1/S6K1 signaling process [39,40]. Instead of eIF4E being merely a downstream target of mTORC1, it has been hypothesized that the interaction of the mTORC1—phosphorylated form of eIF4E and the S6K1 TOS motif facilitates the phosphorylation of S6K1 as well as the relief of the autoinhibited state of S6K1—a role previously thought to be restricted to multisite priming phosphorylations.

This new paradigm shift now establishes a clear feed-forward mechanism whereby the translational machinery eIF4E serves as a physical scaffold that directly stimulates the activation of the kinase S6K1, which promotes translation.

I.3.2. The Hydrophobic Motif Controversy: mTORC1 vs. mTORC2

One of the greatest mechanistic controversies regarding the identity of the kinase that is responsible for critical Step 2, HM phosphorylation of S6K1, continues to revolve around the fact that mTORC1 remains the accepted kinase within the classical model [41].

Recent research using precise genetic mutants and specific mTOR inhibitors (e.g., Torin-1) has begun to undermine the idea of mTORC1 being the only kinase. Truncation mutants of S6K1 (lacking N-terminal and Raptor-binding domains) can still undergo Thr389 phosphorylation, which is also resistant to rapamycin (mTORC1-specific inhibitor), has dependence upon Rictor, and is sensitive to Torin-1 (which inhibits both complexes) [41].

The studies in this area demonstrate evidence supporting “tandem” or “fail-safe” activation models, whereby:

- **Step 1:** mTORC1 can be considered an accessibility kinase for S6K1, facilitating either via the eIF4E-initiated mechanism or by freeing the autoinhibitory constraints of S6K1.
- **Step 2:** mTORC2 has been proposed as an alternative kinase that may catalyzes the phosphorylation of Thr389 once it is accessible, though this remains an emerging hypothesis rather than the established model; mTORC2's role as the HM kinase for Akt (Ser473) and PKC, by contrast, is well established.

This framework must, however, be reconciled with contemporary structural insight into how mTORC2 regulates its other AGC-kinase clients. For Akt and PKC, mTORC2 doesn't phosphorylate the hydrophobic motif directly. Instead, it targets a separate, evolutionarily conserved site called the TOR-Interaction Motif (TIM). TIM phosphorylation relieves an autoinhibitory conformation, which lets PDK1 phosphorylate the activation loop; the kinase then autophosphorylates its own hydrophobic motif intramolecularly [42]. Sequence-conservation analyses show that S6K family kinases carry this same TIM sequence, but whether mTORC2 phosphorylates this site in S6K1, or whether it drives hydrophobic motif autophosphorylation as it does for Akt and PKC, has not been experimentally tested [43]. Proposals that mTORC2 phosphorylates S6K1's Thr389 directly therefore sit outside the mechanism now established for Akt and PKC, raising the possibility that S6K1 either undergoes the same TIM-dependent priming mechanism or reflects a distinct, direct mode of mTORC2 regulation.

This model explains why catalytic inhibitors of mTOR are more potent suppressors of S6K1 activity than rapamycin. It suggests that the rapamycin sensitivity of S6K1 HM phosphorylation might be an indirect effect of blocking the priming/accessibility step (Step 1) rather than inhibiting the direct kinase [41].

Resolving the identity of this kinase holds direct therapeutic implications. If mTORC2 genuinely contributes to Thr389 phosphorylation, then inhibitors capable of blocking both complexes should achieve more complete suppression of S6K1 survival signaling than rapamycin alone, consistent with the improved potency of catalytic mTOR inhibitors observed in resistant tumors [44].

I.4. The Mechanics of Crosstalk: Feedback Loops and Regulatory Crosstalk

The signaling output of the mTOR–p70S6K axis is not unidirectional; it does not simply end at the ribosome.

One of the most critical and defining functions of activated p70S6K is its capacity to provide retrograde feedback to upstream signaling nodes. In oncology, the disruption of these specific feedback loops is the central mechanism

driving targeted therapy failure. When pharmacological agents suppress mTOR, they inadvertently release these biological brakes, triggering the hyperactivation of parallel pathways that sustain tumor survival. While these loops evolved to maintain systemic metabolic homeostasis, in malignant tissues they instead function as robust engines for adaptive drug resistance, driving the same dysregulation that underlies insulin resistance in Type 2 Diabetes (Figure 1).

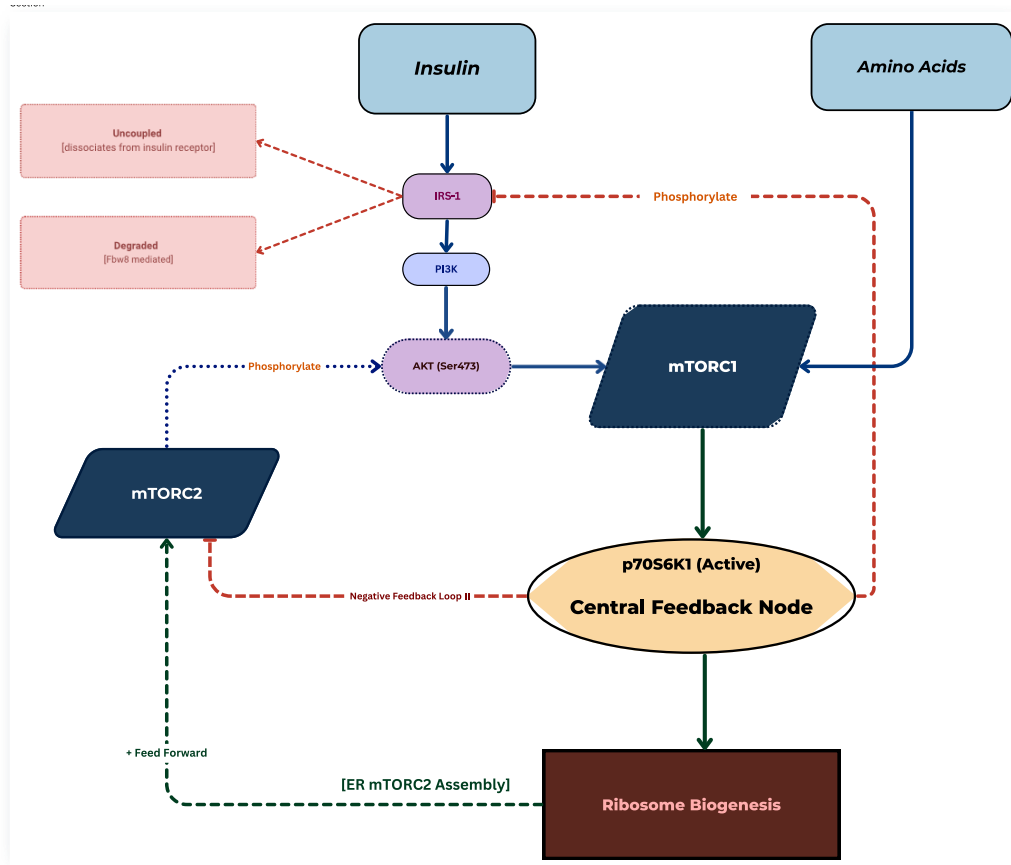


Figure 1. mTOR-p70S6K1 signaling cascade.

