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Original article

Modulation of γδ T Cell Activity by Bisphosphonates in Neoplasms Resistant to Conventional Immunotherapy: An Update

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Abstract

Bisphosphonates (BPs) are traditionally used to manage bone diseases, but recent evidence suggests they may play a significant role in cancer immunotherapy by modulating γδ T cell activity. These agents, particularly nitrogen-containing bisphosphonates, activate Vγ9Vδ2 T cells, enhancing their cytotoxicity against tumor cells. However, gaps remain in understanding the differential effects of bisphosphonates on various γδ T cell subsets, optimal dosing regimens, and their impact on the broader tumor microenvironment. This review synthesizes knowledge on bisphosphonate-induced γδ T cell modulation, explores potential resistance mechanisms, and evaluates clinical applicability across different tumor types. It identifies biomarkers for patient stratification, considers synergistic effects with other therapies, and discusses the economic and practical implications of integrating bisphosphonates into standard oncology practice. Addressing these gaps is essential for optimizing bisphosphonates as immunomodulatory agents, advancing cancer immunotherapy strategies, and improving patient outcomes.

Keywords: ***Diphosphonates; Immunomodulation; Immunotherapy; Tumor Microenvironment; Neoplasms; Immunologic Factors.***

Introduction

Bisphosphonates (BPs) are widely recognized for their critical role in managing bone diseases such as osteoporosis, Paget's disease, and bone metastases. These agents, particularly nitrogen-containing bisphosphonates like zoledronic acid, primarily function by inhibiting the farnesyl pyrophosphate synthase in the mevalonate pathway, which reduces osteoclast-mediated bone resorption [1-3].

However, recent studies have uncovered a potential immunomodulatory role for bisphosphonates, specifically their ability to modulate γδ T cell activity, presenting new opportunities in cancer immunotherapy. The γδ T cells, particularly the Vγ9Vδ2 subtype, are a unique subset of T lymphocytes that bridge innate and adaptive immunity [4-6].

Unlike αβ T cells, they do not require antigen presentation through major histocompatibility complex (MHC) molecules, allowing them to recognize and eliminate tumor cells directly. This property makes γδ T cells promising candidates for immunotherapy, especially against tumors that evade conventional immune recognition [7]. Evidence suggests that nitrogen-containing bisphosphonates can act as potent activators of Vγ9Vδ2 T cells, enhancing their proliferation, activation, and cytotoxic functions against various malignancies [8].

The primary mechanism by which bisphosphonates activate γδ T cells involves the inhibition of farnesyl pyrophosphate synthase in the mevalonate pathway. This leads to the accumulation of isopentenyl pyrophosphate (IPP), a phosphoantigen recognized by the T cell receptor (TCR) on Vγ9Vδ2 T cells [9].

This interaction triggers the activation and expansion of these cells, suggesting that bisphosphonates might bridge innate and adaptive immune responses and provide dual benefits: reducing bone resorption and enhancing antitumor immunity [10].

Despite promising results, several knowledge gaps remain. Firstly, the differential effects of various bisphosphonates on γδ T cell subsets, such as naïve, memory, and effector populations, are not fully elucidated [11].

While some studies suggest robust activation of γδ T cells by bisphosphonates in vitro, little is known about how these effects translate in vivo, particularly in the complex, often immunosuppressive tumor microenvironment. Understanding how bisphosphonates differentially affect these subsets could lead to more precise and effective therapeutic strategies [12,13].

There is a lack of consensus regarding the optimal dosing and administration schedules for bisphosphonates to maximize their immunomodulatory effects while minimizing toxicity [14]. Some studies have explored low-dose regimens of zoledronic acid in combination with interleukin-2 (IL-2) to promote γδ T cell

expansion. Still, no standardized protocol considers the variability in cancer types or stages. This gap in standardization presents a significant challenge for incorporating bisphosphonates into broader immunotherapy regimens [15-17].

Another critical area of uncertainty is the impact of bisphosphonates on the broader immune landscape within the tumor microenvironment. While bisphosphonates are known to activate γδ T cells, their effects on other immune cells, such as dendritic cells, macrophages, and regulatory T cells, remain poorly understood [18-20].

