ENTREMED

Drug Discovery and Development of Innovative Therapeutics

Boston, MA

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ENMD-981693: An Oral, Aurora Kinase – Angiogenesis Inhibitor

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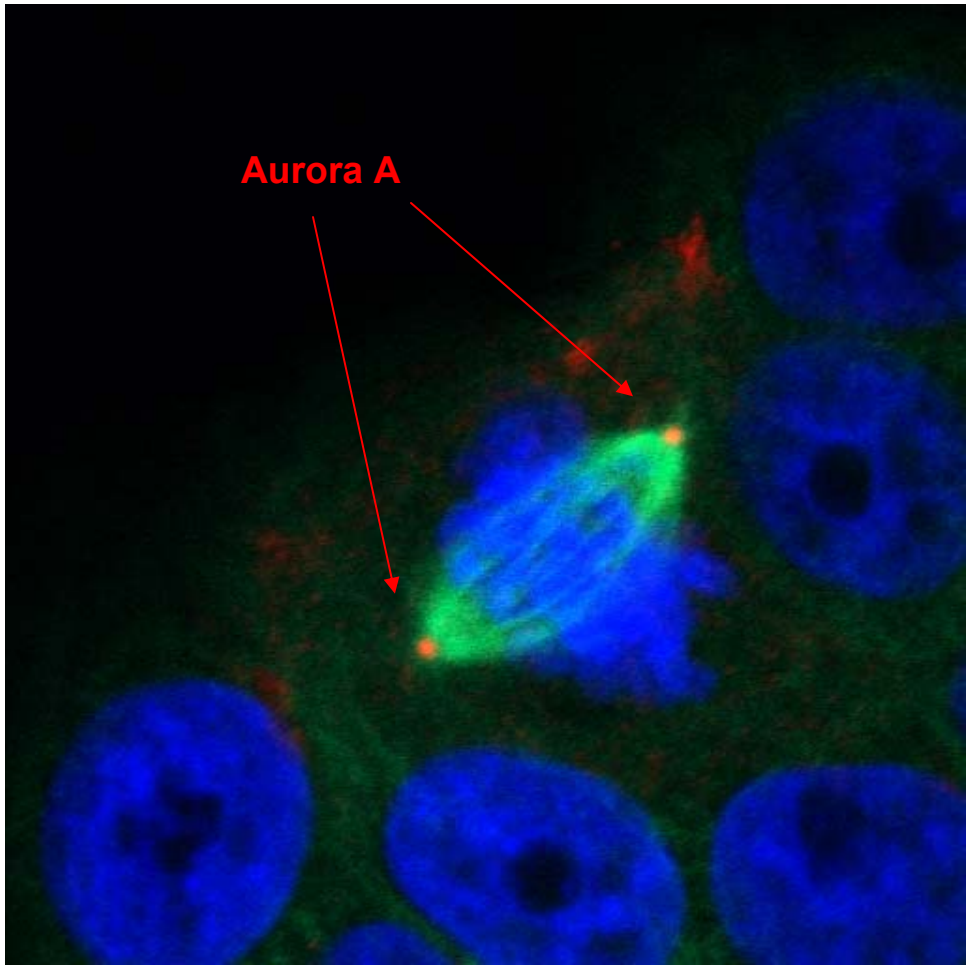
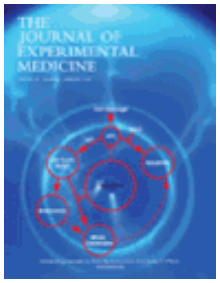
Aurora Kinases May Contribute to Oncogenesis

- The Auroras are mitotic kinases essential for cell division
- Aurora isoforms A and B are over expressed and/or amplified in many tumor types
- Aurora A and B have different localizations during the cell cycle and different roles
- Aberrant Aurora levels can lead to genomic instability, transformation
- Inhibition of Auroras leads to growth arrest and cell death



Aurora Kinase Inhibitor Program

- **Strong interest in Aurora/survivin control of mitotic pathways**
  - ***Tak W. Mak, J. Exp. Med. 2004 199: 399-410***



- **Medicinal chemistry expertise in kinase inhibitors, heterocycles**

- **Evolving target, no marketed products**
  - **Oral, broad single-agent activity, tolerability**

MedChem Approaches to Aurora Kinase Inhibitor Design

- **Incorporate existing SAR and structure-based approaches**
- **Retain key inhibitor-enzyme interactions (multiple H-bonds and π–π interactions)**
- **Structurally distinct, synthetically tractable, low MW heterocycles**
  - **New cores and/or new substituents**
- ***In vitro* potency and stability comparable to (or better than) benchmark compounds**

Aurora Kinase Inhibitor Program Overview

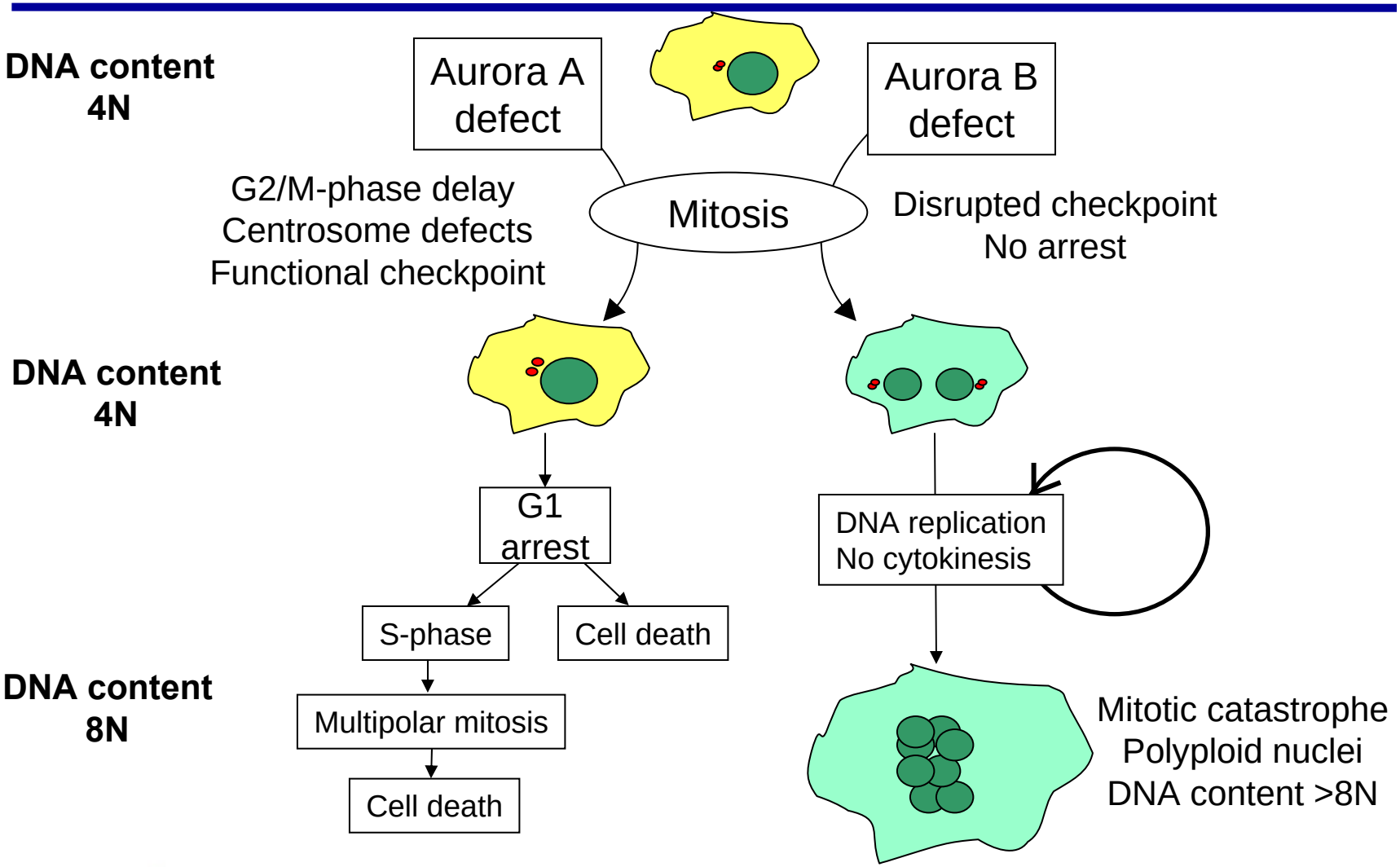
- **Multiple lead series established**
  - **> 300 Analogs prepared**
  - **> 40 Compounds with IC_{50} < 100 nM & GI_{50} < 1 μM**
  - **Strong IP position: four U.S. and International patent applications**

