Poisoning the Clot: Dominant-Negative Fibrinogen Permits Osteosarcoma Metastasis

Stephen W. Chenard, MSc[1,2,3,4]; Benjamin C. Asbury, MS[2,4]; Rachel M. McKee[2,4]; J. Court Reese, BS[2,4]; Herbert S. Schwartz, MD[4]; Rachelle W. Johnson, PhD[2,3]; & Jonathan G. Schoenecker, MD, PhD[2,3,4,5,6,7,8]*

[1]*Vanderbilt University School of Medicine, Nashville, TN*
[2]*Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN*
[3]*Program in Cancer Biology, Vanderbilt University, Nashville, TN*
[4]*Department of Orthopaedic Surgery, Vanderbilt University Medical Center, Nashville, TN*
[5]*Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN*
[6]*Department of Pharmacology, Vanderbilt University, Nashville, TN*
[7]*Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, TN*
[8]*Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN*

**Corresponding Author:*
Stephen W. Chenard, MSc
Vanderbilt University School of Medicine
Nashville, TN 37232
Telephone Number: 541-908-6825
Email: stephen.w.chenard.1@vumc.org; chenard.stephen@gmail.com

Significance & Hypothesis: Lung metastases remain the principal driver of mortality for patients with osteosarcoma. Five-year survival is below 30% for these patients – a statistic that has not improved in over 50 years. There is a well-documented relationship between cancer and coagulation, yet the role of coagulation in osteosarcoma metastases remains underexplored. It was recently demonstrated that pharmacologic depletion of fibrinogen accelerates osteosarcoma growth and angiogenesis *in vivo*, suggesting that fibrin acts as a tumor-suppressive barrier. Whether fibrin integrity governs *metastatic* dissemination remains unknown. We hypothesized that disrupting fibrin polymerization would unmask a metastatic phenotype in an osteosarcoma model that has historically never shown spontaneous metastasis.

Innovation: This study presents the first reported spontaneous lung metastases in the MOSJ osteosarcoma model in over 25 years of published literature, establishing the only spontaneous metastatic osteosarcoma model compatible with immunocompetent C57BL/6 mice. This unlocks access to the full repertoire of genetically engineered animals on the C57BL/6 background, enabling tools for unprecedented mechanistic dissection of osteosarcoma metastases. Conceptually, we identify a dominant-negative mechanism in which structurally compromised fibrin is more pro-metastatic than either intact fibrin or complete fibrin absence—a "worst of both worlds" phenotype with direct clinical implications for patients receiving anticoagulant or antifibrinolytic therapy.

Approach: MOSJ osteosarcoma cells were orthotopically injected into the proximal tibia of three fibrinogen genotypes on the C57BL/6 background: wild-type ($Fga^{+/+}$), AEK heterozygous ($Fga^{+/EK}$; producing a 50:50 mixture of normal and non-polymerizable fibrinogen), and AEK homozygous ($Fga^{EK/EK}$; producing only non-polymerizable fibrinogen). Mice were monitored by serial radiographs for primary tumor progression and survival was tracked to humane endpoint. At necropsy, lungs were evaluated histologically for metastatic burden. In parallel, cell-mediated clot lysis assays were performed using MOSJ cells seeded on pre-formed fibrin matrices.

Results: There was no difference in primary tumor growth or overall survival between $Fga^{+/+}$ and $Fga^{EK/EK}$ mice. In striking contrast, $Fga^{+/EK}$ heterozygous mice had dramatically accelerated primary tumor growth (Figure 1) and significantly shorter overall survival (Figure 2). Histologic analysis of $Fga^{+/EK}$ lungs revealed extensive spontaneous metastases, the first ever reported in the MOSJ model (Figure 3). In vitro, MOSJ cells demonstrate robust plasminogen-dependent fibrinolytic activity, degrading fibrin matrices in a time- and plasminogen-dose-dependent manner, findings that are inhibited by the anti-fibrinolytic agent tranexamic acid (TXA).

Discussion & Impact: These findings suggest that fibrin matrix integrity is a critical determinant of osteosarcoma metastatic competence. They also reveal a paradoxical dominant-negative mechanism in which a structurally compromised fibrin barrier is more permissive for metastasis than either a robust barrier or complete fibrin absence.

We propose that tumor-mediated fibrinolysis of clots generates pro-metastatic signals, such as liberation of matrix-sequestered growth factors and activation of downstream proteolytic cascades, which drive metastatic outgrowth. This positions anti-fibrinolytic agents such as TXA, already familiar to orthopaedic oncologists, as candidate therapies for prevention of osteosarcoma metastasis. The establishment of a C57BL/6-compatible osteosarcoma model with spontaneous metastases also enables future mechanistic studies using genetically engineered models with targeted alterations in coagulation and fibrinolysis.

