ACCSCIENCE PUBLISHING

Cancer Plus

REVIEW ARTICLE

Ammonia-induced cell death: A novel immunometabolic vulnerability for cancer therapy

Xixi Wang[1†], Jing Li[1†], Xiaojun Shu[1†], Ning Hu[1], Shuang Liu[1], Yifei Fan[1], Qin Wang[1], Yue Wu[1], Junyi Lin[2*], Jun Xu[1*], and Song Nie[1*]

[1]Department of Thyroid and Breast Surgery, Jingmen Central Hospital, Jingmen Central Hospital Affiliated to Jingchu University of Technology, Jingmen, Hubei, China

[2]Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, Nebraska, United States of America

Abstract

Ammonia, a by-product of glutamine metabolism, has emerged as a key immunometabolic regulator in the tumor microenvironment. Recent studies reveal that excessive ammonia accumulation impairs effector $CD8^+$ T cell viability through a distinct form of organelle-centered cell death, characterized by lysosomal alkalinization, mitochondrial dysfunction, and autophagy inhibition. This phenomenon, termed ammonia-induced cell death (AICD), contributes to immune suppression and tumor progression. Here, we comprehensively review the molecular pathways governing ammonia production, transport, and detoxification, with a focus on the roles of GLS1, CPS1, Rh-family transporters, and associated solute carriers. We further highlight advances in chemical biology tools—such as ammonia-sensitive probes and isotope-labeled metabolomics—that enable the functional dissection of ammonia metabolism in immune and tumor cells. Emerging therapeutic strategies that combine ammonia metabolism modulators with immune checkpoint inhibitors offer promising avenues to enhance anti-tumor immunity. Despite recent progress, key challenges remain, including the incomplete understanding of ammonia clearance in T cells, limited data on other immune cell types, and concerns about metabolic toxicity. Targeting AICD thus represents a chemically tractable and clinically relevant approach at the interface of metabolism, immunity, and cancer therapy.

Keywords: Ammonia-induced cell death; Tumor microenvironment; Immunotherapy; Immune evasion; Metabolism

[†]These authors contributed equally to this work.

***Corresponding authors:**
Junyi Lin
(julin@unmc.edu)
Jun Xu
(854862195@qq.com)
Song Nie
(112839848@qq.com)

Citation: Wang X, Li J, Shu X, *et al*. Ammonia-induced cell death: A novel immunometabolic vulnerability for cancer therapy. *Cancer Plus*. 2025;7(4):1-15. doi: 10.36922/CP025360054

Received: September 1, 2025

Revised: September 15, 2025

Accepted: November 3, 2025

Published online: December 16, 2025

1. Introduction

The tumor microenvironment (TME) plays a critical role in tumor progression and immune evasion, with metabolic disruptions significantly impacting both tumor and infiltrating immune cells.[1,2] One major metabolic alteration is the enhanced glutamine metabolism in tumor cells, leading to elevated ammonia levels within the TME.[3] Under normal physiological conditions, ammonia, a by-product of glutamine metabolism, is tightly regulated;[4,5] however, in the TME, this regulation is disrupted, resulting in toxic ammonia concentrations that can negatively affect cellular processes. Emerging research is uncovering the mechanisms behind ammonia metabolism dysregulation, revealing

its influence on both tumor proliferation and immune dysfunction, contributing to immune evasion.

The role of ammonia accumulation in immune suppression, particularly its detrimental effects on effector T cells, is garnering more attention. Effector $CD8^+$ T cells are vital for anti-tumor immunity, but excessive ammonia buildup can damage their mitochondria and lysosomes, leading to their death. This process, now known as ammonia-induced cell death (AICD), compromises the anti-tumor capabilities of T cells, diminishing their ability to effectively target tumor cells. The discovery of this mechanism provides new insights into how tumors evade immune detection.

AICD is a key factor in tumor immune evasion, as tumors exploit metabolic disturbances, such as ammonia buildup, to incapacitate infiltrating T cells, preventing the immune system from effectively targeting tumors. This metabolic disruption contributes to the immunosuppressive nature of the TME, allowing tumors to bypass immune surveillance and sustain malignant growth. These findings highlight that metabolic imbalances in the TME not only serve tumor cell survival but also facilitate immune evasion.

Given the suppressive impact of AICD on immune function, targeting ammonia metabolism offers new therapeutic opportunities. By inhibiting glutamine metabolism or neutralizing excess ammonia, it may be possible to restore T cell function and bolster anti-tumor immunity.[6] This approach could be particularly valuable in cases where tumors exhibit resistance to immunotherapy. Targeting ammonia metabolism presents a promising strategy for combination therapies aimed at enhancing T cell-mediated anti-tumor responses. This review will explore ammonia metabolism, the biological implications of AICD, and the potential for developing immunotherapeutic strategies targeting this pathway, while summarizing the latest research in the field.

2. Molecular mechanisms of ammonia metabolism

2.1. Production and metabolic pathways of ammonia

Ammonia metabolism plays a fundamental role in cellular processes, particularly in protein and amino acid metabolism, where ammonia is produced as a metabolic by-product. Its generation is primarily linked to two metabolic pathways: glutamine hydrolysis and amino acid deamination.[7] These pathways are highly active in rapidly dividing cells, such as tumor cells and effector T cells.[8]

2.1.1. Glutamine hydrolysis

Glutamine, one of the most abundant amino acids in cells, is essential for energy production and nitrogen regulation.[9] During glutaminolysis, glutamine serves as an energy source for rapidly dividing cells.[10] The enzyme glutaminase (GLS) catalyzes the breakdown of glutamine into glutamate and ammonia.[11,12] This reaction occurs within mitochondria, where GLS hydrolyzes glutamine to generate glutamate, releasing one ammonia molecule.[13] Glutamate then enters the tricarboxylic acid (TCA) cycle, contributing to energy production and biosynthetic processes.[14] Although normal cells can efficiently detoxify the ammonia produced by glutaminolysis, rapidly proliferating cells like tumor and effector T cells experience elevated ammonia accumulation due to increased metabolic demands.[15]

2.1.2. Amino acid deamination

Amino acid deamination also produces ammonia through the removal of amino groups (-NH2) from amino acids. This process occurs primarily in the liver and kidneys but is also vital in metabolically active cells, including tumor cells and T cells. Deamination follows two primary pathways:

(i) Oxidative deamination: Glutamate is oxidized to α-ketoglutarate with ammonia release, catalyzed by glutamate dehydrogenase in the mitochondria.[16]
(ii) Non-oxidative deamination: Enzymes like serine dehydratase catalyze reactions such as the conversion of serine to pyruvate, with concurrent ammonia release.

These processes contribute to the complex production of ammonia in tumor and immune cells with high metabolic activity, leading to intracellular ammonia buildup.

2.1.3. Ammonia storage and detoxification

Ammonia is toxic at high levels, and cells have developed mechanisms to store and detoxify it to prevent damage, particularly to organelles such as mitochondria and lysosomes. In effector T cells and tumor cells, ammonia is transported to lysosomes, where it combines with protons to form ammonium ions (NH_4^+), temporarily neutralizing it.[17] This buffering system helps maintain intracellular ammonia balance. However, excessive ammonia accumulation can overwhelm this system, leading to lysosomal dysfunction, cellular injury, and eventual cell death.[18] In contrast, the liver manages ammonia through the urea cycle, converting it to urea for excretion. Nonetheless, localized environments like the TME may experience ammonia buildup, contributing to metabolic disturbances and cell damage.

Recent studies have elucidated new pathways of ammonia metabolism and their impact on T cell function, with evidence derived from both *in vitro* and *in vivo* models. *In vitro*, $CD8^+$ T cells isolated from OT-I transgenic mice were activated with the SIINFEKL

peptide and interleukin-2 (IL-2) to mimic the effector T cell state following antigen stimulation. These activated T cells displayed rapid proliferation, making them a robust model to investigate ammonia production and its role in cell death. Complementary *in vivo* studies used C57BL/6J mice intravenously infected with *Listeria monocytogenes* expressing ovalbumin (Lm-OVA) to simulate a physiological infectious environment. Activated $CD8^+$ OT-I T cells were adoptively transferred into these mice, revealing substantial ammonia accumulation and significant effector T cell death, indicating a strong *in vivo* effect of ammonia on T cell survival (moderate-to-strong evidence, animal model).