- ***In vivo* efficacy study of > 10 analogs (po & iv): ENMD-981693**
  - **Activity through both iv and oral routes**
  - **Tumor regression in multiple models**
  - **Aurora A IC_{50} 14 nM, Aurora B IC_{50} 290 nM**
  - **HCT116 GI_{50} 0.2 μM**

ENMD-981693 is an Aurora A and Angiogenic Kinase Inhibitor

- **High-potency activity against oncogenic RTKs and cytoplasmic TKs in addition to Aurora A**
- **Potent activity towards a broad spectrum of targets linked to angiogenesis/lymphangiogenesis**
  - **KDR/VEGFR2, VEGFR3, PDGFRα, FGFR1 and 2**
- **Potential oncology indications include both hematological and solid tumor malignancies**

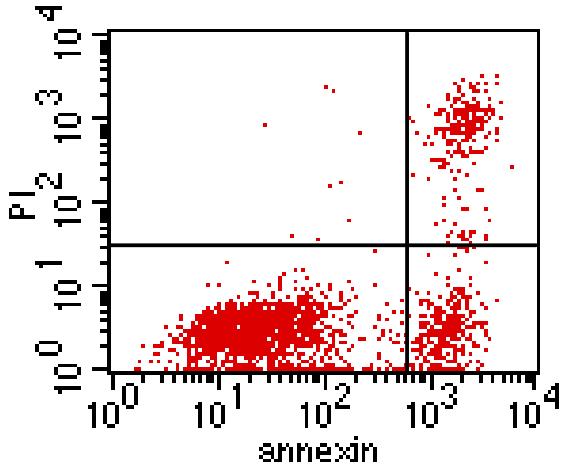
Cellular Outcome Differs Following Aur A *versus* Aur B Inhibition



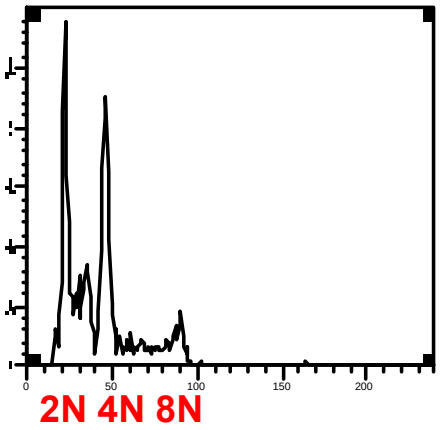
ENMD-981693 Induces Apoptosis Following G2/M Cell Cycle Arrest

U937 Cells
(24h Incubation)

29.%



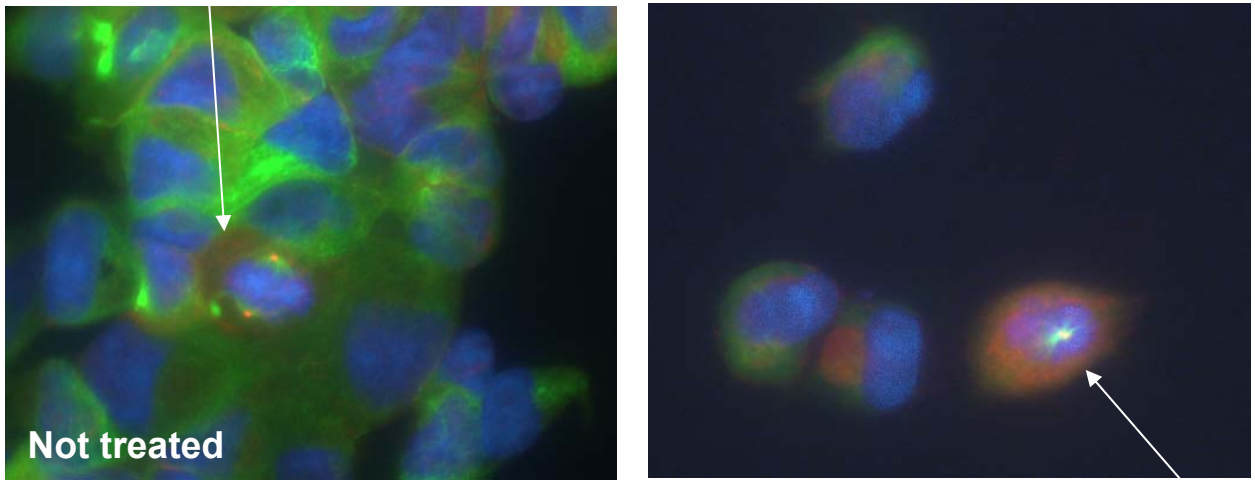
0.4 µM 981693



2N 4N 8N

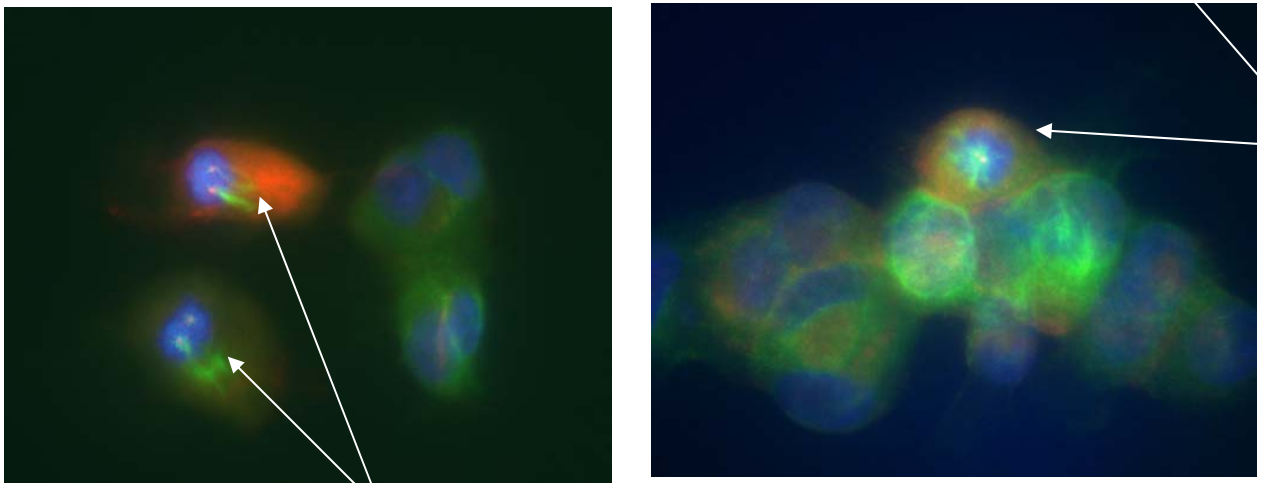
ENMD-981693 Induces a Monospindle Phenotype and Abnormal Mitosis