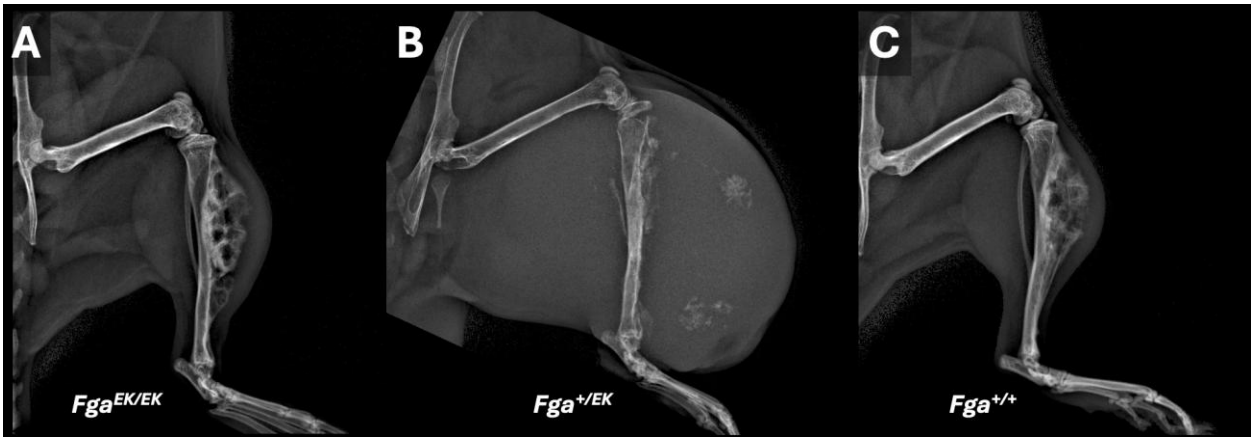

Figure 1. Representative radiographic images 6-weeks post tumor inoculation, demonstrating differences in tumor burden between *Fga*$^{EK/EK}$ (A), *Fga*$^{+/EK}$ (B), and *Fga*$^{+/+}$ (C) mice.

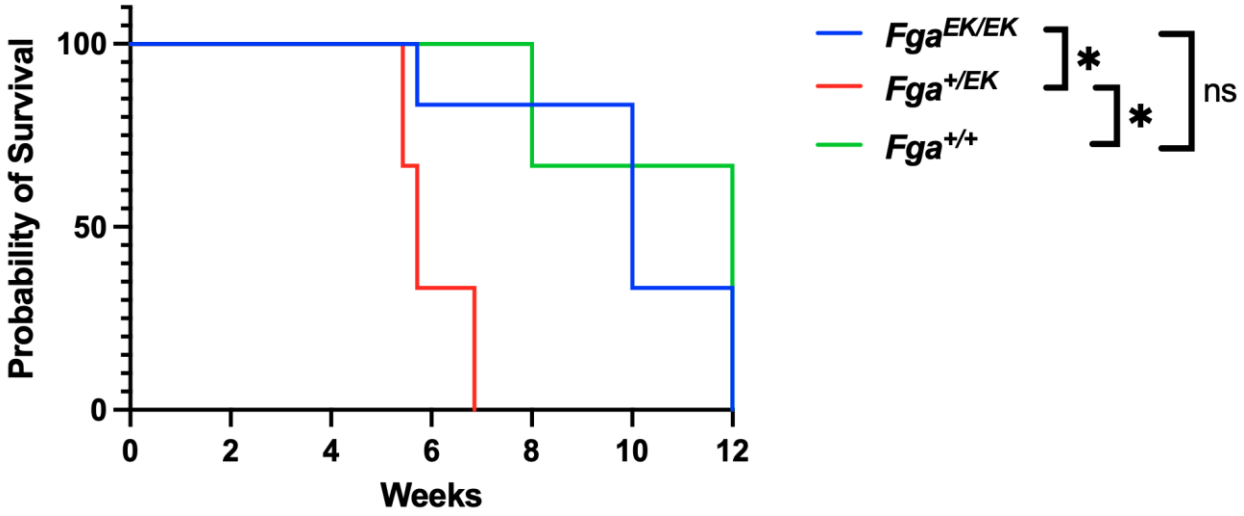

Figure 2. Kaplan-Meier curves demonstrating significantly shorter overall survival for MOSJ-tumor-bearing *Fga*$^{+/EK}$ mice compared to *Fga*$^{EK/EK}$ and *Fga*$^{+/+}$ mice. * Indicates p-value < 0.05.

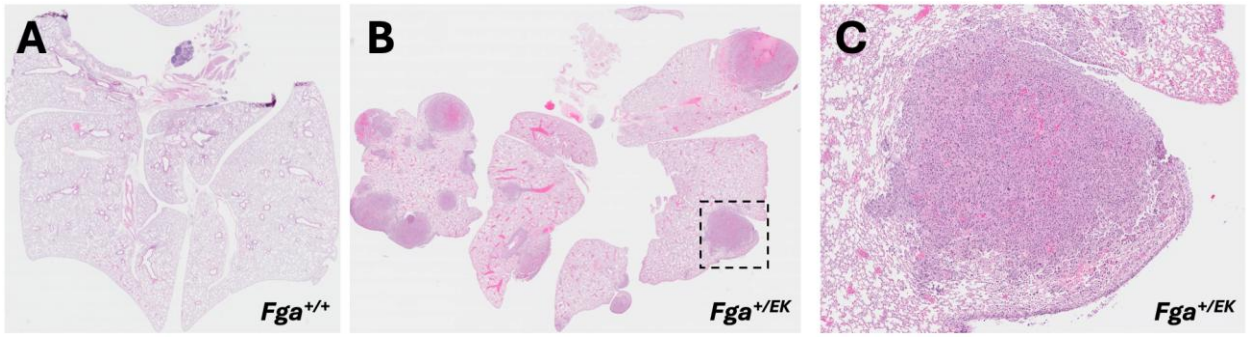

Figure 3. Representative histologic sections of lungs from MOSJ-tumor-bearing *Fga*$^{+/+}$ mice (A), vs *Fga*$^{+/EK}$ mice (B; black dashed rectangular region magnified in C).