Given that the efficacy of immunotherapy often depends on coordinated responses from multiple immune cell types, understanding how bisphosphonates interact with these cells is crucial [21]. Emerging evidence suggests that bisphosphonates may enhance tumor antigen presentation by dendritic cells, amplifying the overall antitumor response, but this requires further investigation[22].

The long-term safety profile of bisphosphonates as immunomodulatory agents in cancer therapy is another area requiring exploration [23]. While these agents are generally well-tolerated in short-term use, prolonged administration has been associated with severe adverse effects, such as osteonecrosis of the jaw and atypical femoral fractures. Through well-designed clinical trials, comprehensive studies are needed to evaluate the risk-benefit ratio of bisphosphonates in this new therapeutic context [24-26].

Moreover, there is a lack of comprehensive studies examining the effects of bisphosphonates on γδ T cells across different tumor types, particularly those resistant to conventional immunotherapies [27]. For instance, while some cancers, such as multiple myeloma and breast cancer, have shown responsiveness to γδ T cell activation by bisphosphonates, other malignancies remain underexplored. Identifying which tumors most likely benefit from this approach is essential for developing tailored treatment strategies [28-30].

A significant gap in the literature pertains to the potential synergistic effects of bisphosphonates when combined with other immunotherapies, such as immune checkpoint inhibitors or chimeric antigen receptor (CAR) T-cell therapy [31]. Preliminary studies indicate that bisphosphonates may enhance the efficacy of these treatments by modifying the immune microenvironment to be more conducive to antitumor activity. Still, this hypothesis remains to be rigorously tested in both preclinical and clinical settings [32].

The current literature lacks detailed insights into the specific clinical applications of bisphosphonates in modulating γδ T cell activity for cancer treatment. While evidence suggests a beneficial role, the contexts in which they are most effective, such as specific cancer types, stages, or patient populations, are not well-defined. This represents a significant barrier to the broader adoption of bisphosphonates as a standard adjunctive therapy in oncology (**Figure 1**) [33-35].

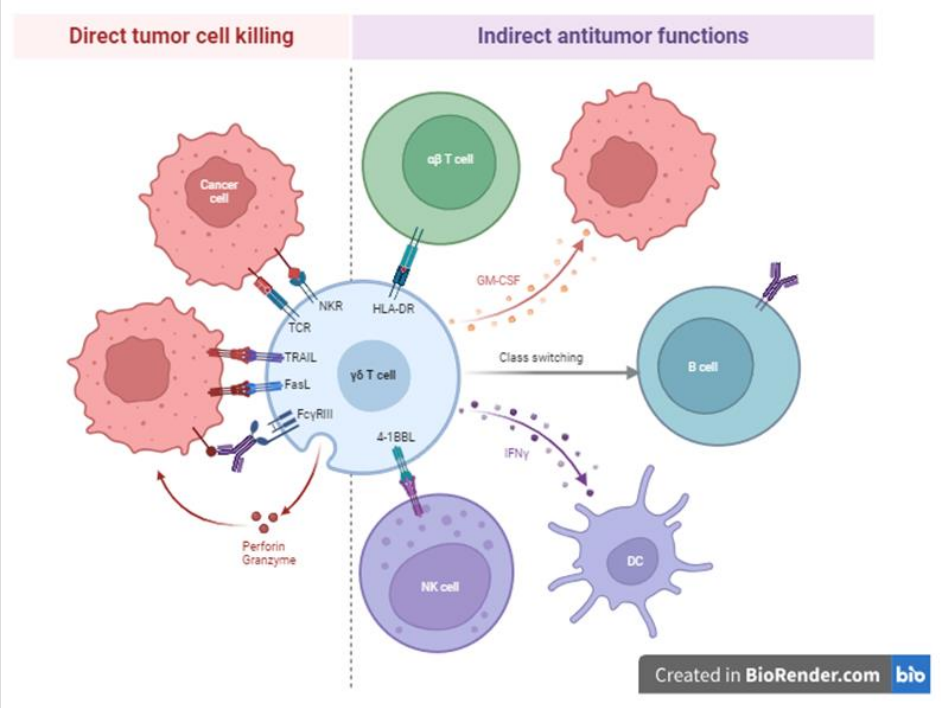

Figure 1. γδ T cells directly kill tumor cells and indirectly enhance antitumor immunity by activating other immune cells.
Source: https://www.nature.com/articles/s41568-019-0153-5