Normal mitosis



PANC-1 Cells treated with 2 μM ENMD-981693 for 6 hours

Aurora A - Red
DNA - Blue
Ac-tubulin - Green

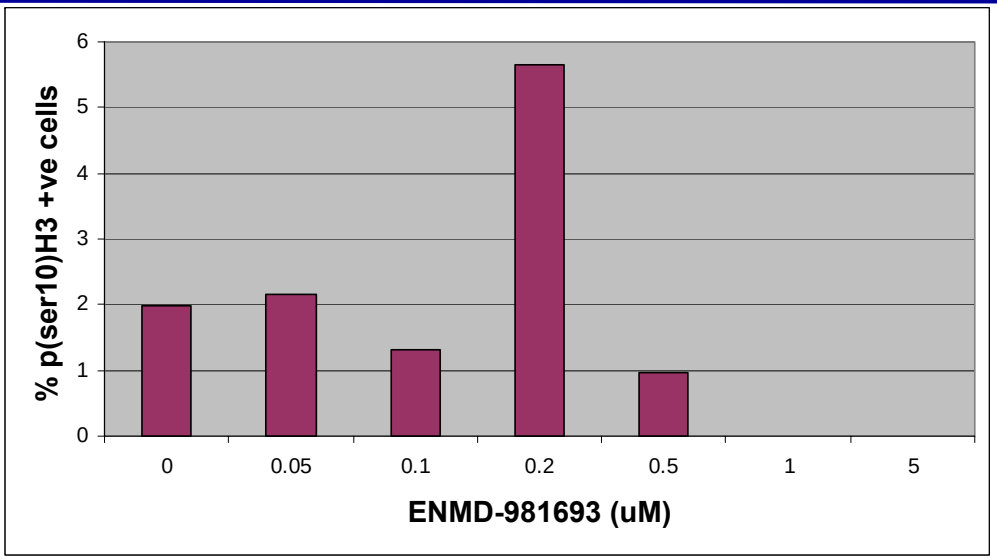


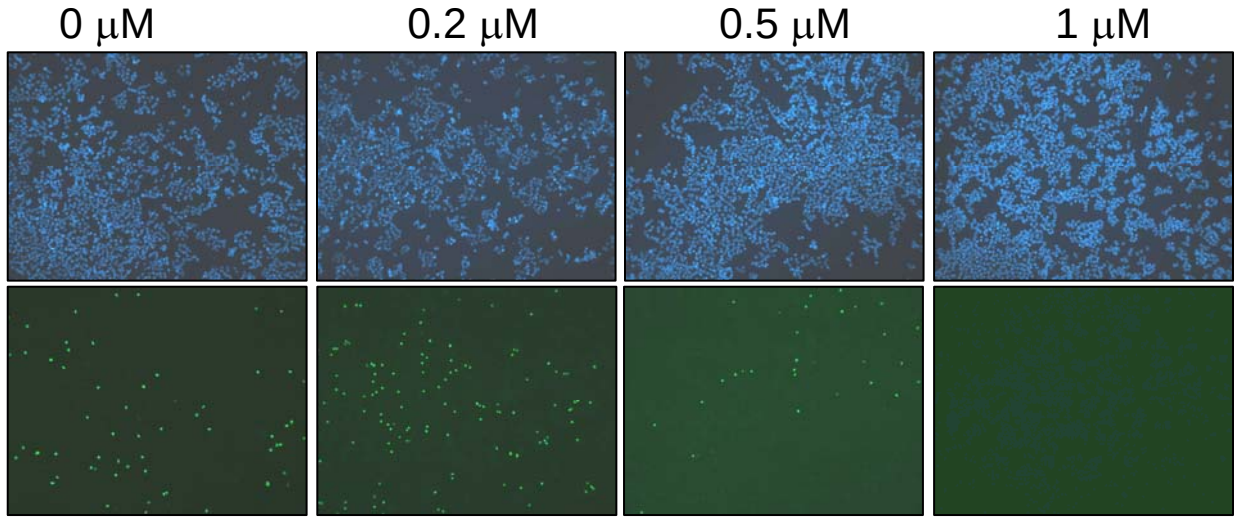
Monospindle cells

Abnormal mitosis

ENMD-981693 Increases Proportion of Phospho-Histone H3 Positive Cells at ~IC_{50}

- HCT116 cells
- 6 hour incubation





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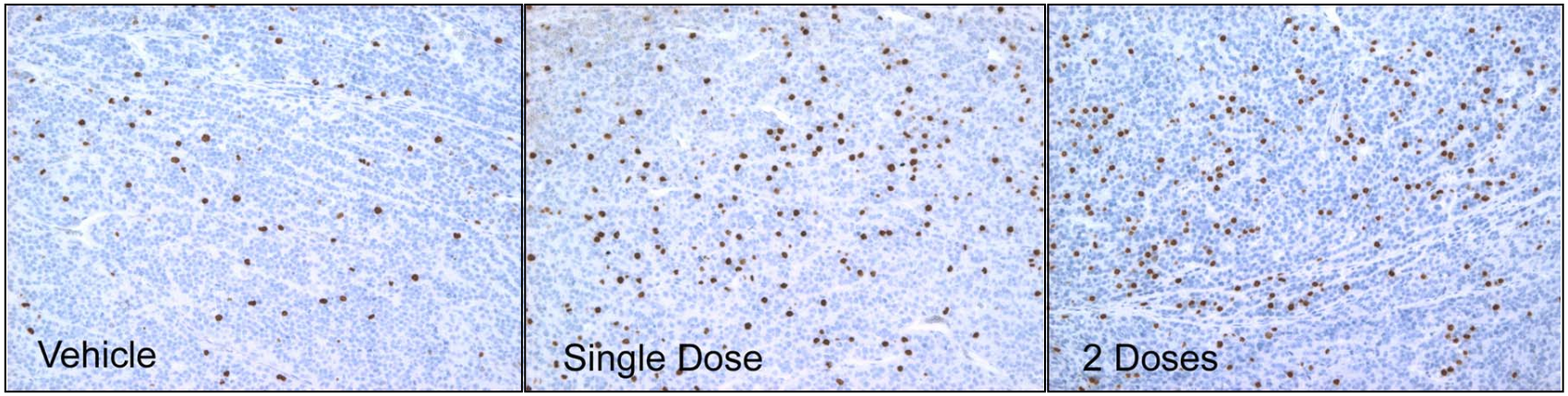
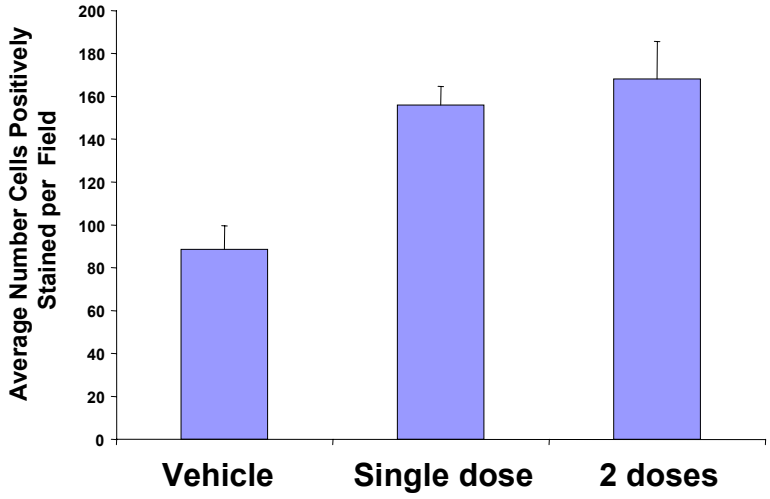
ENMD-981693 Increases Proportion of Phospho-Histone H3 Positive Cells *In Vivo*



