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Pretreatment of Bone Anabolic Agent Delays *or* Prevents Myeloma Progression

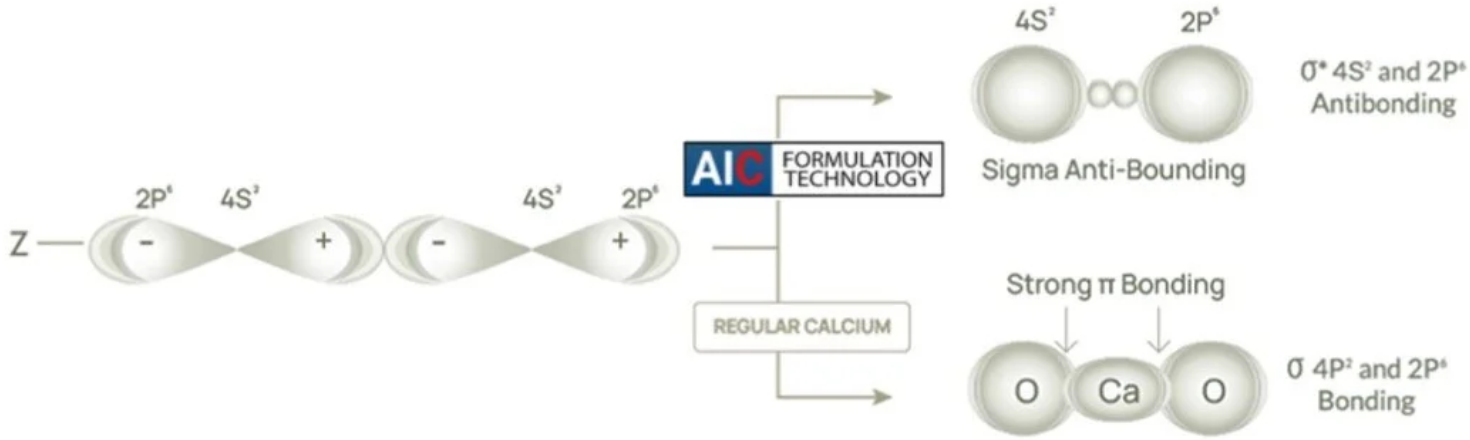
CBHI Calcium & Bone Health Institute · UAMS University of Arkansas for Medical Sciences · ACRI Advanced Calcium Research Institute

INTRODUCTION

Anti-Orbital Ionic Calcium(AIC) is a chemically modified calcium carbonate with a unique weak bond structure (Fig. 1). Due to this weak sigma anti-bonding, AIC maintains a loose hold of the calcium ion to its carbonate group, improving water solubility and allowing for easy absorption into the body without requiring digestion, vitamin D, or peptides. In addition, AIC improves the equilibrium constant by 200-fold, which increases the calcium ion concentration at a given Calcium carbonate concentration. It triggers unique hormonal responses that activate osteoblasts and stimulate self-healing mechanisms, such as reversing calcification and promoting natural healing reactions within the body.

Multiple myeloma (MM) is the second most common blood cancer. It remains an incurable disease since most patients will eventually relapse and die of resistant disease. The bone serves as a home for myeloma to grow but is also targeted by MM, resulting in MM-bone disease (MMBD). MMBD occurs in 90% of MM patients and is one of the defining features of MM characterized by bone destruction.

Many attempts, including ours, have been made to test novel or existing bone anabolic drugs for anti-MMBD; however, they have shown minimal improvement in myeloma progression, although bone formation has been enhanced. Surprisingly, we found that the mice that survived the previous treatment became tolerable to subsequent tumor transplantation.