In tumor contexts, particularly under hypoxic conditions, glucose metabolism primarily produces lactate, limiting available carbon sources. Glutamine then serves as the main carbon and nitrogen donor, supporting biosynthesis, especially lipid synthesis. While the nitrogen from glutamine was historically assumed to convert into ammonia, recent findings in tumor cell models indicate no detectable ammonia accumulation within tumor cells (*in vitro* tumor model), suggesting that ammonia dynamics differ between immune and tumor cells. This discrepancy highlights context-dependent effects and underscores the importance of considering experimental design when interpreting ammonia-related cell death.[14]

A novel ammonia metabolism pathway has been discovered, wherein glutamine is converted into dihydroxyacetone phosphate and secreted from cells.[19] This pathway is crucial in hypoxic environments where tumor cells rely on this mechanism to dispose of excess carbon and nitrogen while continuing to use glutamine for biosynthesis. Animal studies confirm that inhibiting this pathway leads to ammonia buildup and restricts tumor growth.[20]

This research highlights the coordinated metabolism of carbon and nitrogen from glutamine, with dihydroxyacetone phosphate and lactate offering a safe means of ammonia disposal. These insights enhance our understanding of glutamine metabolism and present potential biomarkers and therapeutic targets for cancer diagnosis and treatment.[21]

Maintaining a balance in ammonia metabolism is critical for cellular health. When production exceeds the clearance capacity, ammonia accumulation can cause physiological and pathological consequences. In tumor cells, excessive ammonia not only disrupts their own metabolism but also impairs the function of immune cells in the TME, promoting immune evasion. This metabolic dysregulation offers a promising target for cancer therapies, where modulating ammonia metabolism could improve immunotherapy efficacy.[22]

2.1.4. Ammonia transport and homeostasis regulation

Ammonia, a metabolic by-product, poses toxicity risks to cells if allowed to accumulate. As such, its intracellular concentration is tightly controlled by intricate transport mechanisms. Transmembrane proteins are key players in maintaining ammonia homeostasis, as they either expel ammonia from intracellular compartments or relocate it to designated storage locations, thereby safeguarding cellular organelles from potential harm.

2.1.5. Transmembrane ammonia transport proteins

Ammonia transport across cell membranes is primarily facilitated by specific transmembrane proteins, among which the Rhesus (Rh) protein family is the most prominent.[23] These proteins regulate ammonia transport across membranes, contributing to nitrogen metabolism and overall homeostasis. The Rh family is widely expressed across cellular and organelle membranes, including those of mitochondria, lysosomes, and the plasma membrane.

Key Rh proteins, such as RhAG, RhBG, and RhCG, mediate the transport of ammonia or ammonium ions (NH_4^+) across these membranes. Their primary role is to enable ammonia diffusion across plasma membranes, particularly in rapidly dividing or metabolically active cells, such as tumor cells and effector T cells. By regulating this transport efficiently, Rh proteins prevent excessive ammonia build-up within cells.

The RhCG is predominantly found in tissues involved in ammonia excretion, like the kidneys and liver, playing a crucial role in ammonia export and transport from mitochondria to lysosomes to avert cytoplasmic ammonia build-up.[24] The RhBG is present in various epithelial cells, aiding in ammonia transport across membranes to help maintain local ammonia concentrations.[25] Although primarily identified in some eukaryotes and prokaryotes, the Mep/Amt proteins may play roles in ammonia transport in mammals, similar to Rh proteins.[26] Members of the solute carrier (SLC) family, particularly SLC32 and SLC38, have been implicated in ammonia metabolism. For instance, SLC38 family proteins indirectly contribute to ammonia generation and transport through glutamine transport.[27]

3. Disbalanced ammonia homeostasis and cellular function

Ammonia transport and its homeostatic control are critical for maintaining cellular metabolism and survival.[28] Effective ammonia storage and removal depend on the coordinated action of transport proteins like the Rh family, which help cells manage ammonia levels and avoid organelle

toxicity. However, disruptions in ammonia metabolism can severely threaten cellular function, especially in the TME and proliferating cells, where excessive ammonia accelerates cell death and damages organelles.[29]

Dysregulated ammonia metabolism, leading to excess accumulation, can alter intracellular pH, particularly raising lysosomal pH and impairing their function.[30] Understanding how ammonia transport affects cellular homeostasis offers potential for therapeutic interventions targeting ammonia metabolism in cancer.

In highly metabolically active cells, ammonia is produced through glutaminolysis and transported from mitochondria to lysosomes for storage.[31] The acidic lysosomal environment neutralizes ammonia, converting it into ammonium ions (NH_4^+) and preventing free ammonia from causing cytotoxicity.[32]

Ammonia generated by mitochondria is transported into lysosomes through Rh proteins, ensuring low ammonia concentrations in mitochondria to prevent dysfunction.[33] However, when lysosomal storage capacity is compromised, ammonia build-up can increase lysosomal pH, damaging membrane integrity and risking rupture. This may result in the release of toxic enzymes that harm other cellular structures.

Excess ammonia may also re-enter mitochondria, disrupting their function. Mitochondria rely on membrane potential for ATP production through oxidative phosphorylation.[34] As a basic compound, ammonia neutralizes the acidic mitochondrial environment, reducing membrane potential.[6] This hampers ATP production and forces the cell into an energy-deficient state, which may lead to swelling, membrane damage, oxidative stress, and, ultimately, apoptosis or necrosis.[35] In addition, mitochondrial damage leads to the release of pro-apoptotic proteins like cytochrome c, activating cell death pathways. Furthermore, mitochondrial dysfunction exacerbates reactive oxygen species (ROS) production, which causes additional damage to DNA, proteins, and lipids, intensifying oxidative stress.[36]

Ammonia-induced mitochondrial dysfunction is a core mechanism underlying ammonia-related cell death, notably in studies involving tumor and effector T cells. This suggests that impaired ammonia metabolism could be pivotal in determining cell fate. In their experiments, an Ammonia Assay Kit (Abcam) was used for quantification. Briefly, cells were collected and lysed on ice, and an equal number of $CD8^+$ T cells (approximately 1x10on. Briefly, cells were collected and lysed on ice, and an equal number of CD8ected and lysed on ice, and an equal nuctor T cells. This suggests that impaired ammonia metabolism could be pivotal in determining cell according to the manufacturer's protocols.

3.1. Autophagy and AICD

Autophagy, the process of degrading and recycling damaged organelles, is essential for cellular homeostasis, especially under stress.[37] In the context of AICD, both lysosomal alkalinization and mitochondrial dysfunction inhibit autophagy, preventing the removal of damaged organelles.[38] While autophagosomes may still form, their fusion with lysosomes is blocked, leading to an accumulation of waste and further burdening the cell. Consequently, autophagic flux—the overall autophagy activity—declines significantly, preventing effective stress response and organelle repair.[39]

To counteract excess ammonia, cells use Rh proteins to expel ammonia and restore balance.[40] Systemically, the liver and kidneys convert ammonia into urea for excretion through the urea cycle. However, this system may become overwhelmed during mitochondrial dysfunction or liver disease, leading to elevated ammonia levels. In localized microenvironments, particularly in tumors, ammonia removal may be insufficient, leading to local accumulation.

3.2. Ammonia homeostasis in the TME

In the TME, disruptions in ammonia homeostasis significantly affect both tumor cell metabolism and immune evasion.[41] High metabolic activity in tumor cells produces large amounts of ammonia, and abnormal expression of transport proteins can exacerbate local ammonia build-up. Ammonia imbalance not only physically damages cellular structures but also triggers signaling pathways leading to programmed cell death. This process is characterized by lysosomal damage, mitochondrial dysfunction, and autophagy inhibition.

Research led by Huang Bo has identified a specific form of cell death induced by excessive ammonia accumulation, termed "AICD".[42] This novel death mechanism, defined by dual damage to lysosomes and mitochondria, is distinct from traditional apoptosis and necrosis. While classic cell death pathways rely on factors like cytochrome c release, AICD centers on organelle dysfunction, highlighting the unique role of ammonia metabolism in cellular demise.

Tumor cells exhibit heightened ammonia production through glutaminolysis, and impaired lysosomal or mitochondrial function can hinder proliferation or lead to cell death.[43] Ammonia imbalance also significantly affects immune cells in the TME, such as effector T cells and natural killer (NK) cells, weakening anti-tumor immunity. In highly metabolic environments, immune cells are

particularly vulnerable to ammonia-induced dysfunction, providing insight into the self-limiting nature of immune responses during tumor progression.

4. AICD

4.1. Molecular mechanism of ammonia cell death

Upon antigen stimulation, $CD8^+$ naïve T cells differentiate into effector cells. After antigen clearance, over 95% of these effector T cells undergo rapid apoptosis, known as T cell contraction, which is crucial for immune homeostasis and the prevention of autoimmunity.[44] However, the specific processes behind effector T cell death are still not fully understood. Research by Huang *et al.* has shown that metabolic pathways involving ketone bodies, gluconeogenesis, and glycogen can activate the pentose phosphate pathway, enabling the reduction of reactive oxygen species in $CD8^+$ T cells.[45,46] This process helps in the formation and maintenance of memory T cells and involves the urea cycle to lower intracellular ammonia levels, contributing to memory T cell survival.[44]

Ammonia is mainly detoxified in the liver by converting it into urea through the urea cycle. If liver function is impaired, ammonia clearance becomes inefficient, leading to hyperammonemia, which can result in neurological damage such as hepatic encephalopathy.[47] While ammonia toxicity is well documented, the specific mechanisms by which ammonia induces cellular damage remain unclear.