- **1A9 ovarian cell xenograft**
- **5 hours after single 150 mg/kg treatment or two treatments 24 hours apart**



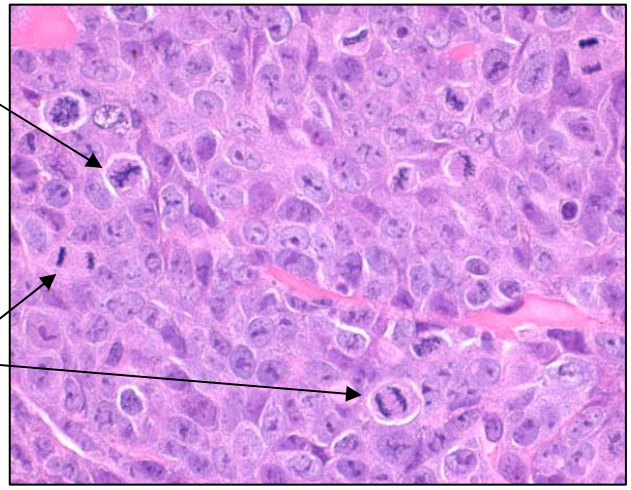
ENMD-981693 Inhibits Aurora A *In Vivo*

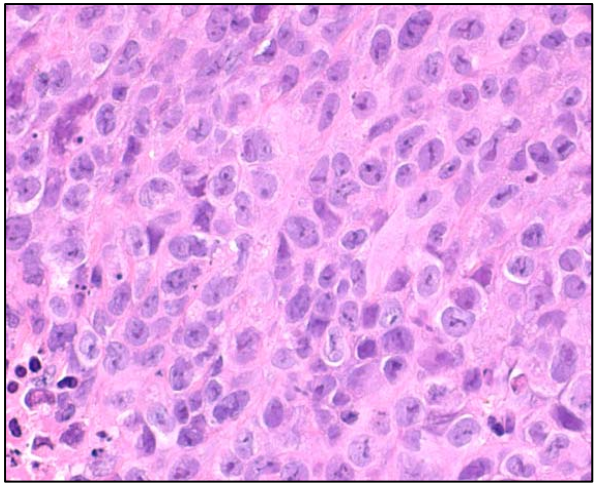
H and E Stained HCT-116 sections

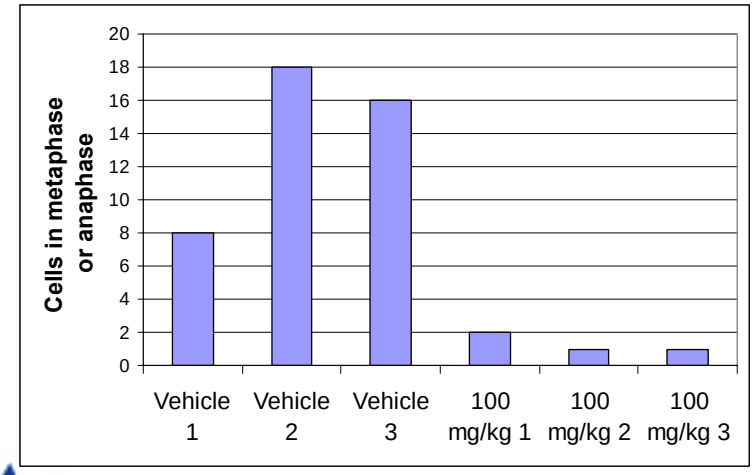
Vehicle **ENMD-981693**

Metaphase

Anaphase





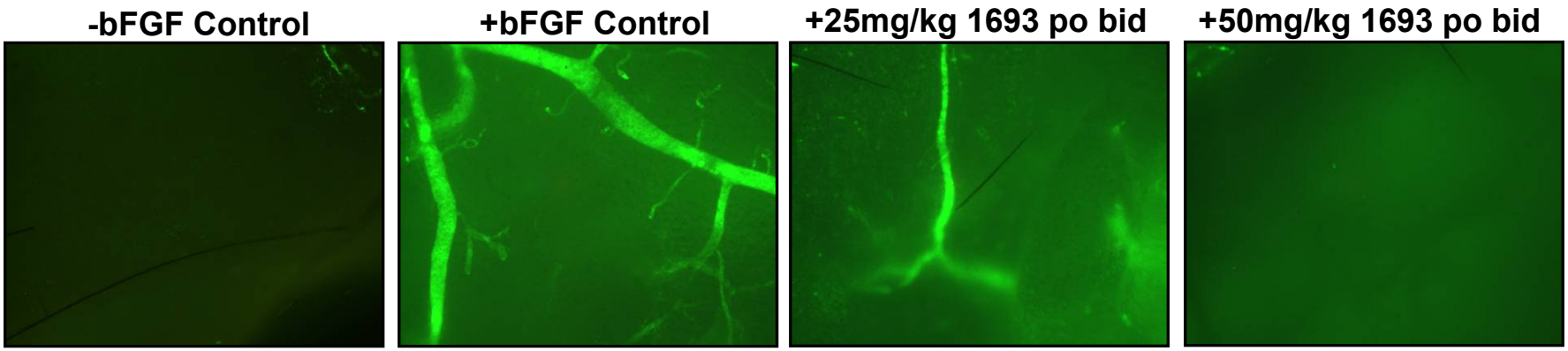


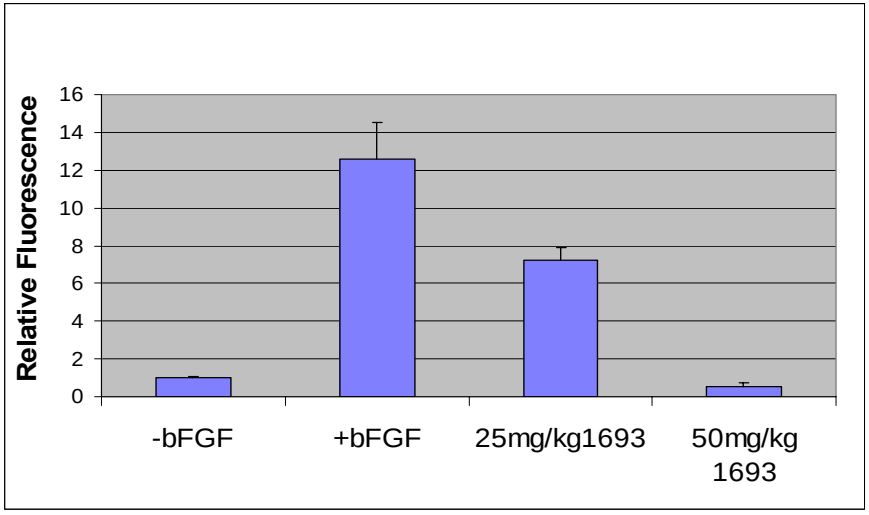
- ENMD-981693 effect *in vivo* consistent with inhibition of Aurora A

ENMD-981693 Inhibitory Activity in Cell-Free and Cellular Assays *In Vitro*

	IC_{50} nM Recombinant Protein	Cellular IC_{50}