AIC FORMULATION TECHNOLOGY

(Calcium) (Oxygen)

$$2Ca^{2+}CO_3 \rightleftharpoons 2Ca^{2+} + O_2 + 2CO_2$$

$$*Ksp = 7.5 \times 10^{-7} \text{ to } 8.7 \times 10^{-7} \text{ at } 25℃$$

Regular Calcium

$$CaCO_3 \rightleftharpoons Ca^{2+} + CO_3^{2-}$$

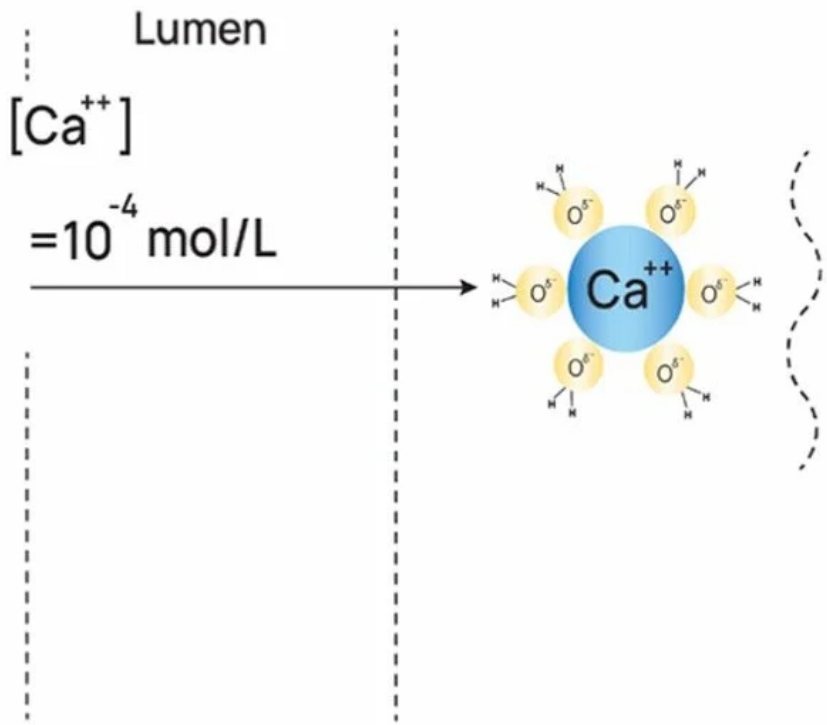
$$*Ksp = 3.7 \times 10^{-9} \text{ to } 8.7 \times 10^{-9} \text{ at } 25℃$$

*equilibrium constant

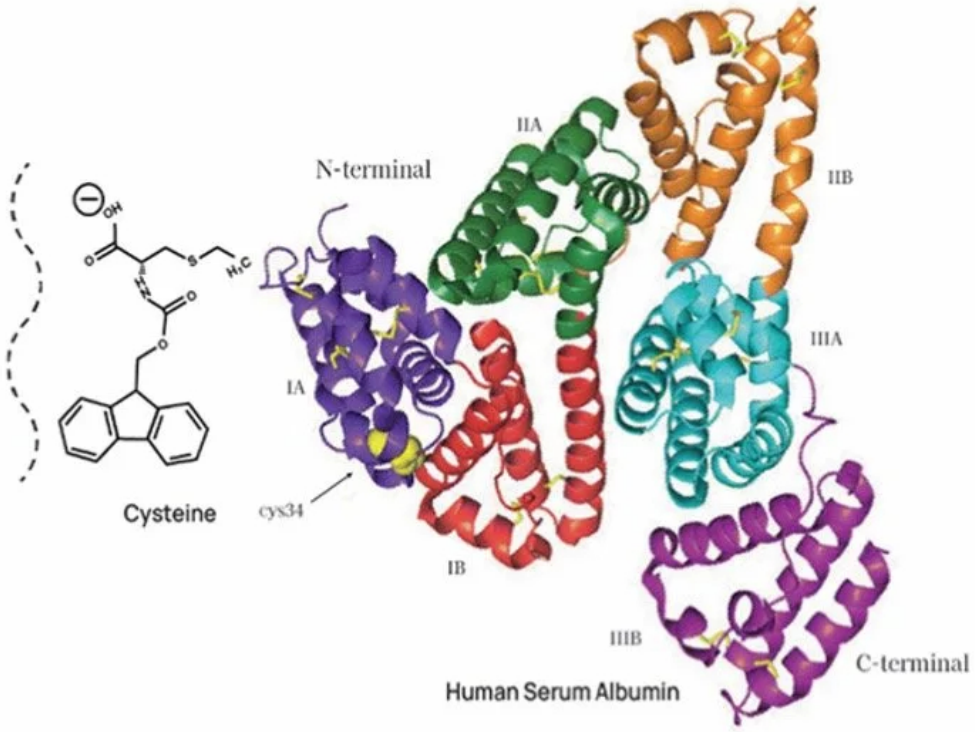
Figure1. Demonstration of Anti-Orbital bonding and Equilibrium Contant Rate of AIC Compared to Regular Calcium.