1.4.1. The IRS-1 Negative Feedback Loop (The Insulin Brake)

There is one well-known biological feedback mechanism where insulin signaling through Insulin Receptor Substrate-1 (IRS-1) is inhibited by S6K1. This occurs when there are high amounts of nutrients and growth factors available and results in hyperactivation of S6K1.

At this point, S6K1 returns up the signaling cascade to phosphorylate IRS-1 on multiple serine residues (e.g., Ser307, Ser636, Ser1101 in humans) [5,6,45]. The phosphorylation of serine residues on IRS-1 causes a large conformational change, which creates two primary consequences:

- **Uncoupling**—The phosphorylation of IRS-1 on serine residues prevents the binding of the Phosphotyrosine Binding domain on IRS-1 to the juxtamembrane region of the Insulin Receptor (IR). This uncouples the receptor from the substrate and therefore prevents the essential tyrosine phosphorylation required for the forward signaling from IR.
- **Degradation**—The phosphorylation of IRS-1 on specific serine residues acts as a signal for the recruitment of E3 ubiquitin ligases (like Fbw8 (F-box and WD repeat domain-containing 8) to mark IRS-1 for degradation via the proteasome.

Furthermore, this degradation involves a spatial component; after Fbw8 is phosphorylated by mTORC2, Fbw8 increases in stability, translocates from the nucleus to the cytosol, and degrades IRS-1. This demonstrates that both mTOR complexes actively work together to enforce this negative feedback mechanism [46].

Physiological vs. Pathological Context: The mechanism described above provides short-term desensitization to continuous stimulation of cells in a healthy physiological state, preventing unregulated, abnormal cell proliferation.

In contrast to the normal physiological state, in pathological states such as chronic hypernutrition or obesity, once S6K1 is continuously and constitutively activated, IRS-1 will continuously be degraded, leading to systemic insulin resistance and inability of cells to respond to insulin even when insulin is present in high concentrations, ultimately resulting in Type 2 Diabetes Mellitus.

I.4.1.1. Inter-Complex Crosstalk: Silencing mTORC2

The regulatory segment of S6K1 doesn't just shape upstream sensors; it also drives direct engagement with mTORC2, revealing a dual inhibitory mechanism inside the mTOR signaling web.

- **Phosphorylation of Rictor at Thr1135:** S6K1 phosphorylates Rictor at Thr1135. This modification does not disrupt mTORC2 assembly or its in vitro kinase activity; however, cells expressing a non-phosphorylatable Rictor T1135A mutant show increased mTORC2-dependent Akt phosphorylation relative to wild-type, indicating that Thr1135 phosphorylation modestly restrains Akt signaling output without dismantling the complex itself [47,48].
- **Sin1 Loop Alteration:** S6K1 also phosphorylates Sin1 at Thr86 and Thr398. These modifications reduce Sin1's association with mTOR and Rictor, destabilizing mTORC2 integrity.

Since mTORC2 activation drives Akt signaling, this feedback loop is one mechanism constraining Akt activity [47,48].

I.4.1.2. MAPK/ERK Crosstalk: Coincidence Detection

The signaling web also serves multiple signal integration points; the p70S6K integrates signals from additional primary pathways.

The MAPK/ERK signaling pathway is most known for its role in regulating cell division and mitogenesis; however, S6K1 receives signals from ERK as well. ERK can phosphorylate S6K1 at its C-terminal autoinhibitory sites (Ser421/Thr424); however, this priming phosphorylation does not fully activate S6K1 [37].

This node has direct clinical relevance in non-small-cell lung cancer, where Raf-1 sustains p70S6K expression to drive tumor proliferation, nominating the Raf-1/p70S6K axis as a therapeutic target [49].

This priming mechanism allows for cross-talk between the MAPK/ERK and mTOR pathways to coordinate cellular energy use to support protein synthesis. Only when nutrient sensors (mTOR signaling) indicate nutrient abundance and cell growth signals (MAPK signaling) confirm a desire to divide does the energy of a cell get expended for maximal synthesis of protein.

I.5. Spatial and Context-Specific Signaling

For many years, the cellular interior has been regarded as a chaotic and disorganized "cytosolic soup," a nonuniform mixture in which kinases and substrates inadvertently collide to propagate their signals.

Contemporary studies on the mTOR-p70S6K axis show that, rather than merely a chemical cascade, it is fundamentally regulated by the spatial compartmentalization of all molecules involved and by their physical state.

I.5.1. Liquid-Liquid Phase Separation (LLPS) and Stress Granules

When environmental insults (such as oxidative stress, proteotoxicity, or severe heat shock) occur, cells must quickly stop energy-consuming processes if they are to survive.

During these times, translation is rapidly ceased, and free mRNA-protein complexes condense into droplet-like structures called Stress Granules (SGs) through an interesting biophysical phenomenon called liquid-liquid phase separation (LLPS).

Within this newly formed, phase-separated environment, the two isoforms of S6K exhibit distinct and specialized behaviors:

- **S6K1 Promotes Granule Assembly:** S6K1 does not remain diffuse throughout the cytosol but actively promotes granule assembly by its physical localization within them. This mechanism of spatial feedback is very unique because it effectively quarantines the enzyme away from its anabolic substrates on the ribosome, thereby enforcing translational arrest of all substrates by physically sequestering the kinase inside these droplet-like structures. This capability enables the cell to quickly allocate its limited resources from growth activities to immediate repair and survival activities [50].
- **S6K2 and the Anchor of Persistence:** Persistent S6K2 serves as the structural support for the survival mechanism; S6K2 is essential for both persistence and maintenance of SG. If S6K2 is knocked down, then the granules become unstable and rapidly dissolve, resulting in the cell becoming more susceptible to continuing stress [50].

1.5.2. The Spatial Paradox: NOP14 and ER-Bound Ribosomes

The concept of a negative feedback mechanism regulating ribosome production was already understood until researchers discovered the critical role that NOP14 had in enhancing ribosome biogenesis.

NOP14, a target of mTORC1 via S6K, has established a new positive feedback mechanism for stimulating ribosome biogenesis. S6K, which has been shown to inhibit mTORC2 by phosphorylating Sin1 in the cytoplasm, also stimulates the expression of NOP14. NOP14 mediates the assembly of a large number of new ribosomes and directs them to bind to the rough endoplasmic reticulum (ER). There is evidence to suggest that the ribosomes that are bound to the ER activate and recruit mTORC2; however, this does not apply to free ribosomes in the cytoplasm [51].

This complicates our understanding of the mTOR pathway as follows:

- Activating mTORC1 leads to an increase in p70S6K.
- Increased p70S6K leads to increased NOP14 expression and ribosome biogenesis.
- Newly formed ribosomes bind to the ER.
- Ribosomes bound to the ER recruit and activate mTORC2.
- mTORC2 phosphorylates Akt, resulting in the continued support and activation of the entire system.

This new discovery completely changes the way that we think about crosstalk by removing the idea that there is a proximity element to crosstalk functions. Instead, there are two distinct functions in the two different places mentioned above.

In the free cytosol, S6K acts as a strong brake (inhibiting IRS and Sin), while at the endoplasmic reticulum membrane, ribosomes driven by S6K act as a very strong accelerator of survival. The presence of NOP14 at high levels in cancer cells serves to stabilize the mTORC2 that is bound to the endoplasmic reticulum, which leads to artificially increased levels of Akt activation.