Flt3	3.0	20 nM (IP using THP-1 cells)
KDR/VEGFR2	36	<80 nM (Western using HUVECs)
FGFR1	93	600 nM (FRS2 Western using HUVECs)
cKit	120	<40 nM (IP using MO7e cells)
FAK/PTK2	55	1-5 uM (IP using MDA-MB-231 cells)
PDGFRα	56	1-5 uM (ICC using Panc1 and MDA-MB-231 cells)
Abl (T315I)	81	~ 25 uM (CrkL Western using patient samples)
Abl	295	> 25 uM (CrkL Western using K562 cells)

ENMD-981693 Inhibits Angiogenesis in Matrigel *In Vivo*





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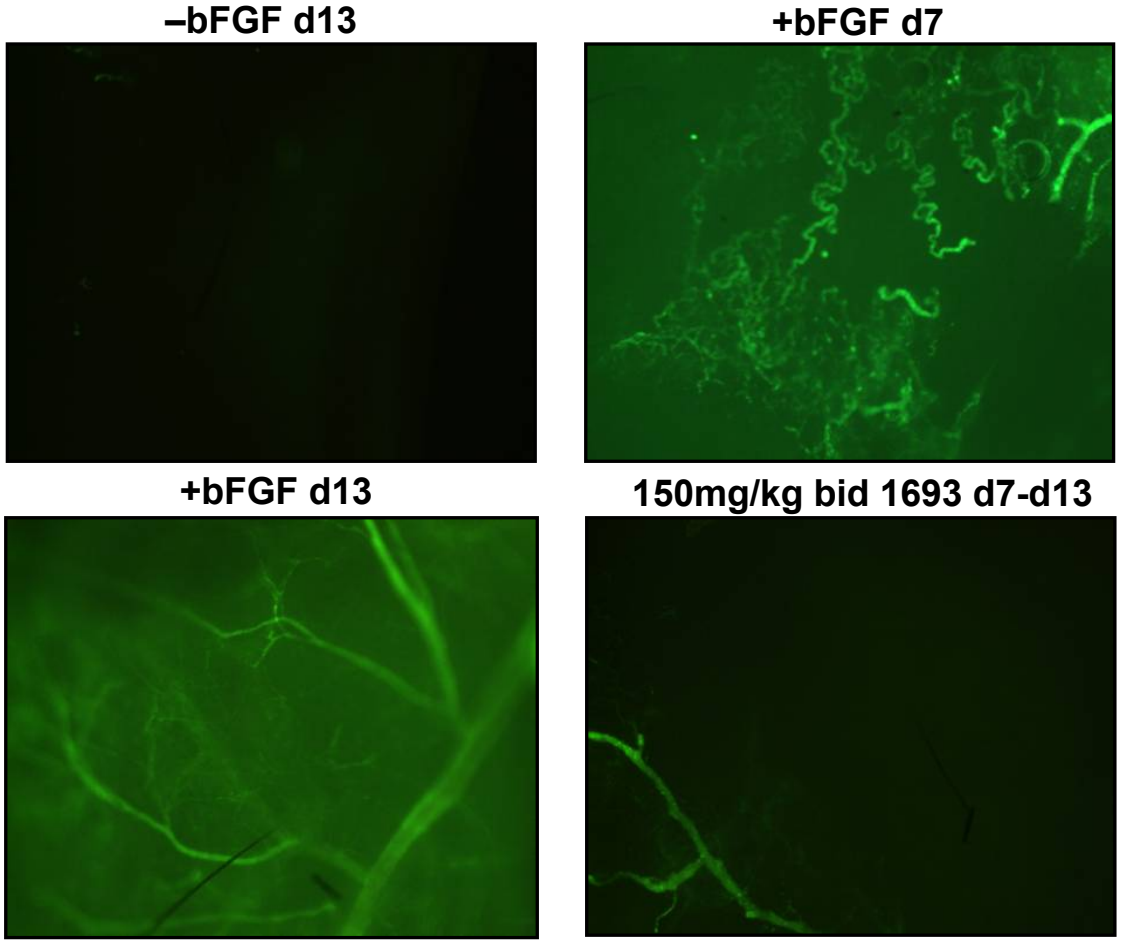
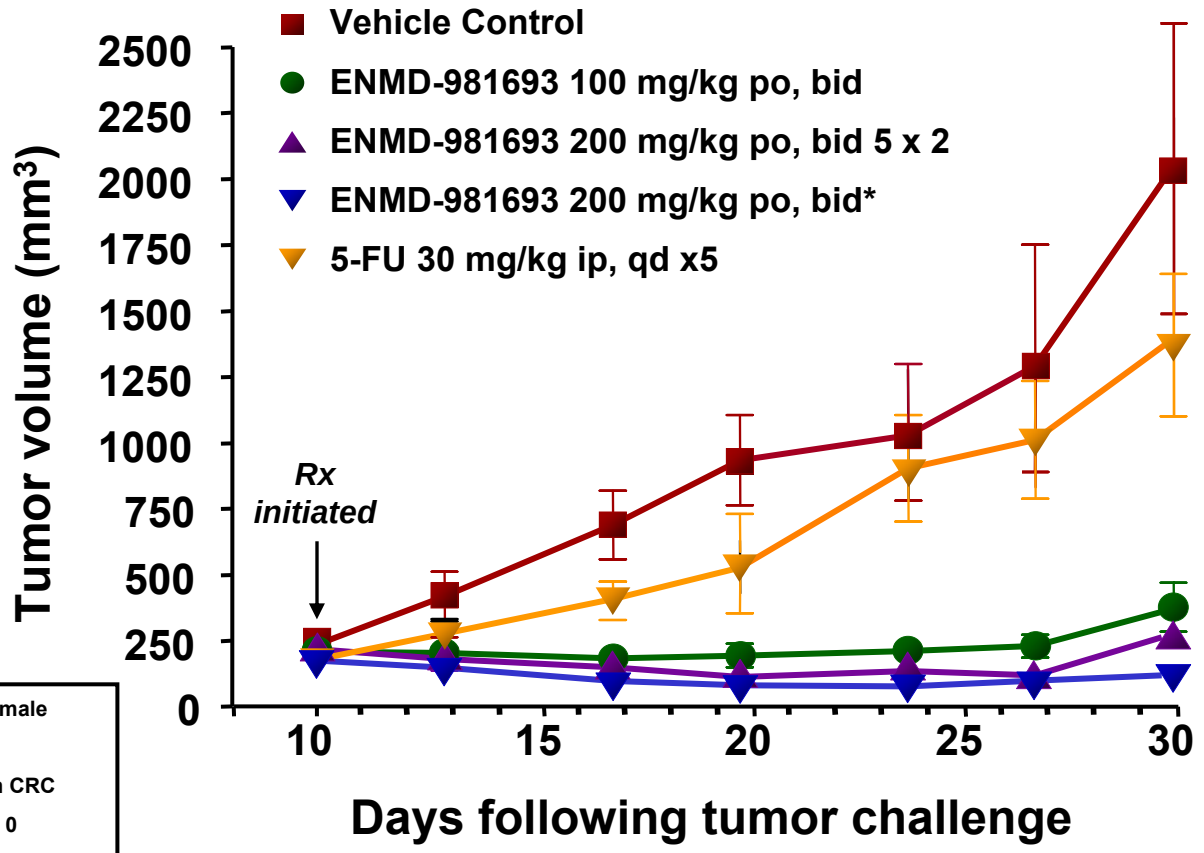
ENMD-981693 Regresses Newly Formed Vessels in Matrigel *In Vivo*