Calcium transport pathways with Serum albumin

AIC Calcium Passive Transport Pathways



AIC Calcium Ion in Human Serum



Calcium transport pathways with Serum albumin

General Calcium Active Transport Pathways

Lumen

$[Ca^{++}]$

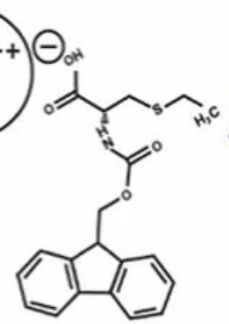
$=10^{-3}$ mol/L

$[Ca^{++}] = 10^{-7}$ mol/L^{-1}

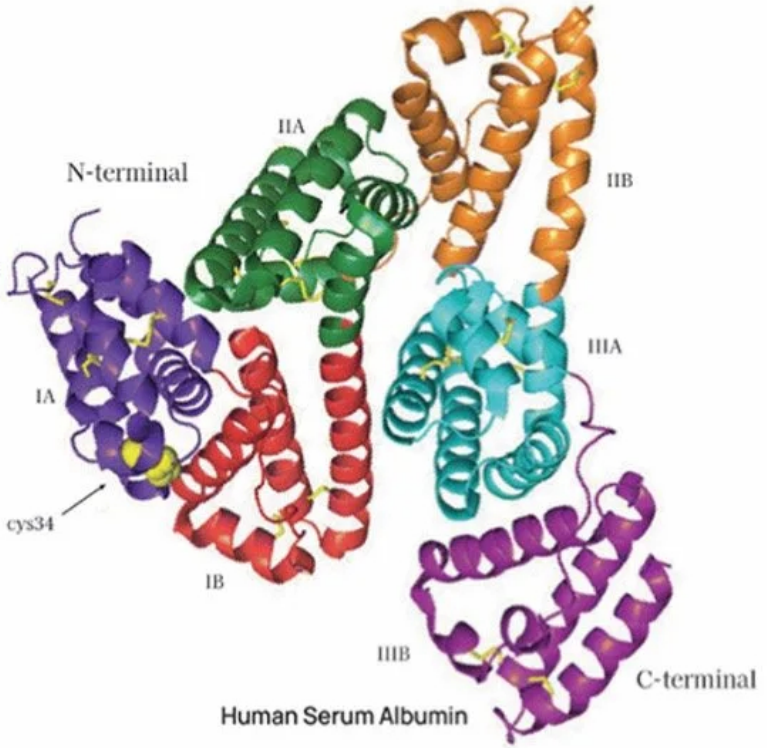
CaBp

Calbindin

Calciferol
(Vitamin D)