This review aims to provide a comprehensive overview of the existing evidence regarding the modulation of γδ T cell activity by bisphosphonates in cancer immunotherapy. By synthesizing findings from recent studies, we seek to identify areas of consensus and controversy, highlight existing gaps in understanding, and propose directions for future research to optimize the use of bisphosphonates as immunomodulatory agents in oncology.

Methods

This review was meticulously designed to evaluate the modulation of γδ T cell activity by bisphosphonates in cancer immunotherapy. It employed a comprehensive search strategy across multiple high-impact databases. The selected databases included PubMed, Scopus, Embase, Web of Science, and SciELO, which were chosen for their extensive collections of peer-reviewed studies. Google Scholar was also utilized to access gray literature, ensuring the inclusion of significant studies unavailable in conventional academic databases. This broad approach aimed to capture a wide range of evidence pertinent to the research objectives. The search strategy employed a combination of Boolean operators and specific keywords such as "diphosphonates," "immunomodulation," "immunotherapy," "tumor microenvironment," "neoplasms," and "immunologic factors." boolean operators (AND, OR) were applied to refine the search

parameters and optimize the retrieval of relevant studies. Inclusion criteria encompass various study designs, including randomized controlled trials, cohort studies, case-control studies, cross-sectional analyses, case series, and systematic reviews. Eligible studies required a minimum sample size of 10 participants to investigate the immunomodulatory effects of bisphosphonates on γδ T cells or their potential synergistic effects with other immunotherapies. Only studies with sufficient methodological detail for qualitative synthesis were included. Two independent reviewers blindedly performed the study selection process, screening titles and abstracts to ensure relevance to the study's aims. Subsequently, full-text articles were reviewed in detail, with any discrepancies resolved by a third independent reviewer, maintaining a robust and unbiased selection process. This blinding ensured that the reviewers' assessments were not influenced by study details, thus enhancing the objectivity of the selection. Data extraction was conducted using a standardized form to capture key study characteristics, including authorship, year of publication, study design, sample size, population details, interventions, and outcomes related to γδ T cell activity and interactions with the tumor microenvironment. The primary focus was identifying bisphosphonates' differential effects on γδ T cell subsets, optimal dosing regimens, and the impact on other immune cells within the tumor microenvironment. A qualitative synthesis was employed, given the heterogeneity in study designs, populations, and outcomes. Thematic analysis was used to categorize and integrate findings across different studies, providing a comprehensive overview of existing evidence. This synthesis aimed to identify key findings, gaps, and controversies surrounding the role of bisphosphonates in modulating γδ T cell activity in cancer. The review explored economic and practical considerations, such as the cost-effectiveness and feasibility of incorporating bisphosphonates into routine clinical practice. The conclusions drawn from this review are grounded in a critically evaluated body of scientific evidence, offering meaningful insights into the potential of bisphosphonates as immunomodulatory agents in oncology. By incorporating these detailed methodological elements, this review ensures a transparent, rigorous, and systematic approach to understanding the complex interactions between bisphosphonates, γδ T cell activity, and the tumor microenvironment in cancer immunotherapy.

Results and Discussion

The specific impacts of bisphosphonates on different γδ T cell subsets, including naïve, memory, and effector populations, are not fully understood. While bisphosphonates, particularly nitrogen-containing types, activate γδ T cells by increasing isopentenyl pyrophosphate (IPP) accumulation, the effects may differ among these subsets [36].

The role of bisphosphonates (BPs) in modulating γδ T cell activity within the context of cancer immunotherapy presents several complexities and areas requiring further exploration [14,37]. A comprehensive understanding of these aspects is vital to enhance the effectiveness of bisphosphonate-based therapies and identify opportunities for improved patient outcomes [6,38].