However, this complete reliance on NOP14 creates a unique hypersensitivity of malignant cells to mTOR inhibitors. When S6K activity is clinically inhibited, NOP14 levels drop dramatically, resulting in a loss of ribosomes from the endoplasmic reticulum and a loss of stability of mTORC2, thus breaking the loop that shields the cancer cell from apoptosis [51].

1.5.3. The Uncharted Nuclear Domain of S6K2

The nuclear space is distinguished from the cytoplasmic compartment. While S6K1 is primarily present in the cytosol and modulates translation, S6K2 predominantly resides in the nucleus and carries out non-translational duties as an epigenetic regulator and as an RNA processing protein.

- **Epigenetic Modifications:** S6K2 directly phosphorylates histone H3 at residue Threonine 45 (Thr45), resulting in an epigenetic modification of the nuclear architecture that makes DNA more “open” or accessible, thereby facilitating the transcription of many genes associated with cellular growth and survival [52].
- **RNA Processing and Transport:** S6K2 also directly impacts the distribution of newly synthesized RNA in the nucleus before it exits to the cytosol. Specifically, S6K2 phosphorylates hnRNPA1 at Serines 4 and 6. This post-

translational modification of hnRNPA1 is required to facilitate transport of this protein and the specific mRNA species associated with that particular protein (e.g., Bcl-xL and XIAP) [52].

This nuclear mechanism is of paramount clinical importance. It reveals S6K2 as a formidable, independent driver of therapeutic resistance in malignancies such as Small Cell Lung Cancer (SCLC) and breast cancer.

By ensuring that these specific survival transcripts are safely exported and shielded, S6K2 actively protects the cancer cell from death, operating entirely independently of the global protein synthesis rates governed by its sibling, S6K1 [53,54].

1.6. Crosstalk with Other Signaling Pathways

The mTOR-p70S6K pathway serves as an integration hub that aligns local nutrient availability with systemic signals from unrelated signal transduction pathways. By creating a way for signals from other pathways to communicate with mTORC1, this allows growth to be perfectly timed with the needs of development and survival.

1.6.1. Hedgehog (Hh) Signaling and the Primary Cilium

The primary cilium is often referred to as the antenna of the cell and is the main point of interaction between the mTOR pathway and Hh signaling. During embryonic development and maintenance of stem cells, the cilium plays an important role in mediating Hh signaling via ciliary functions. There is also strong evidence now that the primary cilium creates a robust two-way communication between the Hh pathway and the mTOR pathway.

- **Upstream Stimulation:** When Hh signaling is activated, the transcription factor NKX2.2 is expressed, which inhibits the negative regulators of mTORC1 and therefore enhances the output of mTORC1.
- **Reciprocal Amplification:** Similarly, p70S6K phosphorylates GLI1, the primary transcriptional effector of the Hh signaling cascade, and causes GLI1 to translocate to the nucleus; thereby increasing transcriptional activities.

The development of this loop allows for both self-perpetuating and self-sustaining effects as a function of an ongoing cycle. This is significant because there are many scenarios involving Shh-driven malignancies (such as medulloblastoma and basal cell carcinoma (BCC)) that require this cross-amplification as a means of not only existence but also eventual proliferation.

In this regard, the resultant pharmacological inhibition of both Shh and mTOR tends to exhibit synergistic efficacy [55,56].

2. Dysregulation in Disease and Therapeutic Implications

When the elegant mutual governance of the mTOR-p70S6K axis is corrupted, the consequences reverberate throughout the entire organism. The dysregulation of these specific crosstalk nodes serves as the primary engine for two of the most pervasive modern pathologies: metabolic syndrome and metastatic cancer.

2.1. Breast Cancer and the Therapeutic Paradox

The feedback loops that occur in physiology will continue to evolve into barriers to effective treatment through the drug targets of the targeted therapy. The interaction/communication between the estrogen receptor, CDK4/6, and PI3K/mTOR as it relates to the growth and progression of HR+/HER2-breast cancer will be the primary mechanism of disease progression and resistance to the drug (i.e., drug or targeted therapy). When patients start standard of care (SOC) therapies, such as CDK4/6 inhibitors (i.e., palbociclib), at some point, the drug will fail to have activity. This failure of activity will produce a phenomenon known as feedback relief. As the drug inhibits the cell cycle and inhibits upstream PI3K signaling, there will be a decrease in the amount of active S6K1.

Although there will be an initial inhibition of the tumor growth, there will be simultaneous feedback relief from the block of the IRS-1 and the block of Rictor, which will lead to a compensatory large reactivation of Akt (via mTORC2) and the Ras/MAPK pathways, ultimately allowing the tumor to maintain its survival signaling and continue existing regardless of whether the drug is being administered or not [1,57].

2.2. Targeting the Crosstalk: The VIKTORIA-I Trial

Targeting multiple nodes of this network simultaneously is one therapeutic strategy suggested by this crosstalk model. VIKTORIA-1 is a phase 3 randomized clinical trial that evaluated Gedatolisib, an intravenous pan-PI3K/mTOR inhibitor that targets all class I PI3K isoforms as well as both mTOR complexes, mTORC1 and mTORC2 (Figure 2). The rationale for this treatment strategy was to block upstream PI3K/mTOR signaling while simultaneously limiting the compensatory reactivation of Akt (via mTORC2) and IRS-1 that typically follows single-node inhibition.

In the PIK3CA wild-type cohort of patients with HR+/HER2- advanced breast cancer who had progressed on prior CDK4/6 inhibitor plus aromatase inhibitor therapy, the Gedatolisib triplet (Gedatolisib + Palbociclib + Fulvestrant) achieved a significantly longer progression-free survival than Fulvestrant alone, with a hazard ratio of 0.24 (95% CI, 0.17 to 0.35); $p < 0.001$ versus Fulvestrant alone [58].

These results support the roader principle that effective treatment of complex cancers may require targeting multiple architectural nodes of crosstalk rather than a single linear pathway.