Recent studies have uncovered that ammonia, a by-product of amino acid metabolism, can trigger apoptosis in effector $CD8^+$ T cells (Figure 1). As these cells activate, ammonia accumulates, eventually leading to their death. This discovery reveals the intricate relationship between metabolism and immune cell function, suggesting new strategies to improve T cell-based cancer therapies.

The enzyme carbamoyl phosphate synthetase 1 (CPS1) has been identified as essential for clearing ammonia from cells. It was found that GLS1, a mitochondrial enzyme, produces ammonia from glutamine, significantly influencing effector T cell death.[10,48,49] This challenges the view of ammonia as merely a waste product, showing it plays a regulatory role in immune cell activity. During $CD8^+$ T cell activation, ammonia levels rise progressively, spiking after antigen clearance and resulting in rapid apoptosis.[50] Effector T cells express low levels of CPS1, which limits ammonia conversion to urea, causing its accumulation. Overexpressing CPS1 or using ammonia scavengers can

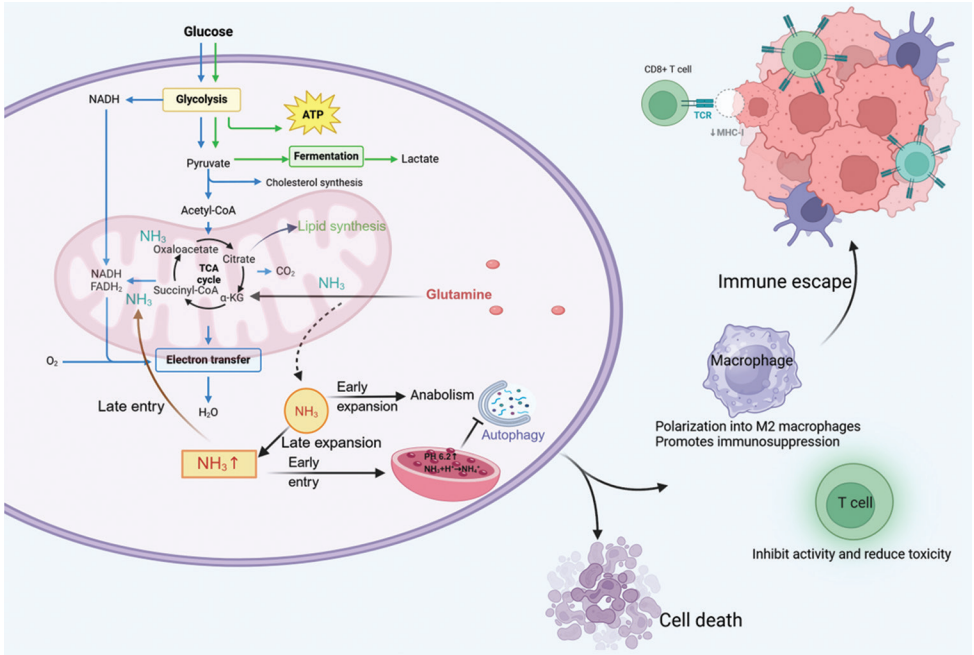

Figure 1. The mechanism of ammonia-induced T cell death and its impact on the immune microenvironment. Glucose metabolism in cancer cells generates pyruvate through glycolysis and lactate through fermentation, accompanied by ATP production. Within mitochondria, the tricarboxylic acid (TCA) cycle produces energy intermediates and releases ammonia (NH_3). Excessive ammonia accumulation disrupts mitochondrial electron transfer, leading to metabolic imbalance in T cells and enhanced autophagy activity. These alterations ultimately promote T cell death, reduce effector function, and impair anti-tumor immunity. As a consequence, cancer cells evade immune surveillance (immune escape). Moreover, ammonia promotes macrophage polarization toward the M2 phenotype, which supports immunosuppression and facilitates tumor growth and progression. Schematic created with BioRender. Lin, J. (2025).

reduce this apoptosis, highlighting ammonia's role in effector T cell death.

Further studies revealed that accumulated ammonia damages lysosomes in $CD8^+$ T cells. In the early stages of T cell activation, glutamine metabolism generates ammonia, which is used in anabolic processes, preventing excessive accumulation. However, during the later stages, proliferation slows, leading to ammonia buildup. This alters lysosomal pH, impairing their function and causing mitochondrial damage, which leads to cell death. This ammonia-induced damage is distinct from known forms of cell death, such as apoptosis, ferroptosis, and pyroptosis.[51]

Ammonia, being weakly basic (pKa 9.3), accumulates in mitochondria and then enters lysosomes, where it binds with protons to form ammonium ions (NH_4^+), raising the lysosomal pH and disrupting function.[52,53] Cytosolic ammonia fails to enter lysosomes effectively and instead returns to mitochondria, leading to mitochondrial dysfunction.[54] The proton consumption by ammonia affects the proton gradient in mitochondria, disrupting energy production. Damaged mitochondria are typically cleared through autophagy, but elevated lysosomal pH inhibits autophagic processes, allowing toxic ammonia and damaged mitochondria to accumulate, leading to effector T cell death.

In adoptive T cell therapy studies, researchers found that ammonia levels also rise in $CD8^+$ T cells within the TME.[55] Inhibiting glutamine metabolism or overexpressing CPS1 in T cells significantly improves their survival in tumors and enhances their anti-tumor activity.[56] These findings not only provide insights into effector T cell death but also present potential therapeutic applications for enhancing T cell-based cancer treatments.

This research suggests new therapeutic strategies for cancer. By blocking AICD pathways in effector T cells, T cell-based therapies could become more effective. Drugs targeting GLS1 or modulating lysosomal pH may help preserve effector T cell function and longevity in the TME. In summary, these findings advance our understanding of ammonia's role in effector T cell metabolism and present promising avenues for therapeutic intervention.[57]

Ammonia metabolism, particularly its by-product ammonia, plays a significant role in both normal cellular functions and pathological processes, including tumorigenesis. In healthy cells, the urea cycle detoxifies ammonia, primarily in the liver. However, when liver function is impaired, hyperammonemia arises, causing conditions like hepatic encephalopathy, though the mechanisms behind ammonia's effects on non-hepatic tissues remain less understood. Recent studies suggest that some non-hepatic cells also produce ammonia, particularly through glutamine hydrolysis, though the process by which cells detoxify this excess ammonia varies, leading to potential toxic accumulation under certain conditions.

Notably, highly proliferative cells, such as cancer cells, rely on glutamine metabolism, producing ammonia as a by-product. While liver cells efficiently remove ammonia, other cell types, including immune cells like $CD8^+$ T cells, have shown variable capacities for ammonia detoxification.[58] Research has indicated that while memory $CD8^+$ T cells can remove ammonia to prolong their survival, effector $CD8^+$ T cells accumulate ammonia, leading to cell death through mitochondrial and lysosomal disruption. This form of AICD is distinct from other known cell death mechanisms and represents a novel target for enhancing anti-tumor immunity.

Cancer cells are known for metabolic reprogramming, a hallmark enabling them to adapt and survive under hostile conditions. Glutamine dependency and dysregulation of ammonia metabolism have emerged as critical aspects of this reprogramming. Tumor cells may store ammonia in lysosomes or export it through specific transporters; however, these mechanisms may fail when metabolic demands are excessively high. The resultant ammonia accumulation in the TME not only exerts direct toxicity on tumor cells but also impairs the anti-tumor immune response by promoting effector T cell dysfunction, contributing to immune escape and tumor progression.

This dual role of ammonia in the TME presents both challenges and opportunities for therapy. While dysregulated ammonia can directly induce tumor cell death, it also impairs effector T cell function through mitochondrial and lysosomal stress, facilitating immune evasion. Therapeutic strategies therefore focus on two complementary approaches: Precisely modulating ammonia levels in tumor cells to selectively induce apoptosis and disrupt metabolic pathways, and protecting immune cells from ammonia-induced apoptosis, for example, through inhibitors of glutamine metabolism or lysosomal function. Together, these strategies highlight the potential of targeting ammonia metabolism to both directly attack tumors and restore anti-tumor immunity, offering a refined avenue for cancer treatment.

4.2. Relationship between ammonia cell death and tumorigenesis

Ammonia is a toxic by-product of amino acid metabolism, necessitating detoxification processes like the urea cycle, which primarily occurs in liver cells. Liver failure can lead to hyperammonemia, a condition linked to hepatic encephalopathy through mechanisms that are still not fully

understood.[59] While the detrimental effects of ammonia in the liver are well-documented, its impact on cells in other tissues remains largely unexplored. It is also unclear whether ammonia produced in non-liver tissues plays a physiological role.

