pH-Sensitive Zinc-Zoledronate Inhalation Therapy for Macrophage Modulation in Lung Cancer

A. Safak, H Smyth
The University of Texas at Austin, Division of Molecular Pharmaceutics and Drug Delivery

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INTRODUCTION

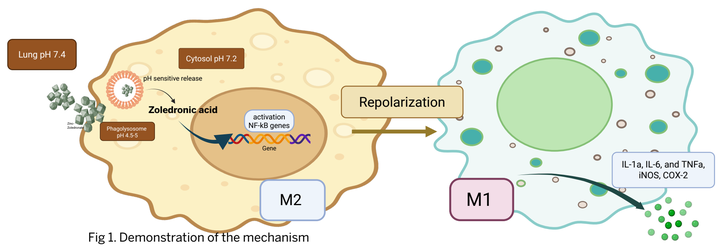

Fig 1. Demonstration of the mechanism

Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for over 1.7 million deaths annually (Siegel et al., 2024). Immunotherapies such as immune checkpoint inhibitors have improved survival outcomes, yet their systemic administration often results in severe immune-related adverse events (irAEs), frequently necessitating treatment discontinuation (Allouchery et al., 2020). Tumor-associated macrophages (TAMs) play a pivotal role in the lung tumor microenvironment, with M2-polarized macrophages promoting tumor progression and immune evasion, whereas M1-polarized macrophages exhibit anti-tumor activity (Yi et al., 2023). Zoledronic acid (ZA), a clinically approved bisphosphonate for bone metastases, has shown potential for TAM reprogramming; however, its systemic use is limited by off-target toxicity (Zheng et al., 2022). The inhalation route offers a unique advantage for lung cancer immunotherapy by enabling local drug delivery directly to the tumor microenvironment, reducing systemic exposure and minimizing immune-related adverse events. This study investigates the development of a pH-sensitive zinc-zoledronate (Zn-Zol) inhalation formulation to target TAMs locally within lung tumors, aiming to repolarize macrophages and enhance therapeutic efficacy.

METHODS

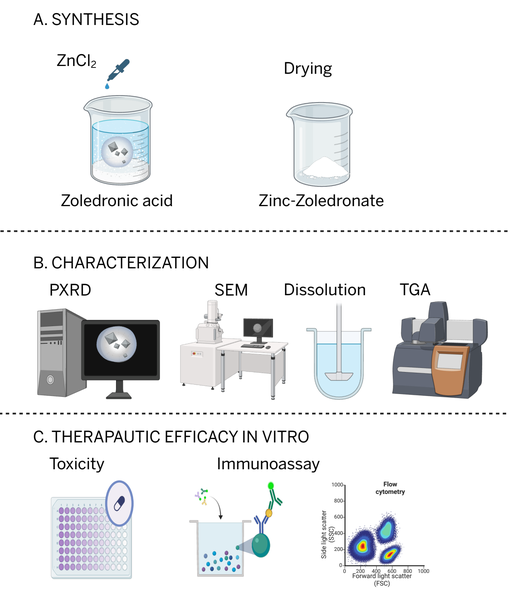

Fig 2. Summary of the methods: A; Salt synthesis of ZincZoledronate (ZincZol), B; Physicochemical characterization methods, C; In-vitro testing of the ZincZol

CONCLUSION

The pH-sensitive zinc-zoledronate inhalation formulation offers a targeted approach to repolarize tumor-associated macrophages (TAMs) within lung tumors. It shifts M2 macrophages to an anti-tumor M1 phenotype, reducing systemic toxicity by delivering the drug directly to the tumor microenvironment. This strategy enhances existing immunotherapies and has potential to improve outcomes in non-small cell lung cancer (NSCLC) by minimizing off-target effects and boosting local immune responses. Additionally, it could synergize with immune checkpoint inhibitors to further amplify anti-tumor efficacy

RESULTS

CHARACTERIZATION OF SYNTHESIS

A.

	Unmilled F1			Milled F1		
	%10	%50	%90	%10	%50	%90
F1 (µm)	2.72	10.71	23.51	1.4	2.2	5.55

Table-1: After air jet milling particle size decreased 10 to 2-3 micron for optimum macrophage uptake

A.1.

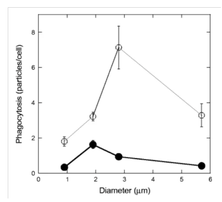

Fig 3.Microspheres by rat alveolar macrophages (After 1 h)[3]

B.