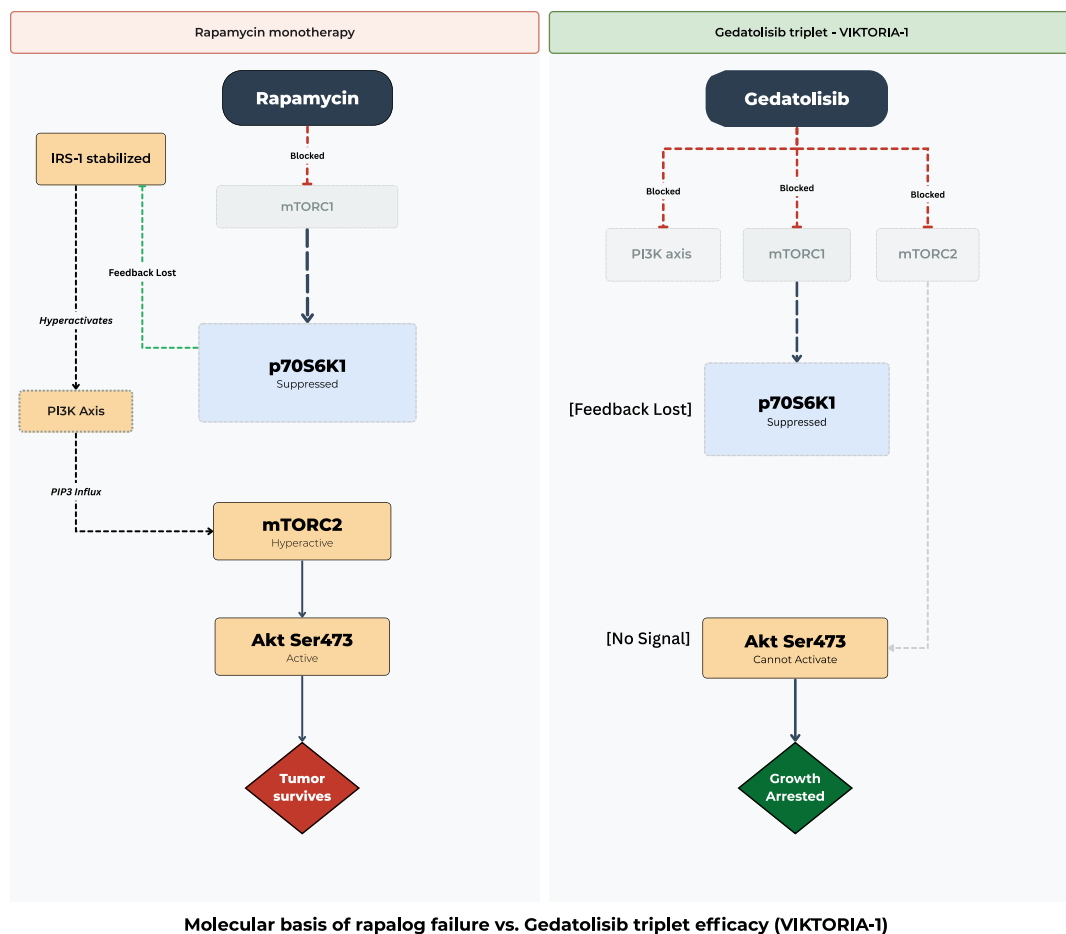


Figure 2. Molecular basis of rapalog failure vs. Gedatolisib triplet efficacy (VIKTORIA-1).

2.3. Type 2 Diabetes and the Broken Brake

There is a strong correlation between the pathophysiology of Type II diabetes and the S6K1/IRS1 feedback loop. There is an abundance of circulating nutrients, and for that reason, a constant and unceasing activation of mTORC1 and S6K1 will lead to excessive phospho-IRS-1, and thus, degradation of IRS-1 allows peripheral tissues to become blind to the action of insulin [5,59].

The relative strength of the biological pathway is exemplified in knockout models. The S6K1 knockout mice have profound protection against diet-induced obesity and insulin resistance. There was also no phosphorylation of

IRS-1, which was stable, and maintained a high level of activity when S6K1-KnockOuts were put on a high-fat diet; they continued to maintain systemic insulin sensitivity [5]. This speaks very clearly too why S6K1 is such a powerful target for metabolic therapeutics; however, systemic side effects of broadly inhibiting cellular protein synthesis necessitate due diligence on the part of pharmacologists.

3. Research Gaps and Discussion

3.1. The Neglected Isoform: S6K2

The other form of the kinase S6K2 is often overlooked by many scientists and researchers. There are actually two genes for the S6 kinase in the mammalian genome, and both genes have multiple splice variants. The great majority of literature published currently focuses only on the cytosolic form of S6K1 (p70-S6K1). Thus, the unique functions of S6K2 have become a significant blind spot in the scientific field. Because S6K2 is located predominantly in the nucleus and has known roles in epigenetics (e.g., via phosphorylation of histones) and in exporting mRNA, S6K2 is probably a key player in mediating the long-term adaptation of cells to the environment in which they exist.

As a result, the research community needs to decouple the functional roles of each of these two kinases to clearly understand how the mTOR pathway regulates transcription primarily through the regulation of mRNA translation globally.

3.2. Spatial Segregation of Opposing Feedback Loops

The most perplexing gap among these regulatory pathways is the spatial separation of these pathways. There is sufficient evidence that S6K1 functions as a powerful negative “brake” feedback mechanism located at the membrane (via IRS-1 and Sin1) [45,46]. On the other hand, a growing body of literature suggests that S6K1 also acts via a positive feed-forward loop from NOP14 and ribosomes located on the ER [51].

The current scientific literature lacks a thorough model to explain how a single kinase can provide the membrane with a “stop” signal and the ER with an “accelerate” signal at the same time. Understanding the spatial compartmentalization of the separate pathways that regulate the two negative feedback loops is of great importance.

3.2.1. The Biomechanics of Stress Granules

Stress-granule biology carries direct therapeutic relevance, as malignant cells may exploit liquid-liquid phase separation (LLPS) to physically shield survival signaling substrates from pharmacological inhibition. However, S6K’s integration into this biophysical phenomenon remains largely unexplored. While its physical partitioning into stress granules during cellular crisis is established, the biochemical state of S6K within these condensates is entirely unknown [50,60]. Future research must determine whether S6K is inhibited to enforce translational arrest, or if it remains active to phosphorylate novel substrates within this phase-separated environment.

3.2.2. The Need for Dynamic Clinical Biomarkers

Creating usable clinical applications out of this complex biology will take more than demonstrating the benefits of targeting feedback networks (like VIKTORIA-1). The Oncologist community is short of standard biomarkers that will assist them in determining whether or not patient loops are working or are stifled in real time [61–64].

With most diagnostic methods still relying on measuring levels of proteins in the first place and, in many cases, measuring one phosphorylation event at a time, there is a need for sophisticated, dynamic biomarkers that can accurately identify how dependent a tumor is on its particular feedback mechanism, thereby enabling clinicians to rationally apply combination therapies prior to any potential resistance developing.

4. Future Directions

To actively address the profound gaps identified above, the next era of signal transduction research must transition from cataloging phosphorylation sites to decoding the biophysical and spatial realities of the cell.

Future investigations should prioritize the following imperatives:

- **Phase Separation Profiling:** Using advanced biophysical techniques such as Fluorescence Recovery After Photobleaching (FRAP) and droplet assays to perform in vitro studies of the phase separating properties of S6K1 and S6K2 is critical for elucidating how their biophysical state and ability to condense when stressed will be modified by phosphorylation from mTORC1 and mTORC2.
- **Isoform-Selective Inhibitors:** The first step is to create isoform-selective inhibitors targeting S6K1 and S6K2. Currently, there is no pharmacologic agent that selectively targets either isoform, and the indiscriminate targeting of both isoforms by pharmacological agents has made it difficult to advance treatment options for cancer patients whose cancers are being driven by nuclear survival signaling. The development of drug agents that selectively target S6K2 may present a potential therapeutic paradigm shift in the treatment of cancers driven by nuclear survival signals, as these drugs would allow clinicians to selectively eradicate malignant epigenetic remodeling while preserving the patients' systemic, S6K1-mediated metabolic control. A first proof-of-concept step in this direction has already been reported, with a covalent, highly isoform-selective S6K2 inhibitor validating the feasibility of this approach [65].
- **Nuclear mTORC2-S6K2 Axis:** The research must focus on the existence of a distinguishable resident pool of mTORC2 in the nucleus. If we can demonstrate that nuclear mTORC2 directly activates S6K2 in the nucleus, then we have identified a completely new class of signaling pathways, which would deliver growth factor signals directly to the chromatin and circumvent the cytoplasm and the translational machinery that converts growth factor signals to protein.