In extrahepatic tissues, amino acid amino groups are thought to be incorporated into glutamine through transamination. This glutamine is transported to the liver for ammonia release, which then enters the urea cycle. However, some non-hepatic cells can produce ammonia through glutamine hydrolysis. Although purine and polyamine synthesis pathways can process ammonia, they do not fully eliminate it. As a result, residual ammonia may accumulate in cells, which has contributed to ongoing controversy in the field. Rapidly proliferating cells, for instance, use alpha-ketoglutaric acid to replenish the TCA cycle, with glutamine hydrolysis producing ammonia as a by-product.[60]

Research has indicated that $CD8^+$ memory T cells, similar to liver cells, use the urea cycle to clear ammonia, potentially extending their lifespan.[44] This suggests that while memory T cells clear ammonia to maintain survival, effector T cells may accumulate ammonia, leading to their death. This study showed that ammonia, derived from glutamine hydrolysis, accumulates in lysosomes and triggers effector $CD8^+$ T cell death by damaging mitochondria. This AICD, characterized by lysosomal alkalization and mitochondrial swelling, differs from known cell death mechanisms. Inhibition of glutaminolysis at early stages in effector T cells has been shown to enhance anti-tumor immunity, potentially by promoting the differentiation of effector T cells into memory-like $CD8^+$ T cells through the reduction of ATP and ROS production.[61]

Cancer metabolic reprogramming, where cancer cells become dependent on specific nutrients or metabolic pathways, is a hallmark of cancer.[62] Ammonia imbalance plays a crucial role in this metabolic reprogramming, which is often targeted to selectively kill cancer cells.[63] Altering the metabolic pathways of immune cells to restore their anti-tumor function or suppress tumor-promoting responses is a major aspect of cancer therapy, often referred to as metabolic reprogramming.[64] In cancer cells, rapid proliferation is supported by glutaminolysis, which generates energy and essential precursors but also produces ammonia.[65] To cope, tumor cells use mechanisms like ammonia transporters, such as Rh family proteins, to transport ammonia to lysosomes or excrete it outside the cell.[66] However, when these regulatory mechanisms fail, ammonia can accumulate within tumor cells and the TME, potentially contributing to immune suppression, potentially contributing to immune suppression and effector T cell dysfunction.[67]

Glucose metabolism has long been recognized as central to cancer, and glutamine addiction is another key aspect of metabolic reprogramming in tumors. Although ammonia's role in cancer has only recently gained attention, emerging studies show it can promote tumor growth and survival. For example, research demonstrated that ammonia released from glutamine after glucose deprivation can induce autophagy, aiding cancer cell survival. In another study, ammonia was shown to activate SREBP-1, a transcription factor involved in lipogenesis, contributing to tumorigenesis.[68] This research revealed that ammonia activates SREBP-1 by dissociating SCAP from Insig, promoting tumor growth through enhanced lipid synthesis.[69] Moreover, AICD plays a critical role in tumor immune editing.[70] Elevated ammonia in the TME induces lysosomal and mitochondrial damage in effector T cells, leading to their dysfunction or apoptosis. This disruption of immune surveillance weakens anti-tumor immunity and facilitates tumor progression.[71,72]

Targeting AICD or restoring ammonia homeostasis, therefore, represents a promising therapeutic approach, combining two complementary strategies: modulating ammonia levels to selectively induce tumor cell death, and protecting immune cells from ammonia-induced apoptosis to enhance anti-tumor immunity. Understanding the molecular mechanisms of this cell death and its role in tumor immunity will offer crucial insights for developing targeted cancer therapies in the future (Table 1).

5. Therapeutic strategies targeting AICD

Cell death is essential for tissue homeostasis, and selective targeting of cancer cell death pathways has emerged as a promising strategy in cancer therapy.[77] While conventional treatments like chemotherapy and radiation can harm normal cells, targeted approaches aim to modulate specific mechanisms such as apoptosis, ferroptosis, necrosis, and disulfidptosis to improve treatment specificity.[78,79] Among these, AICD, driven by the toxic accumulation of ammonia—a by-product of amino acid and glutamine metabolism—represents a novel target for both tumor cells and immune modulation. In effector $CD8^+$ T cells, ammonia accumulation disrupts lysosomal and mitochondrial function, elevating lysosomal pH, impairing autophagy, and inducing apoptosis, distinct from classical forms of cell death.[80] Memory T cells, in contrast, can detoxify ammonia through the urea cycle and survive longer.

In the TME, dysregulated ammonia metabolism contributes to immune suppression, promoting effector T cell dysfunction and facilitating tumor immune evasion.[81,82] Therapeutic strategies targeting ammonia metabolism include inhibiting its production through

Table 1. Summary table of key information

Studies	Models	Main findings	Samples	Strength
Zhang *et al.*[42]	Primary murine CD8$^+$T cells; OT-I adoptive transfer; infection models	Ammonia accumulation in lysosomes disrupts acidification → mitochondrial damage → effector T cell death (AICD). Lysosomal modulation rescues survival.	Cell culture; mouse models	Strong (cellular); moderate (animal); weak (clinical, inferred)
Chen *et al.*[73]	Memory vs. CD8$^+$effector T cells, metabolic assays	Memory cells enhance ammonia clearance (urea/citrulline cycle) → improved survival; effector cells lack this adaptation.	Murine T cells; metabolic flux	Strong (cellular); moderate (animal)
Chengqun *et al.*[74]	Human CRC samples; mouse tumor models	High intratumoral ammonia linked to T cell exhaustion and poor immunotherapy response; clearance reactivates T cells.	Human tumor samples; mice	Moderate (animal+clinical correlation)
Zhao *et al.*[75]	Tumor-bearing mice treated with CB-839	Inhibition of glutamine metabolism reduces ammonia-related immunosuppression→enhances anti-tumor immunity.	Murine tumor models	Moderate (animal); early clinical translation
Polletta *et al.*[76]	Cell lines; biochemical assays	Ammonia stress activates autophagy/mitophagy; SIRT5 regulation implicated.	*In vitro* cell culture	Moderate (cellular mechanistic)

Abbreviations: AICD: Ammonia-induced cell death; CRC: Colorectal cancer.

glutamine metabolism inhibitors (*e.g.*, 6-fluoroglutamine) or enhancing clearance through CPS1 overexpression and ammonia scavengers.[83,84] Modulating lysosomal pH and autophagy pathways can further preserve effector T cell function, enhancing anti-tumor immunity. Such interventions not only directly induce tumor cell death but also restore immune surveillance by improving T cell cytotoxicity, reducing regulatory T cell expansion, skewing tumor-associated macrophages toward a proinflammatory phenotype, and inhibiting tumor angiogenesis and oxidative stress (Figure 2). Overall, integrating ammonia metabolism modulation with targeted cell death strategies offers a refined therapeutic avenue to simultaneously attack tumors and bolster anti-tumor immune responses.

Combining AICD with traditional cancer therapies could yield more effective treatments. For instance, pairing ammonia metabolism inhibitors with chemotherapy could simultaneously kill tumor cells and improve the immune microenvironment, strengthening the immune system's ability to eliminate tumors. Ammonia may also contribute to the limited effectiveness of immunotherapies, such as PD-1/PD-L1 inhibitors.[85] By damaging effector T cells, ammonia diminishes their capacity to attack cancer cells, allowing tumors to escape immune destruction. Inhibiting ammonia accumulation or repairing the damage it causes to immune cells could increase the effectiveness of existing immunotherapies, especially against resistant cancers. PD-1/PD-L1 inhibitors may help restore T cell activity by counteracting suppressive signals, and combining these inhibitors with ammonia metabolism regulators may further enhance T cell function and prevent ammonia-induced T cell death.[86] This combined therapeutic approach could improve the efficiency of tumor immune clearance, particularly in TMEs with high ammonia levels. Research suggests that the co-administration of ammonia metabolism inhibitors and PD-1/PD-L1 inhibitors may synergistically enhance immune responses against tumors, highlighting the potential of this combination therapy strategy.