Antitumor Activity of ENMD-981693 on HCT-116 Tumor Growth



% change in tumor size from Rx initiation
764
686
555
81
45
-54

Mice: NCR nude, female
N : 10/group
Tumor type: human CRC
Tumor site: sc, day 0
Rx initiated: day 10

Vehicle: 15% (0.5% LV-CMC in water)/ 85% (0.1% Tween 80 in water)

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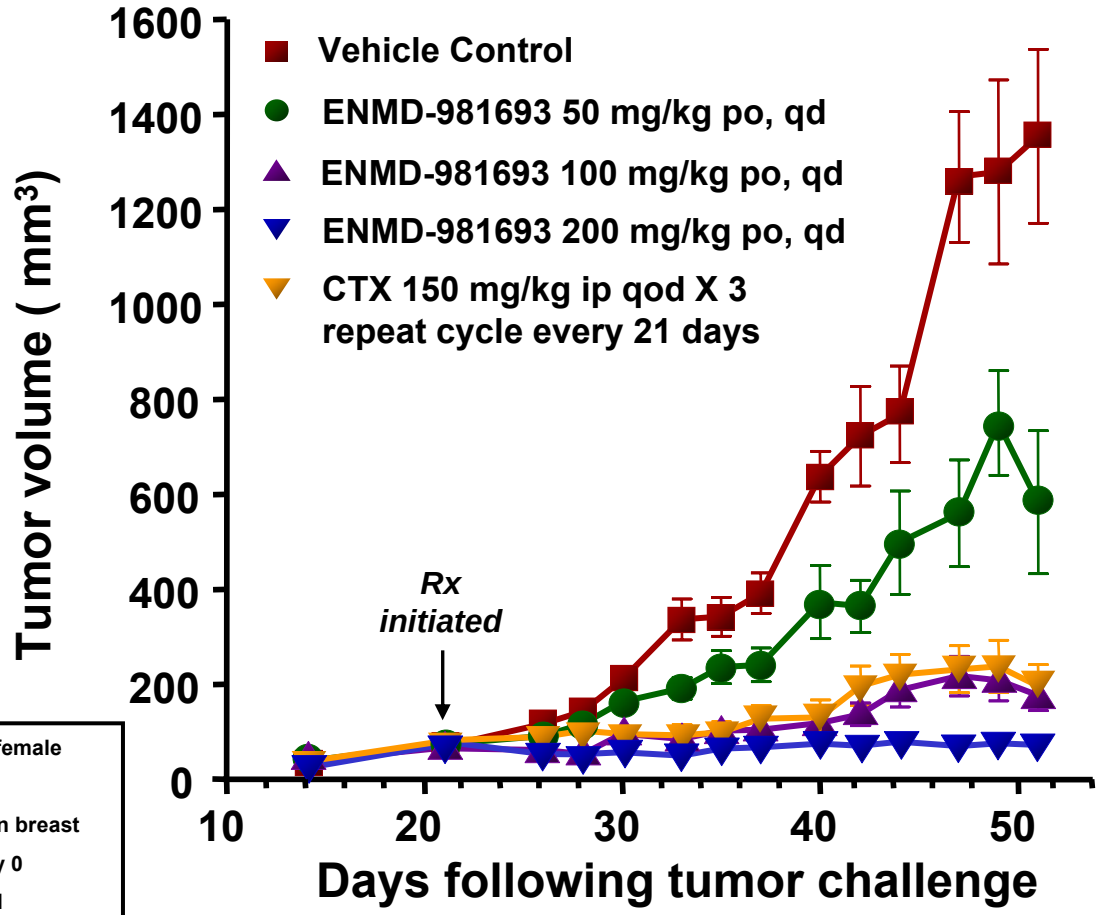
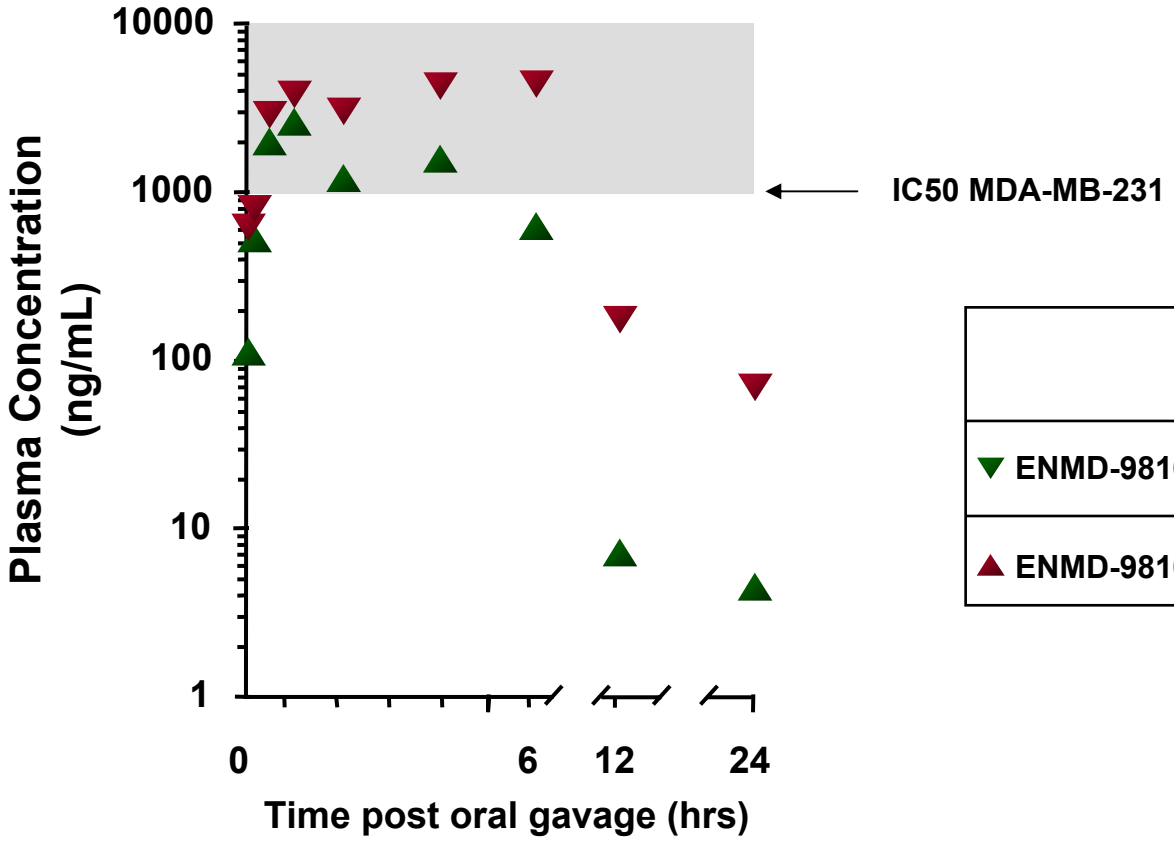
Antitumor Activity of ENMD-981693 on MDA-MB-231 Tumor Growth