5. Conclusions

Cellular metabolism is governed by the structural and functional interactions between mTOR signal transduction pathways and p70S6 kinase (p70S6K). The previous chapters of this review have shown that mTOR and p70S6K exist within a complex interdependent and bidirectional regulatory environment in which both components play significant roles in how the other modulates or shapes its particular signaling environment through numerous potent negative feedback loops that target IRS-1, Rictor, and Sin1.

As such, identifying the networks involved in this interdependence requires a new way of thinking. Specifically, we need to think of the mTOR signaling metabolic junction as an adaptive, dynamically regulated homeostat rather than a rigid, one-dimensional linear “pathway” with clearly defined boundaries.

When one complex is inhibited by applying brakes blindly, a cell will always use its negative feedback loops to reroute survival signals elsewhere. The success of various modern combination therapies (such as those studied in VIKTORIA-1) confirms that by focusing on the crosstalk mechanism (e.g., blocking escape routes instead of just the main road), effective disruption of the tumor's survival network is possible. While the mTOR-p70S6K axis provides an elegant mechanism for ensuring harmonious and balanced growth in healthy physiology, it becomes a powerful mechanism for disease once the mutual regulation between these components is disrupted. With continued research, understanding the spatial and biophysical dimensions of this conversation will be the ultimate avenue for developing the next generation of precision therapies against diseases like cancer.

List of Abbreviations

Akt	Protein Kinase B
BCC	Basal Cell Carcinoma
CDK4/6	Cyclin-dependent kinase 4 and 6
CDKs	Cyclin-dependent kinase
EMT	Epithelial–mesenchymal transition
ER	Endoplasmic Reticulum
ERK	Extracellular Signal-Regulated Kinase

FKBP12/FRB FK506-binding protein 12

FRAP	Fluorescence Recovery After Photobleaching
GLI1	Glioma-associated oncogene homolog 1
HER2	Human epidermal growth factor receptor 2
IRS-1	Insulin Receptor Substrate-1
LLPS	Liquid-liquid phase separation
MAPKs	Mitogen activated protein kinase
mLST8	Mammalian lethal with SEC13 protein 8
mSin1	The mammalian SAPK-interacting protein 1
mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
mTORC2	Mechanistic target of rapamycin complex 2
NOP14	Nucleolar protein 14
p70S6K	p70S6 kinase
PDK1	3-phosphoinositide-dependent protein kinase-1
PI3K	Phosphatidylinositol 3 Kinase
PIKK	Phosphoinositide 3-kinase-related kinase family
PKC	Protein Kinase C
PRAS40	Proline-Rich Akt Substrate of 40 kDa
Raptor	Rapamycin-sensitive mTOR regulatory associated protein
Rictor	Rapamycin-insensitive companion of mTOR complex 2
S6K1	Ribosomal protein S6 kinase beta-1
S6K2	Ribosomal protein S6 kinase beta-2
SGK1	Serum and Glucocorticoid-Regulated Kinase 1
Shh	Sonic hedgehog
SOC	Standard Of Care
TOP	5' terminal oligopyrimidine sequence
TOS	Target of rapamycin

Author Contributions

Conceptualization: S.A.L.; Methodology: S.A.L.; Investigation: S.A.L.; Writing—original draft preparation: T.I., I.R. and S.A.L.; Writing—review and editing: T.I., I.R. and S.A.L.; Visualization: T.I. and S.A.L.; Supervision: S.A.L. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interests

The authors declare no conflict of interest.

Funding

This work received no external funding.

Acknowledgments

The author would like to thank Professor Muktar Mahajan, Hunter Medical Laboratory Sciences, The City University of New York, USA, for constructive criticism of the manuscript.

AI Statement

The authors declare that no generative artificial intelligence technologies were used to generate scientific content, data, analysis, or conclusions presented in this manuscript. AI-assisted writing-support tools, specifically Grammarly, was used during manuscript preparation for language editing, grammar correction, and rephrasing to improve clarity and readability. Graphical abstract and figures were produced by using Canva (Canva Pty Ltd.), depicting no copyrighted or third-party material and prepared without the use of AI image-generation tools.

References

- [1] Jiang, M.; Zhang, K.; Zhang, Z.; Zeng, X.; Huang, Z.; Qin, P.; Xie, Z.; Cai, X.; Ashrafizadeh, M.; Tian, Y.; et al. PI3K/AKT/mTOR Axis in Cancer: From Pathogenesis to Treatment. *MedComm* **2025**, *6*(8), e70295. <https://doi.org/10.1002/mco2.70295>.
- [2] Glaviano, A.; Foo, A.S.C.; Lam, H.Y.; Yap, K.C.H.; Jacot, W.; Jones, R.H.; Eng, H.; Nair, M.G.; Makvandi, P.; Geoerger, B.; et al. PI3K/AKT/mTOR Signaling Transduction Pathway and Targeted Therapies in Cancer. *Mol. Cancer* **2023**, *22*(1), 138. <https://doi.org/10.1186/s12943-023-01827-6>.
- [3] Szwed, A.; Kim, E.; Jacinto, E. Regulation and Metabolic Functions of mTORC1 and mTORC2. *Physiol. Rev.* **2021**, *101*(3), 1371–1426. <https://doi.org/10.1152/physrev.00026.2020>.
- [4] Saxton, R.A.; Sabatini, D.M. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **2017**, *168*(6), 960–976. <https://doi.org/10.1016/j.cell.2017.02.004>.
- [5] Zhang, J.; Gao, Z.; Yin, J.; Quon, M.J.; Ye, J. S6K Directly Phosphorylates IRS-1 on Ser-270 to Promote Insulin Resistance in Response to TNF- α Signaling through IKK2. *J. Biol. Chem.* **2008**, *283*(51), 35375–35382. <https://doi.org/10.1074/jbc.M806480200>.
- [6] Boura-Halfon, S.; Zick, Y. Phosphorylation of IRS Proteins, Insulin Action, and Insulin Resistance. *Am. J. Physiol. Endocrinol. Metab.* **2009**, *296*(4), E581–E591. <https://doi.org/10.1152/ajpendo.90437.2008>.
- [7] Hossain, M.T.; Hossain, M.A. Targeting mTOR Kinase for Cancer Treatment: A Comprehensive Review with Clinical Insights. *Drug Dev. Res.* **2025**, *86*(7), e70167. <https://doi.org/10.1002/ddr.70167>.
- [8] Sridhar, J.; Komati, R.; Kumar, S. Targeting RPS6K1 for Refractory Breast Cancer Therapy. In *Breast Cancer*; Mayrovitz, H.N., Ed.; Exon Publications: Brisbane, Australia, 2022. <https://doi.org/10.36255/exon-publications-breast-cancer-rps6k1>.
- [9] Dorrello, N.V.; Peschiaroli, A.; Guardavaccaro, D.; Colburn, N.H.; Sherman, N.E.; Pagano, M. S6K1- and β TRCP-Mediated Degradation of PDCD4 Promotes Protein Translation and Cell Growth. *Science* **2006**, *314*(5798), 467–471. <https://doi.org/10.1126/science.1130276>.
- [10] Jefferies, H.B.J.; Fumagalli, S.; Dennis, P.B.; Reinhard, C.; Pearson, R.B.; Thomas, G. Rapamycin Suppresses 5'TOP mRNA Translation through Inhibition of p70s6k. *EMBO J.* **1997**, *16*(12), 3693–3704. <https://doi.org/10.1093/emboj/16.12.3693>.
- [11] Montagne, J.; Stewart, M.J.; Stocker, H.; Hafen, E.; Kozma, S.C.; Thomas, G. Drosophila S6 Kinase: A Regulator of Cell Size. *Science* **1999**, *285*(5436), 2126–2129. <https://doi.org/10.1126/science.285.5436.2126>.
- [12] Shima, H.; Pende, M.; Chen, Y.; Fumagalli, S.; Thomas, G.; Kozma, S.C. Disruption of the p70s6k/p85s6k Gene Reveals a Small Mouse Phenotype and a New Functional S6 Kinase. *EMBO J.* **1998**, *17*(22), 6649–6659. <https://doi.org/10.1093/emboj/17.22.6649>.
- [13] Magnuson, B.; Ekim, B.; Fingar, D.C. Regulation and Function of Ribosomal Protein S6 Kinase (S6K) within mTOR Signalling Networks. *Biochem. J.* **2012**, *441*(1), 1–21. <https://doi.org/10.1042/BJ20110892>.