6. Molecular inhibitors targeting ammonia metabolism and transport

6.1. GLS1 inhibitors and CPS1 activators in targeting ammonia accumulation

GLS1, a mitochondrial enzyme catalyzing the conversion of glutamine to glutamate and ammonia, has emerged as a key target in modulating tumor-associated ammonia accumulation.[87] Several GLS1 inhibitors have been developed, including CB-839 (telaglenastat), a potent, selective, orally bioavailable inhibitor that binds the active site of GLS1 and blocks glutaminolysis. CB-839 has demonstrated anti-tumor efficacy in preclinical models and is currently in phase I/II clinical trials in combination with immune checkpoint inhibitors and chemotherapeutics.[88]

On the other hand, carbamoyl phosphate synthetase 1 (CPS1) is a mitochondrial enzyme catalyzing the first step of the urea cycle to convert ammonia into carbamoyl phosphate, thus facilitating detoxification. Pharmacological strategies to upregulate CPS1 activity are still under development. Recent studies have explored CRISPRa-mediated CPS1 overexpression or small-molecule activators that enhance CPS1 transcription through modulation of transcription factors such as HNF4α, showing potential in restoring T cell fitness within ammonia-rich TMEs.[89]

6.2. Modulation of ammonia transport proteins: Rh family and SLC members

The Rh family of ammonia transporters, including RhBG and RhCG, plays a crucial role in maintaining intracellular

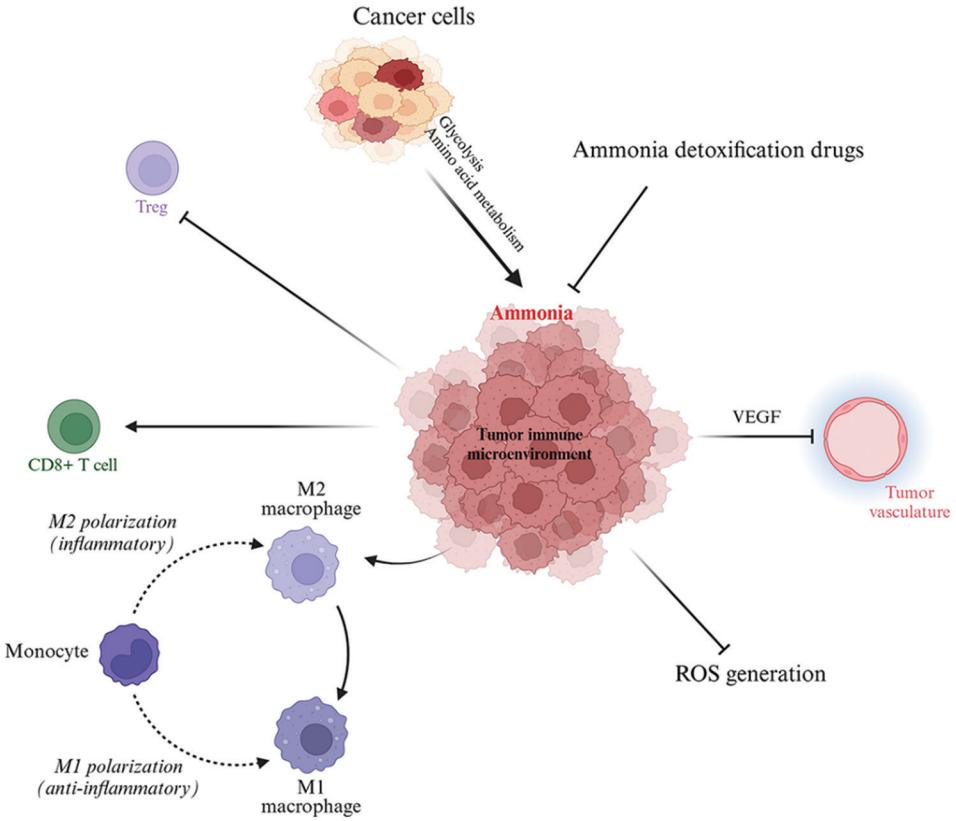

Figure 2. Ammonia-induced immune suppression and detoxification as a therapeutic strategy. Cancer cells release large amounts of ammonia as a by-product of glycolysis and amino acid metabolism, leading to accumulation of ammonia in the tumor immune microenvironment. Elevated ammonia promotes tumor progression through multiple mechanisms: (i) Inhibition of effector $CD8^+$ T cell activity, thereby facilitating immune evasion; (ii) stimulation of regulatory T cell (Treg) recruitment and expansion, enhancing immune tolerance; (iii) skewing monocyte differentiation toward M2 macrophages with pro-tumorigenic and inflammatory properties, while reducing anti-tumor M1 macrophages; (iv) induction of vascular endothelial growth factor (VEGF) production, promoting tumor vasculature formation; and (v) generation of reactive oxygen species (ROS), contributing to tumor progression. In contrast, ammonia detoxification drugs act as a therapeutic intervention by lowering ammonia levels. These agents restore $CD8^+$ T cell function, reduce Treg-mediated immune suppression, and reprogram immune metabolism (*e.g.*, glutamine utilization), thereby enhancing anti-tumor immune responses and preventing immune evasion (74). Schematic created with BioRender. Lin, J. (2025) https://app.biorender.com/illustrations/678de2bb89b3fbc9fe0955b0.

ammonia homeostasis by facilitating ammonia efflux or sequestration into organelles. Although no clinically approved small-molecule modulators of Rh proteins are currently available, recent high-throughput screenings have identified candidate compounds that selectively inhibit RhCG-mediated ammonia flux. These include hydrophobic amine analogs that mimic NH_3/H^+ gradients across lysosomal membranes.[90]

Furthermore, members of the SLC family, especially SLC38A1 (SNAT1) and SLC1A5 (ASCT2), indirectly regulate ammonia production by modulating glutamine uptake. The ASCT2 inhibitor V-9302, designed based on the structural homology of the transporter, reduces glutamine uptake and subsequently lowers intracellular ammonia levels.[91] This strategy simultaneously suppresses tumor growth and alleviates ammonia-induced $CD8^+$ T cell dysfunction.

6.3. Chemical biology tools to probe ammonia dynamics

Chemical biology tools such as ammonia-sensitive fluorescent probes and stable isotope-resolved metabolomics have become indispensable for dissecting ammonia metabolism at the cellular and organelle level.[92] For instance, NH_3-sensitive fluorescent dyes like APG-2 and DAN probes allow for real-time imaging of intracellular pH shifts and ammonia distribution, particularly in mitochondria and lysosomes, enabling visualization of ammonia-induced organelle damage.[93]

In addition, LC-MS/MS-based metabolomics with ^{15}N-labeled glutamine tracing provides quantitative insights into the fate of ammonia in tumor and immune cells. These tools have revealed how glutaminolysis-derived ammonia contributes to immunosuppression and helped

identify potential biomarkers predictive of therapeutic response to ammonia-targeting interventions.[94]

6.4. Combination therapy: Ammonia metabolism inhibitors with PD-1 blockade

Combining metabolic interventions with immune checkpoint blockade is an emerging strategy to overcome resistance in immune-refractory tumors. A recent *in vivo* study in murine melanoma and colorectal cancer models demonstrated that CB-839 administration synergizes with anti–PD-1 therapy, leading to increased intratumoral $CD8^+$ T cell infiltration and reduced exhaustion markers (*e.g.*, PD-1, TIM-3).[95] Mechanistically, GLS1 inhibition lowered ammonia levels in the TME, preserving lysosomal integrity in effector T cells and enhancing their cytotoxic function.

Similarly, overexpression of CPS1 in adoptively transferred $CD8^+$ T cells improved their survival and functionality within ammonia-rich tumors and synergized with PD-L1 blockade to significantly delay tumor progression. These findings underscore the therapeutic potential of targeting ammonia metabolism as a sensitizing strategy for immunotherapy.

7. Future research direction

AICD represents an emerging immunometabolic mechanism with profound implications for cancer biology and therapy. Established findings demonstrate that activated $CD8^+$ T cells produce substantial amounts of ammonia and undergo apoptosis in ammonia-rich environments, supporting the role of ammonia accumulation as a driver of T cell dysfunction. In addition, evidence indicates that glutamine metabolism, ammonia transport, and organelle stress contribute to shaping immune cell fate.

Tumors employ multiple immune evasion strategies, including checkpoint upregulation, immunosuppressive cytokine secretion, and recruitment of regulatory immune cells. AICD has recently emerged as a metabolic mechanism that impairs $CD8^+$ T cell function and contributes to tumor immune escape. Current evidence suggests that AICD is particularly relevant in tumors with high glutamine metabolism and ammonia accumulation, although its prevalence relative to classical evasion mechanisms and across different tumor types remains to be fully elucidated. While preclinical studies indicate that targeting ammonia metabolism can enhance T cell survival and anti-tumor immunity, direct comparisons with other immunotherapeutic strategies, such as checkpoint blockade or adoptive cell therapy, are limited. Integrating AICD-targeting approaches with established therapies may therefore provide complementary avenues to overcome tumor immune evasion.

At the same time, several aspects remain speculative and require further investigation. The precise mechanisms governing ammonia accumulation in immune cells are still unclear, and it is unknown why $CD8^+$ T cells fail to efficiently eliminate or neutralize excess ammonia. Hypotheses include potential deficiencies in clearance pathways, organelle dysfunction, or metabolic bottlenecks. Similarly, while most studies have focused on effector $CD8^+$ T cells, it is uncertain whether AICD extends to $CD4^+$ T cells, regulatory T cells, or natural killer cells, and whether it operates across diverse cancer types and disease stages.