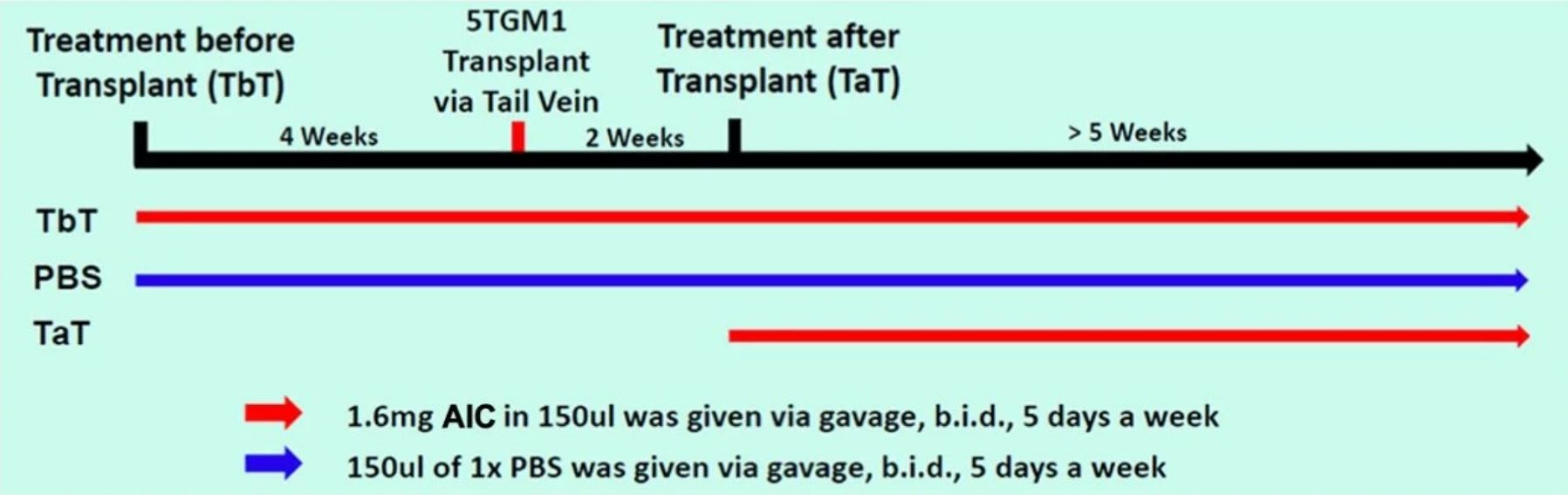
General Calcium in Human Serum



HYPOTHESIS

Collectively, we hypothesized that enhancing the bone environment may delay myeloma growth.

METHODS



- 8-12-week-old NOD SCID gamma mice of equal number of sex were used and transplanted via tail vein with 1×10^6 luciferase-expressing 5TGM1 cells

- Mice were imaged weekly by IVIS imager to assess the myeloma progression and terminated when endpoint criteria were met. The study was terminated after one week of all PBS group mice died due to myeloma progression.

- The spine was extracted from all sacrificed mice, then was performed for DEXA and microCT analyses.