- [14] Nieto, M.A.; Huang, R.Y.; Jackson, R.A.; Thiery, J.P. EMT: 2016. *Cell* **2016**, *166*(1), 21–45. <https://doi.org/10.1016/j.cell.2016.06.028>.
- [15] Zhou, H.Y.; Wong, A.S. Activation of p70S6K Induces Expression of Matrix Metalloproteinase 9 Associated with Hepatocyte Growth Factor-Mediated Invasion in Human Ovarian Cancer Cells. *Endocrinology* **2006**, *147*(5), 2557–2566. <https://doi.org/10.1210/en.2005-1404>.
- [16] Pon, Y.L.; Zhou, H.Y.; Cheung, A.N.; Ngan, H.Y.; Wong, A.S. p70 S6 Kinase Promotes Epithelial to Mesenchymal Transition through Snail Induction in Ovarian Cancer Cells. *Cancer Res.* **2008**, *68*(16), 6524–6532. <https://doi.org/10.1158/0008-5472.CAN-07-6302>.
- [17] Ip, C.K.; Cheung, A.N.; Ngan, H.Y.; Wong, A.S. p70 S6 Kinase in the Control of Actin Cytoskeleton Dynamics and Directed Migration of Ovarian Cancer Cells. *Oncogene* **2011**, *30*(21), 2420–2432. <https://doi.org/10.1038/onc.2010.615>.
- [18] Latoo, S.A.; Andrabi, K.I. Phosphorylation State of S6K1 Is Redundant for Its Blood Interaction with F Actin. *J. Blood Disord. Transfus.* **2023**, *14*(S3), 11. <https://doi.org/10.21203/rs.3.rs-2503726/v1>
- [19] Latoo, S.A. Expression and Purification of p70 (Δ NH2-146/ Δ CT104) S6 K, a 57.3 kDa N- and C-Terminal Truncated p70S6 Kinase-GST Fusion Protein in Bacterial Expression System. *Protein J.* **2026**, *45*(2), 245–253. <https://doi.org/10.1007/s10930-025-10308-2>.
- [20] Lam, S.S.; Ip, C.K.; Mak, A.S.; Wong, A.S. A Novel p70 S6 Kinase-MicroRNA Biogenesis Axis Mediates Multicellular Spheroid Formation in Ovarian Cancer Progression. *Oncotarget* **2016**, *7*(25), 38064–38077. <https://doi.org/10.18632/oncotarget.9345>.
- [21] Ip, C.K.; Yung, S.; Chan, T.M.; Tsao, S.W.; Wong, A.S. p70 S6 Kinase Drives Ovarian Cancer Metastasis through Multicellular Spheroid-Peritoneum Interaction and P-Cadherin/ β 1 Integrin Signaling Activation. *Oncotarget* **2014**, *5*(19), 9133–9149. <https://doi.org/10.18632/oncotarget.2362>.
- [22] Ghosh, J.; Kobayashi, M.; Ramdas, B.; Chatterjee, A.; Ma, P.; Mali, R.S.; Carlesso, N.; Liu, Y.; Plas, D.R.; Chan, R.J.; et al. S6K1 Regulates Hematopoietic Stem Cell Self-Renewal and Leukemia Maintenance. *J. Clin. Invest.* **2016**, *126*(7), 2621–2625. <https://doi.org/10.1172/JCI84565>.
- [23] Noh, W.C.; Kim, Y.H.; Kim, M.S.; Koh, J.S.; Kim, H.A.; Moon, N.M.; Paik, N.S. Activation of the mTOR Signaling Pathway in Breast Cancer and Its Correlation with the Clinicopathologic Variables. *Breast Cancer Res. Treat.* **2008**, *110*, 477–483. <https://doi.org/10.1007/s10549-007-9746-x>.
- [24] Zhou, Y.; Pan, Y.; Zhang, S.; Shi, X.; Ning, T.; Ke, Y. Increased Phosphorylation of p70 S6 Kinase Is Associated with HPV16 Infection in Cervical Cancer and Esophageal Cancer. *Br. J. Cancer* **2007**, *97*, 218–222. <https://doi.org/10.1038/sj.bjc.6603838>.
- [25] Moore, S.M.; Rintoul, R.C.; Walker, T.R.; Chilvers, E.R.; Haslett, C.; Sethi, T. The Presence of a Constitutively Active Phosphoinositide 3-Kinase in Small Cell Lung Cancer Cells Mediates Anchorage-Independent Proliferation via a Protein Kinase B and p70s6k-Dependent Pathway. *Cancer Res.* **1998**, *58*, 5239–5247.
- [26] Nozawa, H.; Watanabe, T.; Nagawa, H. Phosphorylation of Ribosomal p70 S6 Kinase and Rapamycin Sensitivity in Human Colorectal Cancer. *Cancer Lett.* **2007**, *251*, 105–113. <https://doi.org/10.1016/j.canlet.2006.11.008>.
- [27] Grewe, M.; Gansauge, F.; Schmid, R.M.; Adler, G.; Seufferlein, T. Regulation of Cell Growth and Cyclin D1 Expression by the Constitutively Active FRAP-p70s6K Pathway in Human Pancreatic Cancer Cells. *Cancer Res.* **1999**, *59*, 3581–3587.
- [28] Foster, H.; Coley, H.M.; Goumenou, A.; Pados, G.; Harvey, A.; Karteris, E. Differential Expression of mTOR Signalling Components in Drug Resistance in Ovarian Cancer. *Anticancer Res.* **2010**, *30*(9), 3529–3534.
- [29] Qin, Y.; Sekine, I.; Hanazono, M.; Morinaga, T.; Fan, M.; Takiguchi, Y.; Tada, Y.; Shingyoji, M.; Yamaguchi, N.; Tagawa, M. AMPK Activation Induced in Pemetrexed-Treated Cells Is Associated with Development of Drug Resistance Independently of Target Enzyme Expression. *Mol. Oncol.* **2019**, *13*(6), 1419–1432. <https://doi.org/10.1002/1878-0261.12496>.
- [30] Bussenius, J.; Anand, N.K.; Blazey, C.M.; Bowles, O.J.; Bannen, L.C.; Chan, D.S.-M.; Chen, B.; Co, E.W.; Costanzo, S.; DeFina, S.C.; et al. Design and Evaluation of a Series of Pyrazolopyrimidines as p70S6K Inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*(6), 2283–2286. <https://doi.org/10.1016/j.bmcl.2012.01.105>.