% change in tumor size from Rx initiation
1851
684
112
78
-39

Mice: CB17 SCID, female
N : 9/group
Tumor type: human breast
Tumor site: sc, day 0
Rx initiated: day 21

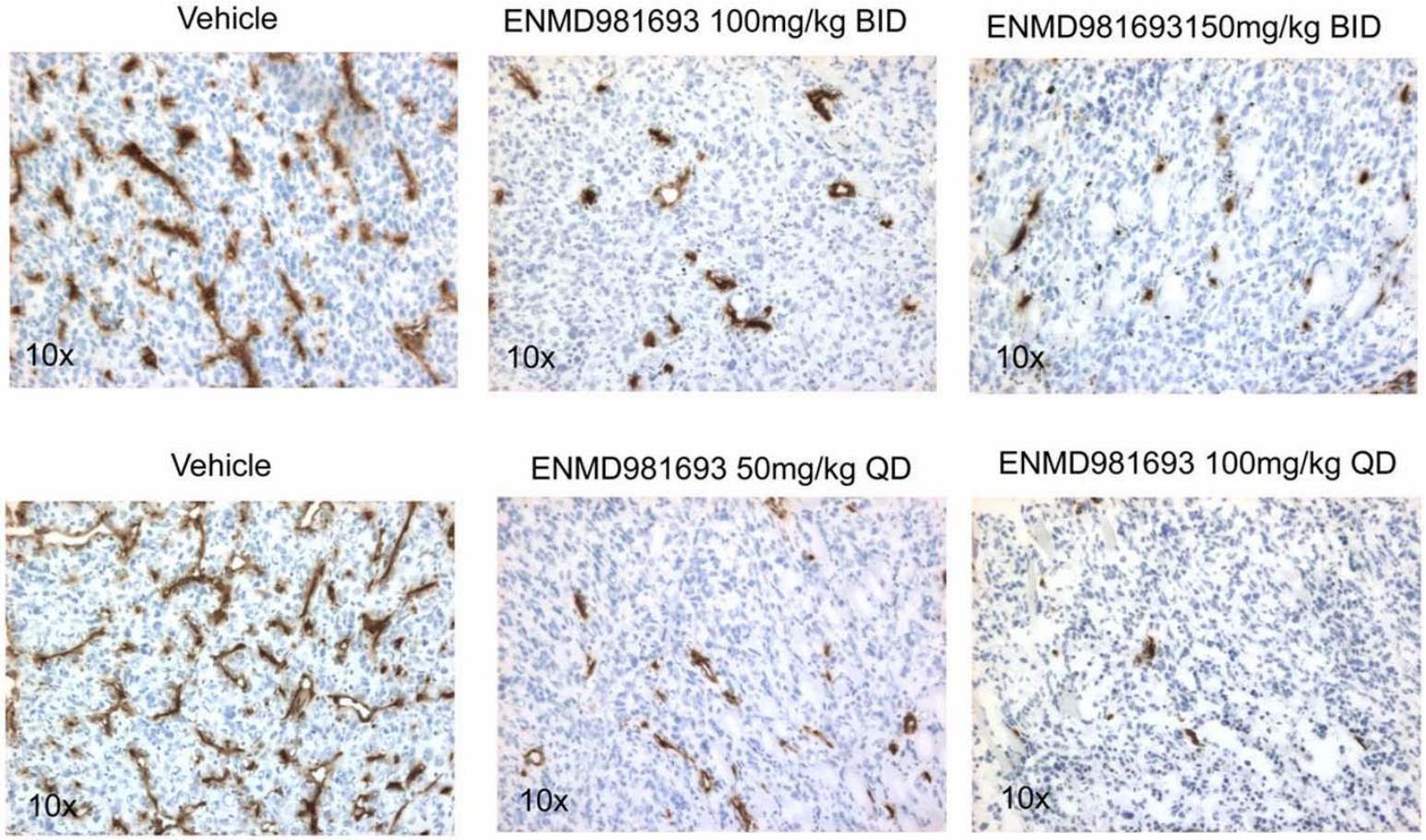
Plasma Concentration of ENMD-981693 Following Oral Gavage to CB17 SCID Mice