Naïve cells may have higher activation thresholds than memory or effector γδ T cells, which could impact their expansion and function. The effector cells may show enhanced cytotoxic responses in a tumor context, which is highly influenced by the tumor microenvironment. Further studies should aim to elucidate these differential effects in vivo [22,39].

The translation of in vitro findings on bisphosphonate-induced γδ T cell activation to in vivo settings remains unclear. Despite in vitro studies demonstrating the potential of bisphosphonates to activate Vγ9Vδ2 T cells, the complex and often immunosuppressive nature of the tumor microenvironment, characterized by factors like hypoxia and regulatory cells, could mitigate these effects [28,40]. However, future research must focus on comprehensive in vivo models and clinical trials to clarify how bisphosphonate-induced γδ T cell activation influences outcomes in real-world conditions (**Figure 2**) [41].

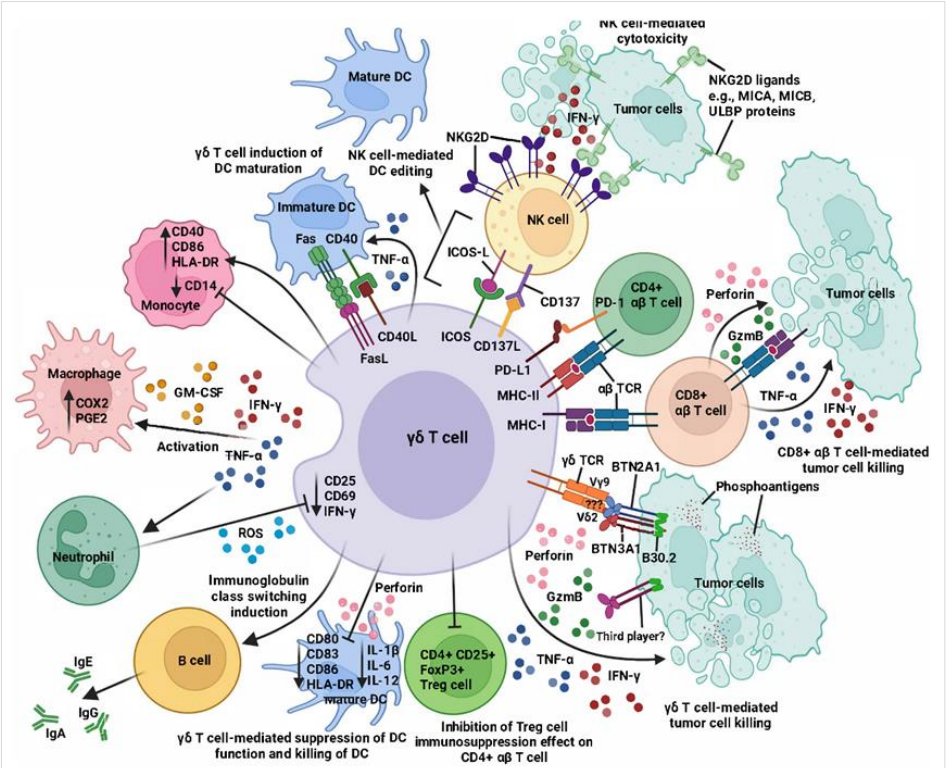

Figure 2. Overview of γδ T Cell Interactions in Cancer Immunity.
Source: https://doi.org/10.3389/fimmu.2022.894315

The broader impact of bisphosphonates on the tumor microenvironment is crucial for maximizing their therapeutic potential. While much attention has focused on their effects on γδ T cells, bisphosphonates also influence other immune cells, such as dendritic cells, macrophages, and regulatory T cells [33,42]. Studies suggest that bisphosphonates may enhance the antigen-presenting capacity of dendritic cells or polarize macrophages toward a pro-inflammatory phenotype, potentially amplifying the overall antitumor response [43].

There is no consensus on the optimal dosing and administration schedules for bisphosphonates to maximize their immunomodulatory effects while minimizing toxicity [35]. Existing studies have investigated low-dose regimens in conjunction with agents like interleukin-2 (IL-2), but variable responses across different cancer types and stages make standardizing protocols challenging [44].