- [31] Azaro, A.; Rodon, J.; Calles, A.; Braña, I.; Hidalgo, M.; Lopez-Casas, P.P.; Munoz, M.; Westwood, P.; Miller, J.; Moser, B.A.; et al. A First-in-Human Phase I Trial of LY2780301, a Dual p70 S6 Kinase and Akt Inhibitor, in Patients with Advanced or Metastatic Cancer. *Invest. New Drugs* **2015**, *33*(3), 710–719. <https://doi.org/10.1007/s10637-015-0241-7>.
- [32] Angevin, E.; Cassier, P.A.; Italiano, A.; Gonçalves, A.; Gazzah, A.; Terret, C.; Toulmonde, M.; Gravis, G.; Varga, A.; Parlavaccchio, C.; et al. Safety, Tolerability and Antitumour Activity of LY2780301 (p70S6K/AKT Inhibitor) in Combination with Gemcitabine in Molecularly Selected Patients with Advanced or Metastatic Cancer: A Phase IB Dose Escalation Study. *Eur. J. Cancer* **2017**, *83*, 194–202. <https://doi.org/10.1016/j.ejca.2017.06.036>.
- [33] Li, J.; Kim, S.G.; Blenis, J. Rapamycin: One Drug, Many Effects. *Cell Metab.* **2014**, *19*(3), 373–379. <https://doi.org/10.1016/j.cmet.2014.01.001>.
- [34] Hasskarl, J. Everolimus. In *Small Molecules in Oncology*; Martens, U.M., Ed.; Springer International Publishing: Cham, Switzerland, 2018; pp. 101–123. https://doi.org/10.1007/978-3-319-91442-8_8.
- [35] Kwitkowski, V.E.; Prowell, T.M.; Ibrahim, A.; Farrell, A.T.; Justice, R.; Mitchell, S.S.; Sridhara, R.; Pazdur, R. FDA Approval Summary: Temsirolimus as Treatment for Advanced Renal Cell Carcinoma. *Oncologist* **2010**, *15*(4), 428–435. <https://doi.org/10.1634/theoncologist.2009-0178>.
- [36] Tsimberidou, A.M.; Shaw, J.V.; Juric, D.; et al. Phase 1 Study of M2698, a p70S6K/AKT Dual Inhibitor, in Patients with Advanced Cancer. *J. Hematol. Oncol.* **2021**, *14*, 127. <https://doi.org/10.1186/s13045-021-01132-z>.
- [37] Lehman, J.A.; Gomez-Cambronero, J. Molecular Crosstalk between p70S6k and MAPK Cell Signaling Pathways. *Biochem. Biophys. Res. Commun.* **2002**, *293*(1), 463–469. [https://doi.org/10.1016/S0006-291X\(02\)00238-3](https://doi.org/10.1016/S0006-291X(02)00238-3).
- [38] Kim, D.; Akcakanat, A.; Singh, G.; Sharma, C.; Meric-Bernstam, F. Regulation and Localization of Ribosomal Protein S6 Kinase 1 Isoforms. *Growth Factors* **2009**, *27*(1), 12–21. <https://doi.org/10.1080/08977190802556986>.
- [39] Majeed, S.T.; Batool, A.; Majeed, R.; Bhat, N.N.; Zargar, M.A.; Andrabi, K.I. mTORC1 Induces Eukaryotic Translation Initiation Factor 4E Interaction with TOS-S6 Kinase 1 and Its Activation. *Cell Cycle* **2021**, *20*(9), 839–854. <https://doi.org/10.1080/15384101.2021.1901038>.
- [40] Batool, A.; Majeed, S.T.; Ashaq, S.; Majeed, R.; Bhat, N.N.; Andrabi, K.I. Eukaryotic Initiation Factor 4E Is a Novel Effector of mTORC1 Signaling Pathway in Cross Talk with Mnk1. *Mol. Cell. Biochem.* **2020**, *465*, 13–26. <https://doi.org/10.1007/s11010-019-03663-z>.
- [41] Majeed, S.T.; Majeed, R.; Malik, A.A.; Andrabi, K.I. MTORC2 Is a Physiological Hydrophobic Motif Kinase of S6 Kinase 1. *Biochim. Biophys. Acta Mol. Cell Res.* **2023**, *1870*(4), 119449. <https://doi.org/10.1016/j.bbamer.2023.119449>.
- [42] Baffi, T.R.; Lordén, G.; Wozniak, J.M.; Feichtner, A.; Yeung, W.; Kornev, A.P.; King, C.C.; Del Rio, J.C.; Limaye, A.J.; Bogomolovas, J.; et al. mTORC2 Controls the Activity of PKC and Akt by Phosphorylating a Conserved TOR Interaction Motif. *Sci. Signal.* **2021**, *14*(678), eabe4509. <https://doi.org/10.1126/scisignal.abe4509>.
- [43] Baffi, T.R.; Newton, A.C. mTOR Regulation of AGC Kinases: New Twist to an Old Tail. *Mol. Pharmacol.* **2022**, *101*(4), 213–218. <https://doi.org/10.1124/molpharm.121.000310>.
- [44] Yu, Z.; Chen, J.; Takagi, E.; Wang, F.; Saha, B.; Liu, X.; Joubert, L.-M.; Gleason, C.E.; Jin, M.; Li, C.; et al. Interactions between mTORC2 Core Subunits Rictor and mSin1 Dictate Selective and Context-Dependent Phosphorylation of Substrate Kinases SGK1 and Akt. *J. Biol. Chem.* **2022**, *298*(8), 102288. <https://doi.org/10.1016/j.jbc.2022.102288>.
- [45] Martínez Báez, A.; Ayala, G.; Pedroza-Saavedra, A.; González-Sánchez, H.M.; Chihu Amparan, L. Phosphorylation Codes in IRS-1 and IRS-2 Are Associated with the Activation/Inhibition of Insulin Canonical Signaling Pathways. *Curr. Issues Mol. Biol.* **2024**, *46*(1), 634–649. <https://doi.org/10.3390/cimb46010041>.
- [46] Kim, S.J.; DeStefano, M.A.; Oh, W.J.; Wu, C.C.; Vega-Cotto, N.M.; Finlan, M.; Liu, D.; Su, B.; Jacinto, E. mTOR Complex 2 Regulates Proper Turnover of Insulin Receptor Substrate-1 via the Ubiquitin Ligase Subunit Fbw8. *Mol. Cell* **2012**, *48*(6), 875–887. <https://doi.org/10.1016/j.molcel.2012.09.029>.
- [47] Dibble, C.C.; Asara, J.M.; Manning, B.D. Characterization of Rictor Phosphorylation Sites Reveals Direct Regulation of mTOR Complex 2 by S6K1. *Mol. Cell. Biol.* **2009**, *29*(21), 5657–5670. <https://doi.org/10.1128/MCB.00735-09>.
- [48] Julien, L.A.; Carriere, A.; Moreau, J.; Roux, P.P. mTORC1-Activated S6K1 Phosphorylates Rictor on Threonine 1135 and Regulates mTORC2 Signaling. *Mol. Cell. Biol.* **2010**, *30*(4), 908–921. <https://doi.org/10.1128/MCB.00601-09>.