There is also a balance between promise and caution. Preclinical studies suggest that inhibiting AICD could enhance anti-tumor immunity, but the long-term safety of such interventions remains unknown. Chronic suppression of T cell death may disrupt immune homeostasis or increase the risk of autoimmunity. Furthermore, because ammonia is integral to both normal and malignant cell metabolism, selective targeting is essential to avoid unintended toxicity.

Looking forward, future directions are necessarily more exploratory. These include investigating the molecular basis of ammonia detoxification in immune cells, enhancing intracellular ammonia elimination, and integrating ammonia modulation with established immunotherapies or metabolic interventions. Evaluating the universality of AICD across tumor types and stratifying patients based on metabolic profiles may enable precision applications.

In summary, AICD is supported by growing experimental evidence as a novel immunometabolic vulnerability, while its mechanistic underpinnings, cell-type specificity, and translational applications remain open questions. Addressing these knowledge gaps will be essential for safely and effectively harnessing ammonia metabolism as a therapeutic target in cancer immunotherapy.

8. Conclusion

AICD represents an emerging immunometabolic mechanism that bridges cancer metabolism and tumor immunity. Recent evidence demonstrates that excessive ammonia accumulation—mainly derived from aberrant glutamine metabolism—disrupts mitochondrial and lysosomal integrity in effector $CD8^+$ T cells, leading to metabolic exhaustion, immune dysfunction, and tumor immune evasion. Rather than being a mere metabolic by-product, ammonia functions as a signaling molecule capable of reshaping immune cell fate and modulating the TME.

Targeting key enzymes and transporters that regulate ammonia metabolism, such as GLS1 and CPS1, provides new therapeutic avenues for restoring immune function and suppressing tumor growth. In particular, pharmacological inhibition of ammonia production or

enhancement of its detoxification may protect T cells from AICD while simultaneously sensitizing tumors to immune attack. Furthermore, combining ammonia metabolism modulators with immune checkpoint inhibitors or metabolic-targeting agents may yield synergistic anti-tumor effects, overcoming immune resistance and improving treatment outcomes.

Despite significant progress, several challenges remain. The precise molecular basis of ammonia detoxification in different immune cell subsets, the reasons for $CD8^+$ T cell vulnerability, and the broader relevance of AICD across tumor types remain incompletely understood. Future research should focus on elucidating these mechanisms, identifying biomarkers that reflect ammonia dysregulation, and developing selective strategies that minimize systemic toxicity.

In summary, AICD defines a novel immunometabolic vulnerability with broad translational potential. By precisely modulating ammonia metabolism, it is possible to simultaneously reprogram tumor metabolism, restore immune surveillance, and enhance the efficacy of current cancer immunotherapies. Targeting AICD thus opens a promising frontier for integrating metabolic and immune-based therapeutic strategies in oncology.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Junyi Lin, Jun Xu, Song Nie
Visualization: Junyi Lin
Writing–original draft: Xixi Wang, Junyi Lin, Jing Li, Xiaojun Shu, Ning Hu, Shuang Liu, Yifei Fan, Qin Wang, Yue Wu
Writing–review & editing: Junyi Lin, Jun Xu, Song Nie

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: Understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol*. 2020;17(8):807-821.

   doi: 10.1038/s41423-020-0488-6

2. Lei X, Lei Y, Li JK, *et al*. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. *Cancer Lett*. 2020;470:126-133.

   doi: 10.1016/j.canlet.2019.11.009

3. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: Metabolism and immune function, supplementation and clinical translation. *Nutrients*. 2018;10(11):1564.

   doi: 10.3390/nu10111564

4. Wu J, Liu W, Qiu X, *et al*. A noninvasive approach to evaluate tumor immune microenvironment and predict outcomes in hepatocellular carcinoma. *Phenomics*. 2023;3(6):549-64.

   doi: 10.1007/s43657-023-00136-8

5. Wang XH, Fu YL, Xu YN, *et al*. Ginsenoside Rh1 regulates the immune microenvironment of hepatocellular carcinoma via the glucocorticoid receptor. *J Integr Med*. 2024;22(6):709-18.

   doi: 10.1016/j.joim.2024.09.004

6. Wise DR, Thompson CB. Glutamine addiction: A new therapeutic target in cancer. *Trends Biochem Sci*. 2010;35(8):427-33.

   doi: 10.1016/j.tibs.2010.05.003

7. De Vitto H, Pérez-Valencia J, Radosevich JA. Glutamine at focus: Versatile roles in cancer. *Tumour Biol*. 2016; 37(2):1541-1558.

   doi: 10.1007/s13277-015-4671-9

8. Rehman U, Gupta G, Sahebkar A, Kesharwani P. Ammonia-induced cell death: A novel frontier to enhance cancer immunotherapy. *Immunology*. 2025;175(1):16-20.

   doi: 10.1111/imm.13918

9. Dimski DS. Ammonia metabolism and the urea cycle: Function and clinical implications. *J Vet Intern Med*. 1994;8(2):73-78.

   doi: 10.1111/j.1939-1676.1994.tb03201.x

10. Yang L, Achreja A, Yeung TL, *et al*. Targeting stromal glutamine synthetase in tumors disrupts tumor microenvironment-regulated cancer cell growth. *Cell Metab*. 2016;24(5):685-700.

    doi: 10.1016/j.cmet.2016.10.011

11. Cyriac R, Lee K. Glutaminase inhibition as potential cancer therapeutics: Current status and future applications. *J Enzyme Inhibit Med Chem*. 2024;39(1):2290911.

doi: 10.1080/14756366.2023.2290911

12. Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: Cell biology, physiology, and clinical opportunities. *J Clin Investig*. 2013;123(9):3678-3684.

doi: 10.1172/jci69600

13. Souba WW. Glutamine and cancer. *Ann Surg*. 1993;218(6):715-728.

doi: 10.1097/00000658-199312000-00004

14. Wang Y, Bai C, Ruan Y, *et al*. Coordinative metabolism of glutamine carbon and nitrogen in proliferating cancer cells under hypoxia. *Nat Commun* 2019;10(1):201.

doi: 10.1038/s41467-018-08033-9

15. Dang CV. Glutaminolysis: Supplying carbon or nitrogen or both for cancer cells? *Cell Cycle*. 2010;9(19):3884-3886.

doi: 10.4161/cc.9.19.13302

16. Hird FJ, Marginson MA. Oxidative deamination of glutamate and transdeamination through glutamate. *Arch Biochem Biophys*. 1966;115(2):247-256.

doi: 10.1016/0003-9861(66)90273-6

17. Weiner ID, Verlander JW. Renal ammonia metabolism and transport. *Compr Physiol*. 2013;3(1):201-220.

doi: 10.1002/cphy.c120010

18. Visek WJ. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutr Rev*. 1979;37(9):273-82.

doi: 10.1111/j.1753-4887.1979.tb06690.x

19. Ju YH, Bhalla M, Hyeon SJ, *et al*. Astrocytic urea cycle detoxifies Aβ-derived ammonia while impairing memory in Alzheimer's disease. *Cell Metab*. 2022;34(8):1104-1120.e8.

doi: 10.1016/j.cmet.2022.05.011

20. Shanware NP, Mullen AR, DeBerardinis RJ, Abraham RT. Glutamine: Pleiotropic roles in tumor growth and stress resistance. *J Mol Med* (*Berl*). 2011;89(3):229-236.

doi: 10.1007/s00109-011-0731-9

21. Cluntun AA, Lukey MJ, Cerione RA, Locasale JW. Glutamine metabolism in cancer: Understanding the heterogeneity. *Trends Cancer*. 2017;3(3):169-180.

doi: 10.1016/j.trecan.2017.01.005

22. Jin L, Alesi GN, Kang S. Glutaminolysis as a target for cancer therapy. *Oncogene*. 2016;35(28):3619-2625.

doi: 10.1038/onc.2015.447

23. Weiner ID, Verlander JW. Ammonia transport in the kidney by Rhesus glycoproteins. *Am J Physiol Renal Physiol*. 2014;306(10):F1107-F1120.

doi: 10.1152/ajprenal.00013.2014

24. Mak DO, Dang B, Weiner ID, Foskett JK, Westhoff CM. Characterization of ammonia transport by the kidney Rh glycoproteins RhBG and RhCG. *Am J Physiol Renal Physiol*. 2006;290(2):F297-F305.

doi: 10.1152/ajprenal.00147.2005

25. Quentin F, Eladari D, Cheval L, *et al*. RhBG and RhCG, the putative ammonia transporters, are expressed in the same cells in the distal nephron. *J Am Soc Nephrol*. 2003;14(3):545-554.