The broader effects of bisphosphonates on the immune landscape within the tumor microenvironment are not well-characterized. While γδ T cell activation is established, the impact on other immune cells, such as dendritic cells, macrophages, and regulatory T cells, needs further exploration [45]. These interactions is vital for predicting therapeutic efficacy, as effective immunotherapy often relies on coordinated responses from multiple immune cells [39].

One critical gap in current research pertains to the mechanisms by which certain tumors exhibit resistance to the immunomodulatory effects of bisphosphonates. Tumors may develop resistance through several pathways, such as altering the expression of phosphoantigens, which are crucial for γδ T cell activation [46,47].

This alteration can occur due to changes in the tumor microenvironment that affect the mevalonate pathway, reducing the accumulation of intermediates like isopentenyl pyrophosphate (IPP) necessary for γδ T cell stimulation [11]. Tumors may increase the expression of immune checkpoint molecules, such as PD-L1, which inhibit the immune response by γδ T cells [21]. These resistance mechanisms could inform new combination therapies, such as using bisphosphonates alongside immune checkpoint inhibitors, to overcome the tumor's evasion strategies and potentiate the immune response [48].

The long-term safety profile of bisphosphonates in cancer therapy is a critical concern. Prolonged use is associated with adverse effects like osteonecrosis of the jaw and atypical femoral fractures, which can limit their application in oncology [20]. These risks, particularly in long-term immunomodulatory use, requires extensive safety monitoring, risk stratification, and mitigation strategies [49].

Long-term safety and tolerability remain significant concerns, particularly given the potential adverse effects associated with bisphosphonates, such as osteonecrosis of the jaw and atypical femoral fractures [44,50]. A deeper exploration of strategies to mitigate these risks, such as adjusting dosing schedules, combining with other therapeutic agents, or developing new bisphosphonate formulations, is essential. Ongoing surveillance and post-marketing studies should assess long-term outcomes and adverse events to refine treatment protocols and ensure patient safety [51].

The potential synergy between bisphosphonates and other immunotherapies, such as checkpoint inhibitors or CAR T cells, remains an exciting yet underexplored area [16]. Preliminary evidence suggests that bisphosphonates modify the tumor microenvironment to enhance the efficacy of these treatments, but the mechanisms remain unclear. Further research is required to understand the effects of bisphosphonates across a broader range of tumor types, particularly those resistant to conventional immunotherapies [34,52].

The response to γδ T cell activation by bisphosphonates may vary significantly depending on the tumor's genetic and molecular characteristics, the presence of specific antigens, or the overall immune landscape [6-8]. Identifying which tumors are most likely to respond to bisphosphonate-based therapies will help refine their use and guide future clinical trials [53].

The potential for bisphosphonates to synergize with other immunotherapies, such as checkpoint inhibitors or CAR-T cell therapies, represents a promising avenue for future research [32]. Preliminary evidence suggests that bisphosphonates may enhance the efficacy of these therapies by modulating the tumor microenvironment to be more conducive to immune activity [46]. However, more detailed investigations are needed to define the optimal combinations, dosing regimens, and sequencing of these therapies to maximize patient outcomes [54].

The clinical applicability of bisphosphonates for modulating γδ T cell activity across different tumor types is still largely undefined. While there is evidence supporting their use in cancers like multiple myeloma and breast cancer, their efficacy in other malignancies has not been thoroughly explored [10,11]. Research should aim to identify which tumor types are most likely to benefit from bisphosphonate-induced γδ T cell activation, considering factors such as tumor microenvironment, immune evasion mechanisms, and genetic profiles [34,55].

A thorough comparison of different bisphosphonates such as zoledronic acid, alendronate, and risedronate is essential to understand their differential effects on γδ T cell activation and interaction with the tumor microenvironment [49]. Current literature suggests that while all these agents can enhance γδ T cell activity, they differ in their potency, duration of action, and safety profiles [56].