RESULTS

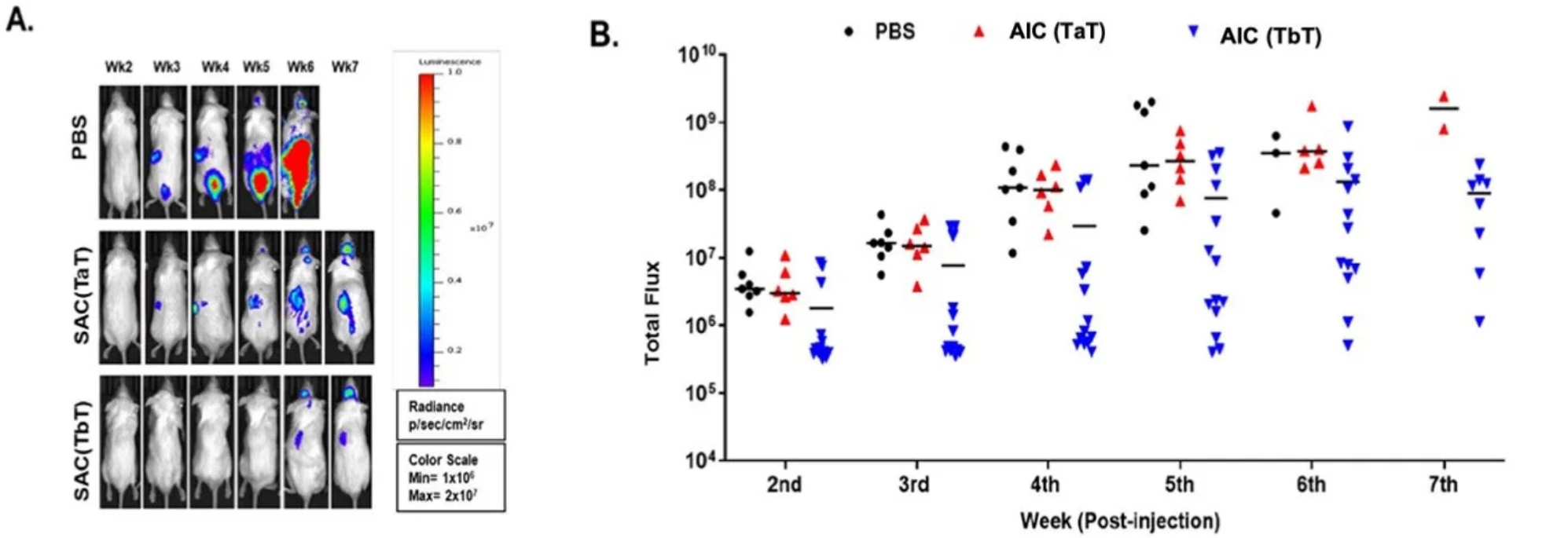
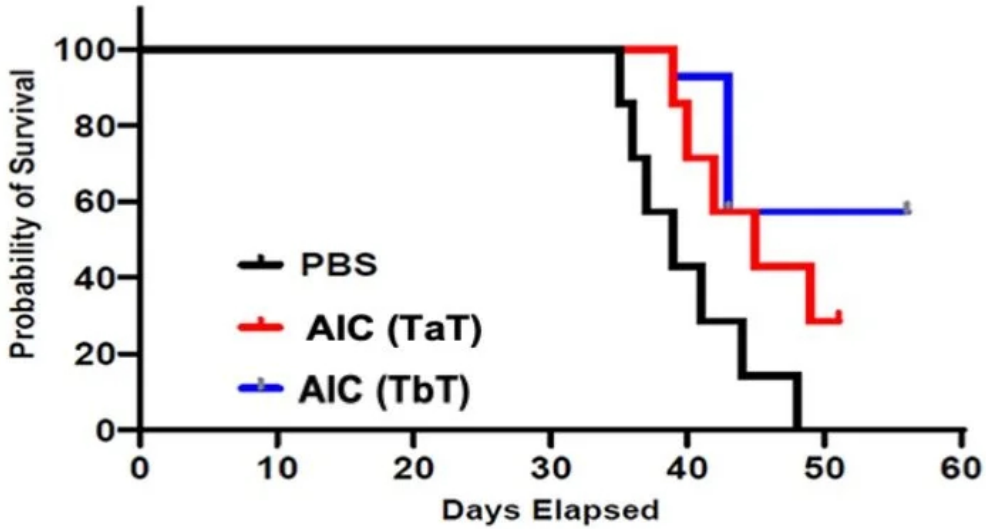


Figure 3. **(A)** Representative BL images of PBS-treated vs AIC-treated 5TGM-1 transplanted NSG mice. All BL images were normalized to a single scale, as shown in the box. **(B)** From the weekly image, a total flux value was obtained from the whole body of the mouse. All total flux values (black dot or red and blue triangles; PBS or AIC-treated) with median (black line) were expressed as a progression of myeloma.