doi: 10.1097/01.asn.0000050413.43662.55

26. Winkler FK. Amt/MEP/Rh proteins conduct ammonia. *Pflugers Arch*. 2006;451(6):701-707.

doi: 10.1007/s00424-005-1511-6

27. Schiöth HB, Roshanbin S, Hägglund MG, Fredriksson R. Evolutionary origin of amino acid transporter families SLC32, SLC36 and SLC38 and physiological, pathological and therapeutic aspects. *Mol Aspects Med*. 2013;34(2-3):571-585.

doi: 10.1016/j.mam.2012.07.012

28. Allen GJP, Quijada-Rodriguez AR, Wilson JM, Weihrauch D. The role of the antennal glands and gills in acid-base regulation and ammonia excretion of a marine osmoconforming brachyuran. *Comp Biochem Physiol A Mol Integr Physiol*. 2024;292:111619.

doi: 10.1016/j.cbpa.2024.111619

29. Meng M, Chen S, Lao T, Liang D, Sang N. Nitrogen anabolism underlies the importance of glutaminolysis in proliferating cells. *Cell Cycle*. 2010;9(19):3921-3932.

doi: 10.4161/cc.9.19.13139

30. Li Q, Abrashev R, Harvey LM, McNeil B. Oxidative stress-associated impairment of glucose and ammonia metabolism in the filamentous fungus, *Aspergillus niger* B1-D. *Mycol Res*. 2008;112(Pt 9):1049-1055.

doi: 10.1016/j.mycres.2008.03.004

31. Shen YA, Chen CL, Huang YH, *et al*. Inhibition of glutaminolysis in combination with other therapies to improve cancer treatment. *Curr Opin Chem Biol*. 2021;62:64-81.

doi: 10.1016/j.cbpa.2021.01.006

32. He X, Li J, An S, Jiang C. pH-sensitive drug-delivery systems for tumor targeting. *Ther Deliv*. 2013;4(12):1499-1510.

doi: 10.4155/tde.13.120

33. Zhang L, Michele Nawata C, De Boeck G, Wood CM. Rh protein expression in branchial neuroepithelial cells, and the role of ammonia in ventilatory control in fish. *Comp Biochem Physiol A Mol Integr Physiol*. 2015;186:39-51.

doi: 10.1016/j.cbpa.2014.10.004

34. Zhang J, Zeng W, Han Y, Lee WR, Liou J, Jiang Y. Lysosomal LAMP proteins regulate lysosomal pH by direct inhibition of the TMEM175 channel. *Mol Cell*. 2023;83(14):2524-2539.e7.

doi: 10.1016/j.molcel.2023.06.004

35. Birsoy K, Wang T, Chen WW, Freinkman E, Abu-Remaileh M, Sabatini DM. An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. *Cell*. 2015;162(3):540-551.

doi: 10.1016/j.cell.2015.07.016

36. Gibson SB. A matter of balance between life and death: targeting reactive oxygen species (ROS)-induced autophagy for cancer therapy. *Autophagy*. 2010;6(7):835-837.

doi: 10.4161/auto.6.7.13335

37. Villar VH, Merhi F, Djavaheri-Mergny M, Durán RV. Glutaminolysis and autophagy in cancer. *Autophagy*. 2015;11(8):1198-208.

doi: 10.1080/15548627.2015.1053680

38. Polletta L, Vernucci E, Carnevale I, *et al*. SIRT5 regulation of ammonia-induced autophagy and mitophagy. *Autophagy*. 2015;11(2):253-270.

doi: 10.1080/15548627.2015.1009778

39. Eng CH, Abraham RT. The autophagy conundrum in cancer: Influence of tumorigenic metabolic reprogramming. *Oncogene*. 2011;30(47):4687-4696.

doi: 10.1038/onc.2011.220

40. Weiner ID, Verlander JW. Molecular physiology of the Rh ammonia transport proteins. *Curr Opin Nephrol Hypertens*. 2010;19(5):471-477.

doi: 10.1097/MNH.0b013e32833bfa4e

41. Oh MH, Sun IH, Zhao L, *et al*. Targeting glutamine metabolism enhances tumor-specific immunity by modulating suppressive myeloid cells. *J Clin Investig*. 2020;130(7):3865-3884.

doi: 10.1172/jci131859

42. Zhang H, Liu J, Yuan W, *et al*. Ammonia-induced lysosomal and mitochondrial damage causes cell death of effector CD8(+) T cells. *Nat Cell Biol*. 2024;26(11):1892-1902.

doi: 10.1038/s41556-024-01503-x

43. Fan Y, Xue H, Li Z, Huo M, Gao H, Guan X. Exploiting the Achilles' heel of cancer: Disrupting glutamine metabolism for effective cancer treatment. *Front Pharmacol*. 2024;15:1345522.

doi: 10.3389/fphar.2024.1345522

44. Tang K, Zhang H, Deng J, *et al*. Ammonia detoxification promotes CD8(+) T cell memory development by urea and citrulline cycles. *Nat Immunol*. 2023;24(1):162-173.

doi: 10.1038/s41590-022-01365-1

45. Ma R, Ji T, Zhang H, *et al*. A Pck1-directed glycogen metabolic program regulates formation and maintenance of memory CD8(+) T cells. *Nat Cell Biol*. 2018;20(1):21-7.

doi: 10.1038/s41556-017-0002-2

46. Zhang H, Tang K, Ma J, *et al*. Ketogenesis-generated β-hydroxybutyrate is an epigenetic regulator of CD8(+) T-cell memory development. *Nat Cell Biol*. 2020;22(1):18-25.

doi: 10.1038/s41556-019-0440-0

47. Auron A, Brophy PD. Hyperammonemia in review: Pathophysiology, diagnosis, and treatment. *Pediatr Nephrol*. 2012;27(2):207-222.

doi: 10.1007/s00467-011-1838-5

48. Kim GW, Lee DH, Jeon YH, *et al*. Glutamine synthetase as a therapeutic target for cancer treatment. *Int J Mol Sci*. 2021;22(4):1701.

doi: 10.3390/ijms22041701

49. Yoshikawa S, Nagao M, Toh R, *et al*. Inhibition of glutaminase 1-mediated glutaminolysis improves pathological cardiac remodeling. *Am J Physiol Heart Circ Physiol*. 2022;322(5):H749-H761.

doi: 10.1152/ajpheart.00692.2021

50. van der Windt GJ, Everts B, Chang CH, *et al*. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity*. 2012;36(1):68-78.

doi: 10.1016/j.immuni.2011.12.007

51. Wang JB, Erickson JW, Fuji R, *et al*. Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell*. 2010;18(3):207-219.

doi: 10.1016/j.ccr.2010.08.009

52. Weiner ID, Verlander JW. Role of NH3 and NH4+ transporters in renal acid-base transport. *Am J Physiol Renal Physiol*. 2011;300(1):F11-F23.

doi: 10.1152/ajprenal.00554.2010

53. Felipo V, Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol*. 2002;67(4):259-279.

doi: 10.1016/s0301-0082(02)00019-9

54. Kasatkina LA, Borisova TA. Glutamate release from platelets: Exocytosis versus glutamate transporter reversal. *Int J Biochem Cell Biol*. 2013;45(11):2585-2595.

doi: 10.1016/j.biocel.2013.08.004

55. Giles JR, Ngiow SF, Manne S, *et al*. Shared and distinct biological circuits in effector, memory and exhausted CD8(+) T cells revealed by temporal single-cell transcriptomics and epigenetics. *Nat Immunol*. 2022;23(11):1600-1613.

doi: 10.1038/s41590-022-01338-4

56. Li T, Copeland C, Le A. Glutamine metabolism in cancer. *Adv Exp Med Biol*. 2021;1311:17-38.

doi: 10.1007/978-3-030-65768-0_2

57. DeBerardinis RJ, Cheng T. Q's next: The diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene*. 2010;29(3):313-324.

doi: 10.1038/onc.2009.358

58. Li Z, Lin J, Yin P. Ammonia death: A novel potential strategy to augment immunotherapy in cancer. *Cancer Gene Ther.* 2024;31(12):1751-1753.

doi: 10.1038/s41417-024-00851-y

59. Lu K. Cellular pathogenesis of hepatic encephalopathy: An update. *Biomolecules*. 2023;13(2):396.

doi: 10.3390/biom13020396

60. Moedas MF, Simões RJM, Silva MFB. Mitochondrial targets in hyperammonemia: Addressing urea cycle function to improve drug therapies. *Biochem Pharmacol*. 2024;222:116034.