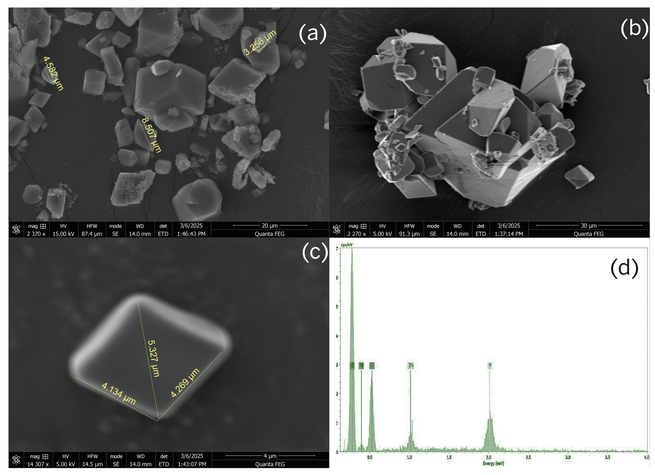

Fig 4. SEM images of Zinc-zoledronate at various magnifications find the magniifications; (a) 2,370x, (b) 2,270x, (c) 14,307x. (d) Energy dispersive spectra of single crystal

C.

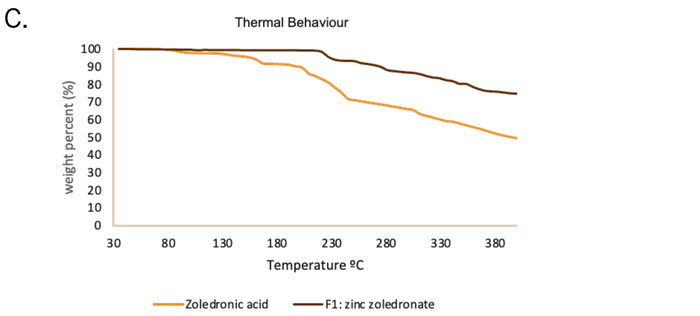

Fig 5. TGA thermograms show the thermal stability of zinc–zoledronate compared to free zoledronic acid. Zinc–zoledronate exhibited reduced weight loss and a higher decomposition onset temperature, indicating improved thermal stability and lower moisture content following complexation.

D.

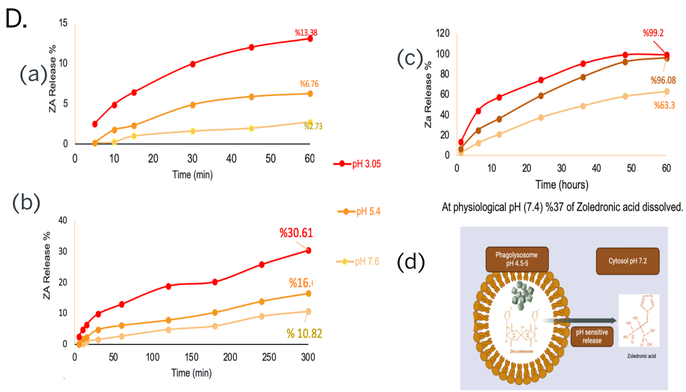

Fig 6. Dissolution behaviour of Zinc-zoledronate at different time point and different pH conditions, (a) 1h, (b) 5h, (c) 80 h, (d) representation of pH dependent solubility

CRYSTALLOGRAPHY

A.

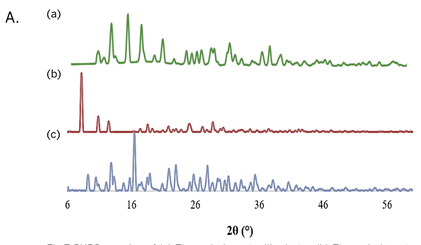

Fig 7. PXRD overlay of (a) Zinc zoledronate dihydrate, (b) Zinc zoledronate anhydrus, (c)Zoledronic acid monohydrate.

B.

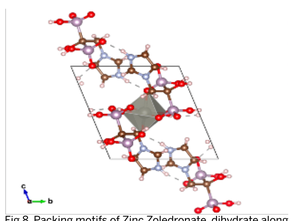

Fig 8. Packing motifs of Zinc Zoledronate dihydrate along the a-axis.

F2/F3: Chitosan matrix for controlled zoledronic acid release

C.

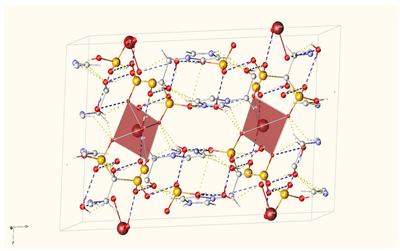

Fig 9. Packing motifs of Zinc Zoledronate dihydrate crystal lattice along the a-axis.