RESULTS



Log-rank (Mantel-Cox) test	
Chi square	4.1
df	1
P value	0.0437
P value summary	*
Are the survival curves sig different?	Yes

	PBS	AIC (TaT)
Median survival	39	45

Log-rank (Mantel-Cox) test	
Chi square	11
df	1
P value	0.0007
P value summary	***
Are the survival curves sig different?	Yes

	PBS	AIC (TbT)
Median survival	39	Undefined

Figure 4. Mouse survivals were scored daily. When the mouse met the endpoint criteria, it was sacrificed and recorded. Survival curves were plotted from 7 mice from PBS (black line), 7 mice from AIC (TaT) (red line), and 14 mice from AIC (TbT) (blue line). Median survival times were 39 days for PBS, 45 days for AIC-TaT, and undefined for AIC-TbT-treated mice. The Mantel–Cox test showed p = 0.0437 for PBS vs AIC-TaT and p= 0.007 for PBS vs AIC (TbT).

RESULTS

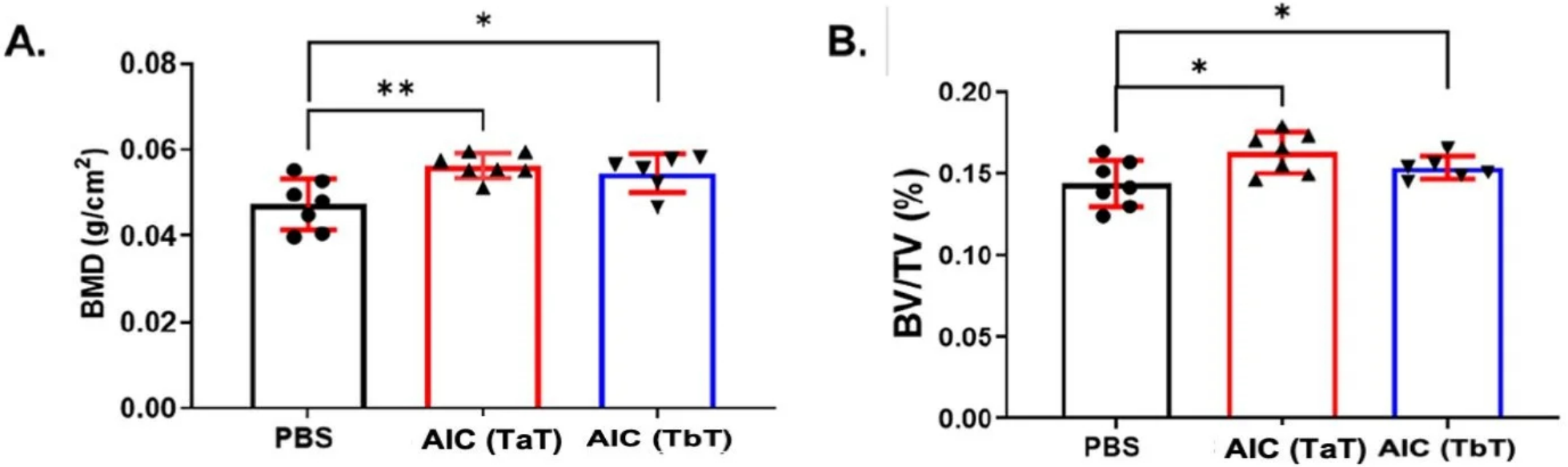
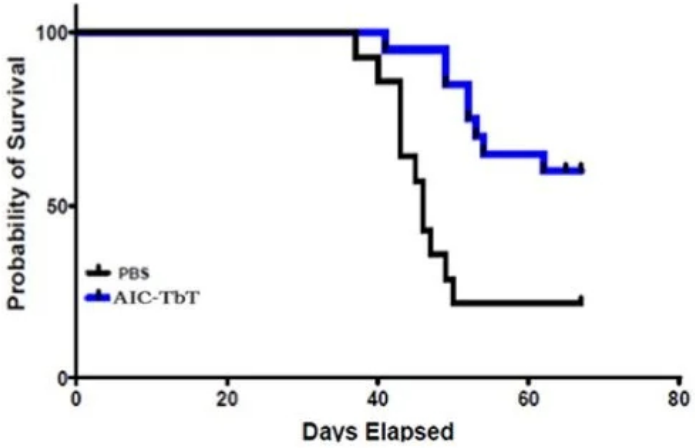