- [49] Qiu, Z.; Ye, B.; Zhao, S.; Li, X.; Li, L.; Mo, X.; Li, W. Non-Canonical Raf-1/p70S6K Signalling in Non-Small-Cell Lung Cancer. *J. Cell. Mol. Med.* **2019**, *23*(11), 7632–7640. <https://doi.org/10.1111/jcmm.14636>.
- [50] Sfakianos, A.P.; Mellor, L.E.; Pang, Y.F.; Kritsiligkou, P.; Needs, H.; Abou-Hamdan, H.; Désaubry, L.; Poulin, G.B.; Ashe, M.P.; Whitmarsh, A.J. The mTOR-S6 Kinase Pathway Promotes Stress Granule Assembly. *Cell Death Differ.* **2018**, *25*(10), 1766–1780. <https://doi.org/10.1038/s41418-018-0076-9>.
- [51] Yan, X.; Kuang, B.H.; Ma, S.; Wang, R.; Lin, J.; Zeng, Y.X.; Xie, X.; Feng, L. NOP14-Mediated Ribosome Biogenesis Is Required for mTORC2 Activation and Predicts Rapamycin Sensitivity. *J. Biol. Chem.* **2024**, *300*(3), 105681. <https://doi.org/10.1016/j.jbc.2024.105681>.
- [52] Khalil, M.I.; Helal, M.; El-Sayed, A.F.; El Hajj, R.; Holail, J.; Houssein, M.; Waraky, A.; Pardo, O.E. S6K2 in Focus: Signaling Pathways, Post-Translational Modifications, and Computational Analysis. *Int. J. Mol. Sci.* **2024**, *26*(1), 176. <https://doi.org/10.3390/ijms26010176>.
- [53] Sridharan, S.; Basu, A. Distinct Roles of mTOR Targets S6K1 and S6K2 in Breast Cancer. *Int. J. Mol. Sci.* **2020**, *21*(4), 1199. <https://doi.org/10.3390/ijms21041199>.
- [54] Sever, N.İ.; Cengiz Şahin, S. S6K2 Promises an Important Therapeutic Potential for Cancer. *Future Oncol.* **2019**, *15*(1), 95–102. <https://doi.org/10.2217/fon-2018-0332>.
- [55] Larsen, L.J.; Møller, L.B. Crosstalk of Hedgehog and mTORC1 Pathways. *Cells* **2020**, *9*(10), 2316. <https://doi.org/10.3390/cells9102316>.
- [56] De Chiara, M.; Sicurella, M.; Melloni, M.; Conti, I.; Neri, L.M. Dual Hedgehog/GLI1 and PI3K/Akt/mTOR Targeting Possesses Higher Efficacy to Inhibit T-Cell Acute Lymphoblastic Leukemia Growth. *Cells* **2025**, *14*(24), 1972. <https://doi.org/10.3390/cells14241972>.
- [57] Dunn, S.; Eberlein, C.; Yu, J.; Gris-Oliver, A.; Ong, S.H.; Yelland, U.; Cureton, N.; Staniszewska, A.; McEwen, R.; Fox, M.; et al. AKT-mTORC1 Reactivation Is the Dominant Resistance Driver for PI3K β /AKT Inhibitors in PTEN-Null Breast Cancer and Can Be Overcome by Combining with Mcl-1 Inhibitors. *Oncogene* **2022**, *41*, 5046–5060. <https://doi.org/10.1038/s41388-022-02482-9>.
- [58] Hurvitz, S.A.; Layman, R.M.; Curigliano, G.; André, F.; Cristofanilli, M.; Kim, S.B.; Martínez Rodríguez, J.L.; Nadal, J.C.; Kim, G.M.; Lo, L.; et al. VIKTORIA-1 Trial of Gedatolisib Plus Fulvestrant with or Without Palbociclib in Hormone Receptor-Positive/HER2-/PIK3CA Wild-Type Advanced Breast Cancer. *J. Clin. Oncol.* **2026**, *44*(12), 1108–1119. <https://doi.org/10.1200/JCO-25-02643>.
- [59] Yarahmadi, A.; Azarpira, N.; Mostafavi-Pour, Z. Role of mTOR Complex 1 Signaling Pathway in the Pathogenesis of Diabetes Complications; A Mini Review. *Int. J. Mol. Cell. Med.* **2021**, *10*(3), 181–189. <https://doi.org/10.22088/IJMCM.BUMS.10.3.181>.
- [60] Jia, Y.; Jia, R.; Dai, Z.; Zhou, J.; Ruan, J.; Chng, W.; Cai, Z.; Zhang, X. Stress Granules in Cancer: Adaptive Dynamics and Therapeutic Implications. *iScience* **2024**, *27*(8), 110359. <https://doi.org/10.1016/j.isci.2024.110359>.
- [61] Study Details | NCT05501886 | Gedatolisib Plus Fulvestrant with or Without Palbociclib vs. Standard-of-Care for the Treatment of Patients with Advanced or Metastatic HR+/HER2- Breast Cancer (VIKTORIA-1). ClinicalTrials.gov. Available online: <https://www.clinicaltrials.gov/study/NCT05501886> (accessed on).
- [62] Layman, R.M.; Han, H.S.; Rugo, H.S.; Stringer-Reasor, E.M.; Specht, J.M.; Dees, E.C.; Kabos, P.; Suzuki, S.; Mutka, S.C.; Sullivan, B.F.; et al. Gedatolisib in Combination with Palbociclib and Endocrine Therapy in Women with Hormone Receptor-Positive, HER2-Negative Advanced Breast Cancer: Results from the Dose Expansion Groups of an Open-Label, Phase 1b Study. *Lancet Oncol.* **2024**, *25*(4), 474–487. [https://doi.org/10.1016/S1470-2045\(24\)00034-2](https://doi.org/10.1016/S1470-2045(24)00034-2).
- [63] Ling, S.; Dexter, A.; Race, A.M.; Sharma, S.; Hamm, G.; Polanska, U.M.; Marshall, J.F.; Takats, Z.; Brindle, K.; Yuneva, M.O.; et al. Use of Metabolic Imaging to Monitor Heterogeneity of Tumour Response Following Therapeutic mTORC1/2 Pathway Inhibition. *Dis. Model. Mech.* **2025**, *18*(2), DMM050804. <https://doi.org/10.1242/dmm.050804>.
- [64] Baxevanis, C.N. Biomarkers in the Era of Precision Oncology. *Cancers* **2023**, *15*(6), 1782. <https://doi.org/10.3390/cancers15061782>.

- [65] Gerstenecker, S.; Haarer, L.; Schröder, M.; Kudolo, M.; Schwalm, M.P.; Wydra, V.; Serafim, R.A.M.; Chaikuad, A.; Knapp, S.; Laufer, S.; et al. Discovery of a Potent and Highly Isoform-Selective Inhibitor of the Neglected Ribosomal Protein S6 Kinase Beta 2 (S6K2). *Cancers* **2021**, *13*(20), 5133. <https://doi.org/10.3390/cancers13205133>.

Disclaimer/Publisher's Note: The views expressed in this article are those of the author(s) and do not necessarily reflect the views of the publisher or editors. The publisher and editors assume no responsibility for any injury or damage resulting from the use of information contained herein.