D. Collaboration Study of Zoledronic acid chitosan particles [4]

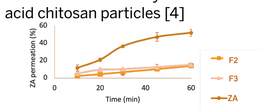

Fig 10. Chitosan particle release profile

Formulation	F2	F3
Zoledronic acid (ZA)	0.250	0.250
Chitosan (Ch)	0.5	0.250
Ch:ZA ratio	2:1	1:1
NaoH 0.1 N	-	-
Acetic acid (1 %)	-	-
DI water	100 mL	100 mL
Final volume	100 mL	100 mL

Table 2. F2/F3: Chitosan matrix for controlled zoledronic acid release

The zinc-zoledronate formulation demonstrated a reaction mass efficiency of 82%, producing stable crystalline particles with a size range of 2-3 µm, suitable for macrophage uptake. PXRD confirmed the formation of a dihydrate crystal structure, and the formulation exhibited pH-dependent solubility. SEM imaging revealed crystals with rough surfaces, indicating successful salt formation. In vitro assays showed that Zn-ZA at 5 µM significantly increased TNF-α secretion by 4.2-fold and IL-6 by 3.8-fold in M2 macrophages compared to untreated controls. Flow cytometry confirmed macrophage repolarization, with a 45% decrease in CD163+ M2 macrophages and a corresponding 52% increase in CD80+ M1 macrophages after 48 hours of treatment. The formulation demonstrated low cytotoxicity, maintaining cell viability above 85% at therapeutic concentrations.

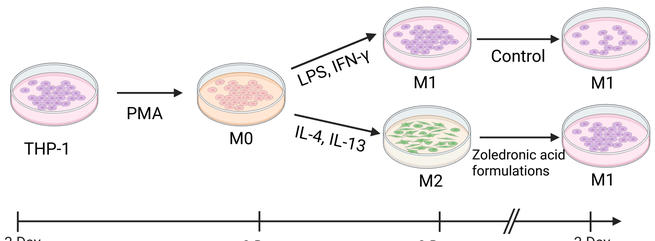

Fig 11. Visual representation of in-vitro experiment protocol

Formulations exhibit low toxicity for target doses (</=5mM)

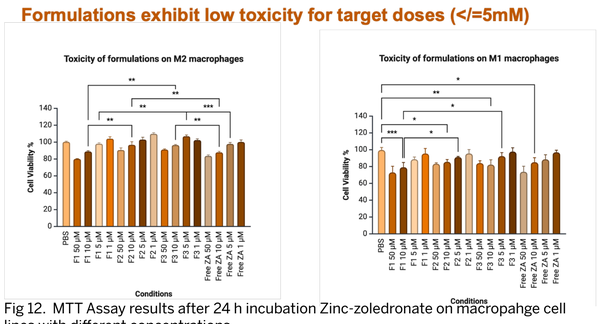

Fig 12. MTT Assay results after 24 h incubation Zinc-zoledronate on macropahge cell lines with different concentrations

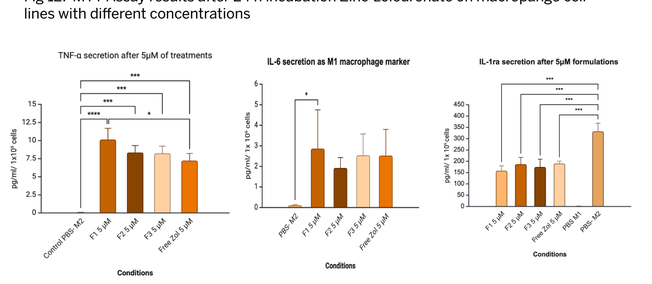

Fig 13. ELISA for cytokine secretion after 24h incubation of zoledronic acid formulations

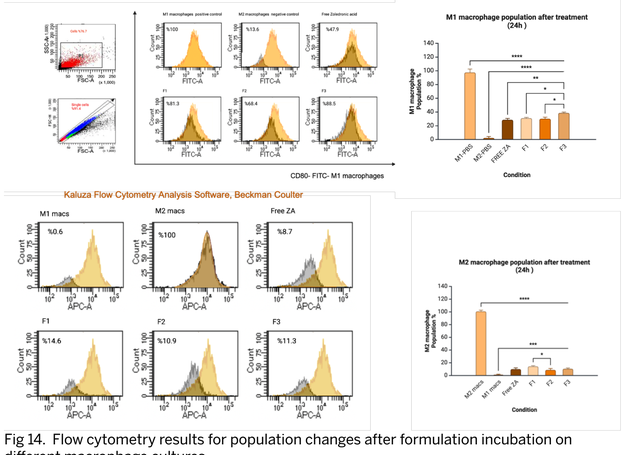

Fig 14. Flow cytometry results for population changes after formulation incubation on different macrophage cultures

References:
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):7-30.
2. Allouchery M, Lombard T, Albiges L. Immune-related adverse events: mechanisms and management. Nat Rev Clin Oncol. 2020;17(2):89-100.
3. Yi H, Guo C, Yu X. Macrophage polarization in the tumor microenvironment. Trends Cancer. 2023;9(1):25-38.
4. Zheng H, Wang J, Zhou X. Zoledronic acid: more than just a bone-targeting drug. Cancer Res. 2022;82(4):567-574.