Figure 5. The mouse was sacrificed and stored in the freezer for more than 5 days. Once the carcass was thawed, the vertebrae were extracted from the frozen carcass and scanned for DEXA using PixiMus. **(A)** Represents the bone mineral density (BMD) changes in AIC-TaT and AIC-TbT mice versus PBS mice in the spine. Myeloma-associated bone lesions were assessed by microCT analysis. The trabecular regions at total lumbar vertebrae (L1–L6) were analyzed and expressed changes of **(B)** bone volume (BV/TV). Statistical differences were tested using Student's t-tests. * denotes $p < 0.05$; * denotes $p < 0.05$.

RESULTS

A.



Log-rank (Mantel-Cox) test		
Chi square		9.903
df		1
P value		0.0017
P value summary		**
	PBS	AIC-TbT
Median survival	46	Undefined

B.

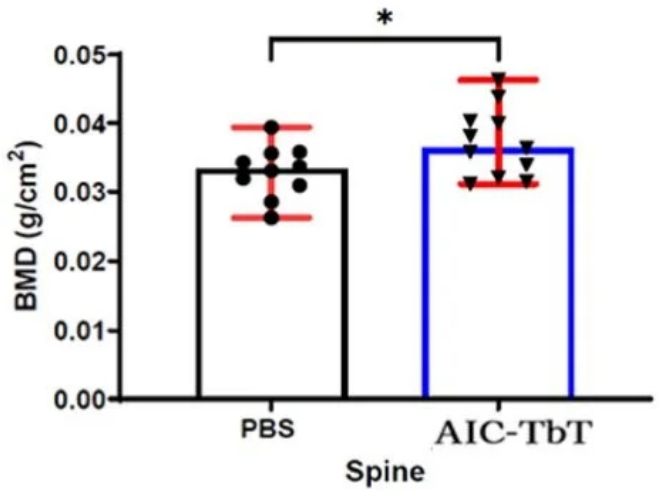


Figure 6. Mouse Survivals and Bone Mineral Contents of PBS vs AIC-treated 5TGM-1 transplanted (TbT) C57/KaLwRij mice. **(A)** Survival curves were plotted for 14 PBS-treated mice (black line) and 20 AIC-TbT mice (blue line). Median survival times were 39 days for PBS and undefined for AIC-TbT mice. **(B)** The vertebrae were extracted from the carcass and scanned for DEXA using PixiMus. The bone mineral density (BMD) changes in the spines of AIC-TbT mice are compared to those of PBS mice. Statistical differences were tested using Student's t-tests. * denotes $p < 0.05$.

CONCLUSION

- Mouse survival has improved in AIC-treated mice compared to control (PBS-treated) mice in both Immune-deficient and Immune-competent Models (NSG and C57/KaLwRij mice).
- DEXA and μCT results demonstrated that the bone mineral density and bone volume-to-total volume ratio increased significantly in the spines of AIC-treated mice compared to control mice. AIC enhanced the bone formation.
- Our results showed that improving the bone microenvironment by AIC pretreatment significantly delays or prevents myeloma cell growth in mice.

- **OUR DATA SUGGEST THAT EARLY TREATMENT WITH BONE ANABOLIC AGENTS IN THE PRE-EMERGING OR REMISSION PHASE**

ACKNOWLEDGEMENTS

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