For instance, zoledronic acid is known for its potent and sustained activation of γδ T cells, but its use is associated with more frequent adverse effects. In contrast, other bisphosphonates may offer a more favorable safety profile but less robust immunomodulatory activity [28-30]. Comparative studies should investigate these differences, focusing on optimizing dosing regimens and administration routes to balance efficacy and safety across various cancer types and stages [57].

The current literature lacks standardization in study designs, which complicates comparisons and limits the generalizability of findings. Variability in patient populations, dosing regimens, outcome measures, and follow-up periods makes it challenging to draw definitive conclusions [58]. The trials should adhere to standardized protocols with well-defined inclusion criteria, consistent dosing schedules, and validated endpoints to allow for more robust meta-analyses and systematic reviews [27].

An area not fully explored is the potential mechanisms of resistance by which certain tumors may evade the immunomodulatory effects of bisphosphonates. Tumor cells may alter the expression of phosphoantigens or upregulate immune checkpoint molecules that inhibit γδ T cell activity [7,16]. For example, tumors could modify the presentation or recognition of IPP, limiting the activation of Vγ9Vδ2 T cells. Understanding these resistance mechanisms is crucial to developing strategies, such as combining bisphosphonates with agents that block these escape pathways [52].

Identifying biomarkers or genetic profiles that could help stratify patients who are most likely to benefit from bisphosphonate-based immunotherapy is necessary [41]. The expression levels of enzymes in the mevalonate pathway, specific γδ T cell receptors, or

the presence of tumor antigens may predict responsiveness to bisphosphonates. Developing reliable biomarkers could guide patient selection and improve clinical outcomes by tailoring treatments to those most likely to benefit [43,54].

The stratification of patients based on biomarkers or genetic profiles represents another area of interest. Identifying which patients are most likely to benefit from bisphosphonate-based immunotherapy could lead to more personalized treatment approaches [18,33].

Potential biomarkers include the expression levels of specific γδ T cell subtypes, such as Vγ9Vδ2, or tumor-specific characteristics like antigen profiles or metabolic signatures linked to the mevalonate pathway [4,6,17]. A detailed discussion is warranted on integrating these biomarkers into clinical practice, optimizing patient selection, and improving therapeutic outcomes. Studies should focus on validating these biomarkers in diverse populations and across various cancer types to establish their predictive value in clinical settings [35-37,44].

Comparative studies are necessary to evaluate the efficacy of different bisphosphonates and their combinations with other therapies across various cancer types and stages. Such studies could provide insights into which bisphosphonates are most effective in specific contexts and help refine treatment protocols [14,52]. They could explore the differential effects of bisphosphonates in combination with existing immunotherapies, like immune checkpoint inhibitors or adoptive cell therapies [23].

Finally, economic and practical considerations are vital for successfully integrating bisphosphonates into routine clinical practice. Evaluating the cost-effectiveness of bisphosphonates as immunomodulatory agents, their accessibility, and logistical challenges, such as administration routes and monitoring requirements, is crucial for broad adoption [36,49].

These factors should be studied in parallel with clinical trials to ensure that bisphosphonates can be feasibly implemented in diverse healthcare settings. Incorporating bisphosphonates into routine clinical practice also raises several economic and practical considerations [3-5]. Cost-effectiveness analysis is needed to determine whether the benefits of bisphosphonate-based immunotherapy outweigh the costs, particularly in resource-limited settings [4,15].

Practical issues such as drug accessibility, administration routes, and the need for regular monitoring must be addressed. These factors will influence the feasibility of integrating bisphosphonates into standard care protocols and may require tailored approaches depending on the healthcare setting [36,58].

Conclusion

In conclusion, these gaps are essential for optimizing bisphosphonates as immunomodulatory agents in cancer therapy. Research should focus on elucidating resistance mechanisms, refining patient stratification methods, conducting comparative studies, and considering economic and practical factors to enhance the clinical utility of bisphosphonates in oncology. We can better understand how to leverage bisphosphonates to improve cancer immunotherapy outcomes by exploring these areas in depth.

Conflict of interest

The authors declare that there is no conflict of interest.

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