doi: 10.1016/j.bcp.2024.116034

61. Vuillefroy de Silly R, Pericou L, Seijo B, Crespo I, Irving M. Acidity suppresses CD8 + T-cell function by perturbing IL-2, mTORC1, and c-Myc signaling. *EMBO J*. 2024;43:4922-4953.

doi: 10.1038/s44318-024-00235-w

62. Arner EN, Rathmell JC. Metabolic programming and immune suppression in the tumor microenvironment. *Cancer Cell*. 2023;41(3):421-433.

doi: 10.1016/j.ccell.2023.01.009

63. Sharma S, Agnihotri N, Kumar S. Targeting fuel pocket of cancer cell metabolism: A focus on glutaminolysis. *Biochem Pharmacol*. 2022;198:114943.

doi: 10.1016/j.bcp.2022.114943

64. Daye D, Wellen KE. Metabolic reprogramming in cancer: unraveling the role of glutamine in tumorigenesis. *Semin Cell Dev Biol*. 2012;23(4):362-369.

doi: 10.1016/j.semcdb.2012.02.002

65. Yoo HC, Park SJ, Nam M, *et al*. A variant of SLC1A5 is a mitochondrial glutamine transporter for metabolic reprogramming in cancer cells. *Cell Metab*. 2020;31(2):267-283.e12.

doi: 10.1016/j.cmet.2019.11.020

66. Anderson NM, Mucka P, Kern JG, Feng H. The emerging role and targetability of the TCA cycle in cancer metabolism. *Protein Cell*. 2018;9(2):216-237.

doi: 10.1007/s13238-017-0451-1

67. Vitale I, Manic G, Coussens LM, Kroemer G, Galluzzi L. Macrophages and metabolism in the tumor microenvironment. *Cell Metab*. 2019;30(1):36-50.

doi: 10.1016/j.cmet.2019.06.001

68. Lee HM, Nayak A, Kim J. A new role for ammonia in tumorigenesis? *Cell Metab*. 2022;34(7):944-946.

doi: 10.1016/j.cmet.2022.06.009

69. Cheng C, Geng F, Li Z, *et al.* Ammonia stimulates SCAP/Insig dissociation and SREBP-1 activation to promote lipogenesis and tumour growth. *Nat Metab*. 2022;4(5):575-588.

doi: 10.1038/s42255-022-00568-y

70. Kouidhi S, Ben Ayed F, Benammar Elgaaied A. Targeting tumor metabolism: A new challenge to improve immunotherapy. *Front Immunol*. 2018;9:353.

doi: 10.3389/fimmu.2018.00353

71. Li C, Zhang G, Zhao L, Ma Z, Chen H. Metabolic reprogramming in cancer cells: Glycolysis, glutaminolysis, and Bcl-2 proteins as novel therapeutic targets for cancer. *World J Surg Oncol*. 2016;14(1):15.

doi: 10.1186/s12957-016-0769-9

72. Bell HN, Huber AK, Singhal R, *et al*. Microenvironmental ammonia enhances T cell exhaustion in colorectal cancer. *Cell Metab*. 2023;35(1):134-149.e6.

doi: 10.1016/j.cmet.2022.11.013

73. Weisshaar N, Ma S, Ming Y, *et al*. The malate shuttle detoxifies ammonia in exhausted T cells by producing 2-ketoglutarate. *Nat Immunol*. 2023;24(11):1921-1932.

doi: 10.1038/s41590-023-01636-5

74. Chen G, Wang C, Huang S, *et al*. Novel roles of ammonia in physiology and cancer. *J Mol Cell Biol*. 2025;17(1):mjaf007.

doi: 10.1093/jmcb/mjaf007

75. Chengqun Q, Xijun W, Yangyang F, Xiaoqin Y, Min C, Jun X. Prognostic and immune modulatory effects of ammonia-induced cell death in head and neck squamous cell carcinoma. *Discov Oncol*. 2025;16(1):1039.

doi: 10.1007/s12672-025-02508-0

76. Zhao L, Lee ZH, Shah YM. Ammonia as a critical metabolic modulator of anti-tumor immunity. *Med Gas Res*. 2025;15(3):446-447.

doi: 10.4103/mgr.MEDGASRES-D-24-00147

77. Newton K, Strasser A, Kayagaki N, Dixit VM. Cell death. *Cell*. 2024;187(2):235-256.

doi: 10.1016/j.cell.2023.11.044

78. Park W, Wei S, Kim BS, *et al*. Diversity and complexity of cell death: A historical review. *Exp Mol Med*. 2023;55(8):1573-1594.

doi: 10.1038/s12276-023-01078-x

79. Wang X, Lin J, Li Z, Wang M. In what area of biology has a "new" type of cell death been discovered? *Biochim Biophys Acta Rev Cancer*. 2023;1878(5):188955.

doi: 10.1016/j.bbcan.2023.188955

80. Chen L, Cui H. Targeting glutamine induces apoptosis: A cancer therapy approach. *Int J Mol Sci*. 2015;16(9):22830-22855.

doi: 10.3390/ijms160922830

81. Eil R, Vodnala SK, Clever D, *et al*. Ionic immune suppression

within the tumour microenvironment limits T cell effector function. *Nature*. 2016;537(7621):539-543.

doi: 10.1038/nature19364

82. Holecek M. Side effects of long-term glutamine supplementation. *J Parent Enteral Nutr*. 2013;37(5):607-616.

doi: 10.1177/0148607112460682

83. Halama A, Suhre K. Advancing cancer treatment by targeting glutamine metabolism-a roadmap. *Cancers (Basel)*. 2022;14(3):553.

doi: 10.3390/cancers14030553

84. Wang Z, Liu F, Fan N, *et al*. Targeting glutaminolysis: New perspectives to understand cancer development and novel strategies for potential target therapies. *Front Oncol*. 2020;10:589508.

doi: 10.3389/fonc.2020.589508

85. Dammeijer F, van Gulijk M, Mulder EE, *et al*. The PD-1/PD-L1-checkpoint restrains T cell immunity in tumor-draining lymph nodes. *Cancer Cell*. 2020;38(5):685-700.e8.

doi: 10.1016/j.ccell.2020.09.001

86. Suarez GV, Melucci Ganzarain CDC, Vecchione MB, *et al*. PD-1/PD-L1 Pathway modulates macrophage susceptibility to *Mycobacterium tuberculosis* specific CD8(+) T cell induced death. *Sci Rep*. 2019;9(1):187.

doi: 10.1038/s41598-018-36403-2

87. Jiang B, Zhang J, Zhao G, *et al*. Filamentous GLS1 promotes ROS-induced apoptosis upon glutamine deprivation via insufficient asparagine synthesis. *Mol Cell*. 2022;82(10):1821-1835.e6.

doi: 10.1016/j.molcel.2022.03.016

88. Wicker CA, Hunt BG, Krishnan S, *et al*. Glutaminase inhibition with telaglenastat (CB-839) improves treatment response in combination with ionizing radiation in head and neck squamous cell carcinoma models. *Cancer Lett*. 2021;502:180-188.

doi: 10.1016/j.canlet.2020.12.038

89. Polfus LM, Khajuria RK, Schick UM, *et al*. Whole-exome sequencing identifies loci associated with blood cell traits and reveals a role for alternative GFI1B splice variants in human hematopoiesis. *Am J Hum Genet*. 2016;99(2):481-488.

doi: 10.1016/j.ajhg.2016.06.016

90. Mayer M, Schaaf G, Mouro I, *et al*. Different transport mechanisms in plant and human AMT/Rh-type ammonium transporters. *J Gen Physiol*. 2006;127(2):133-144.

doi: 10.1085/jgp.200509369

91. Schulte ML, Fu A, Zhao P, *et al*. Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. *Nat Med*. 2018;24(2):194-202.

doi: 10.1038/nm.4464

92. Zhao Y, Zhu Y, Huang J, Song Z, Tang W. Influence of *in situ* biochar capping on microbial dynamics and ammonia nitrogen release in sediment. *J Environ Manag*. 2025;373:123524.

doi: 10.1016/j.jenvman.2024.123524

93. Steichen M, Decrem Y, Godfroid E, Buess-Herman C. Electrochemical DNA hybridization detection using peptide nucleic acids and [Ru(NH3)6]3+ on gold electrodes. *Biosens Bioelectron*. 2007;22(9-10):2237-2243.

doi: 10.1016/j.bios.2006.10.041

94. Esteban R, Ariz I, Cruz C, Moran JF. Review: Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci*. 2016;248:92-101.

doi: 10.1016/j.plantsci.2016.04.008

95. Gouda MA, Voss MH, Tawbi H, *et al*. A phase I/II study of the safety and efficacy of telaglenastat (CB-839) in combination with nivolumab in patients with metastatic melanoma, renal cell carcinoma, and non-small-cell lung cancer. *ESMO Open*. 2025;10(5):104536.

doi: 10.1016/j.esmoop.2025.104536