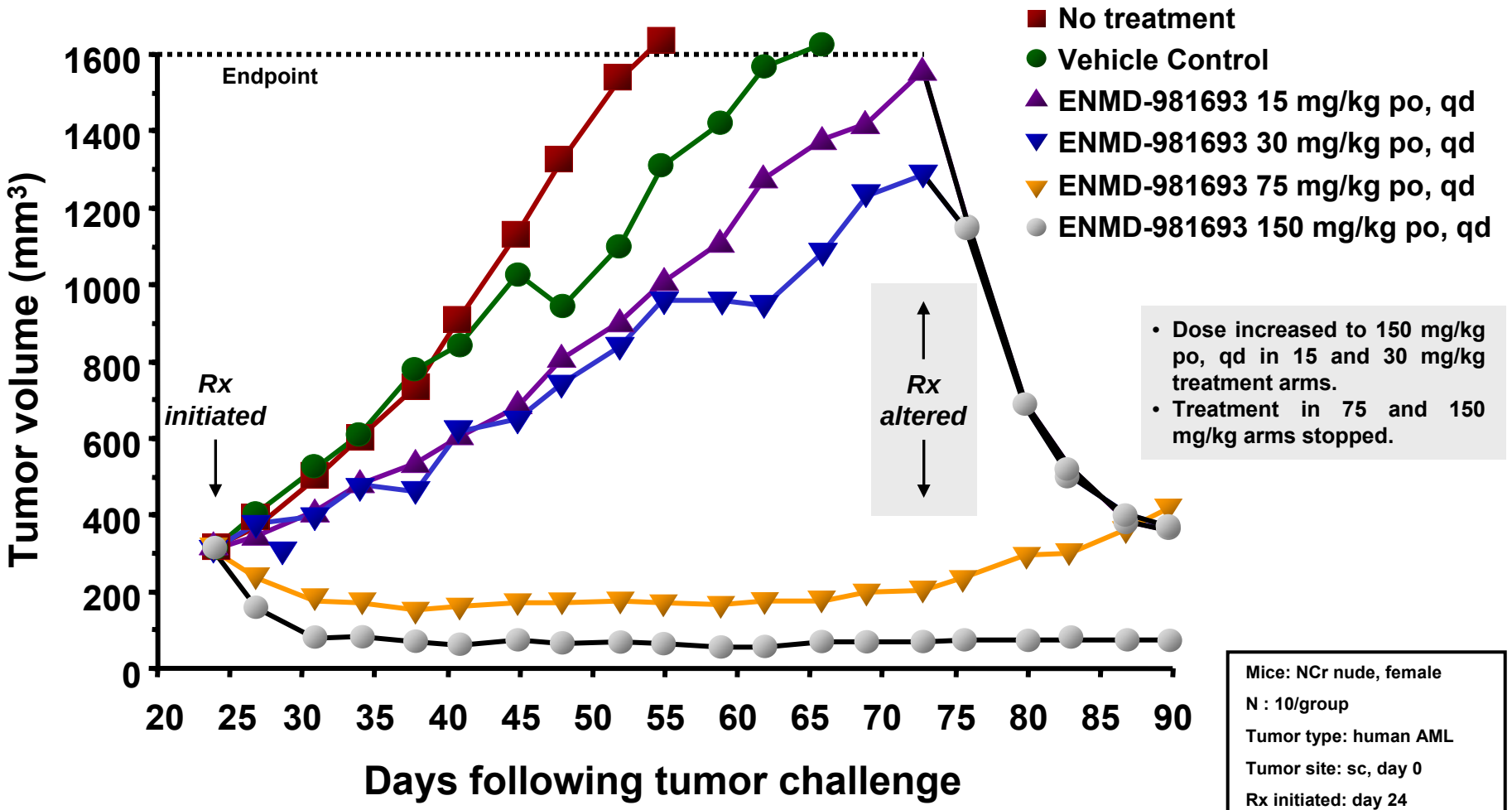
	Dose (mg/kg)	Tumor response
▼ ENMD-981693	100	85% Growth Inhibition
▲ ENMD-981693	200	Regression

ENMD-981693 Reduces Microvessel Density in Established Tumor Xenografts

MDA-MB-231 Sections Stained for CD31



Antitumor Activity of ENMD-981693 on MV4;11 Tumor Growth in Nude Mice



- Dose increased to 150 mg/kg po, qd in 15 and 30 mg/kg treatment arms.
- Treatment in 75 and 150 mg/kg arms stopped.

Mice: NCr nude, female
N : 10/group
Tumor type: human AML
Tumor site: sc, day 0
Rx initiated: day 24

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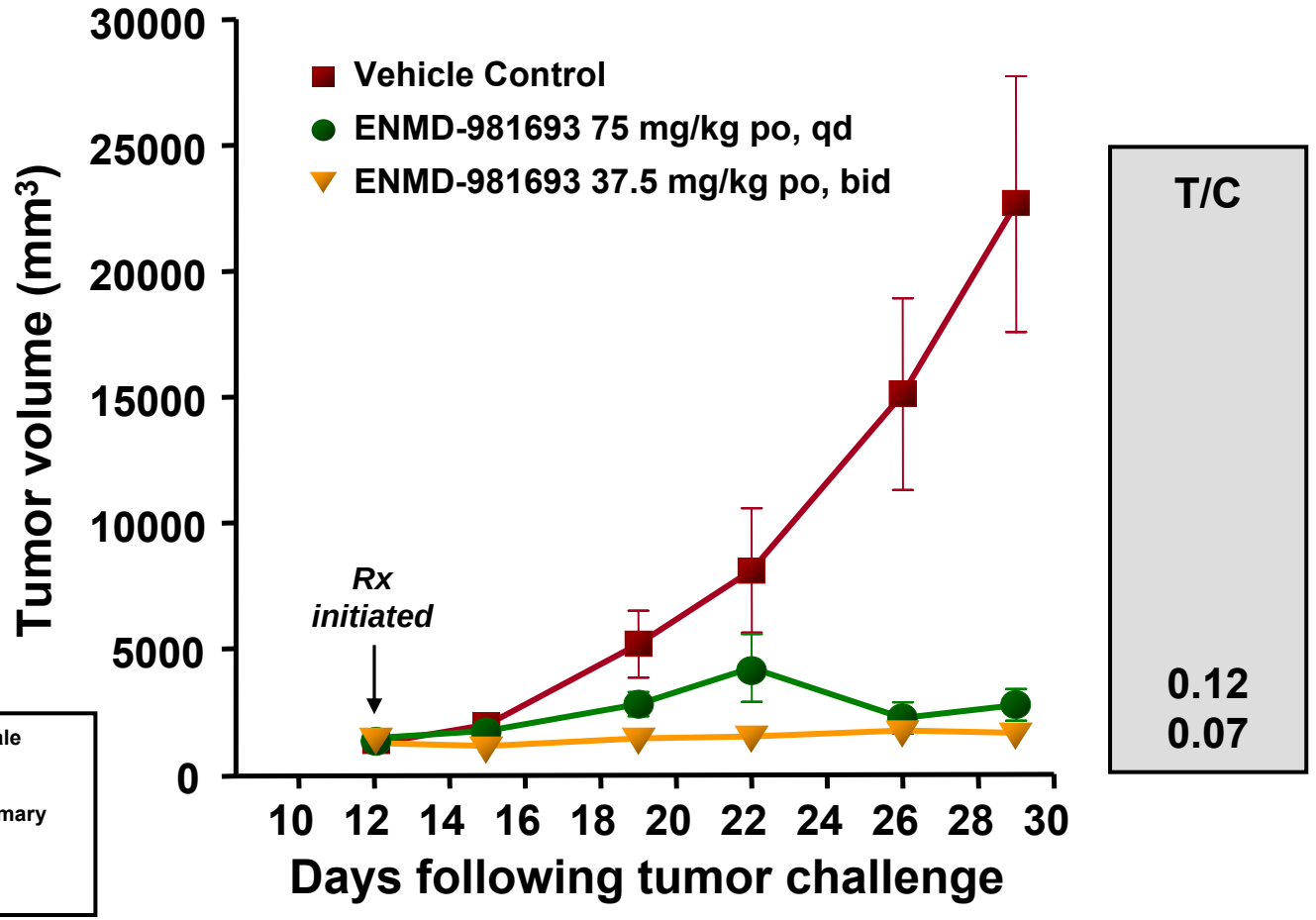
Antitumor Activity of ENMD-981693 on MADB-106 Tumor Growth in Fischer Rats



Rats: Fischer 344, male
N : 8/group
Tumor type: rat mammary
Tumor site: sc, day 0
Rx initiated: day 12

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ENMD-981693: Chemical Properties

- **Proprietary, heterocyclic molecule**
- **Stable, white solid**
- **Not a racemic mixture**
- **Scalable 5-step synthesis**
  - **7 kg non-GMP synthesis of ENMD-981693 completed**
  - **5 kg GMP in 4Q 07**
  - **Pre-formulation and salt screening completed**

ENMD-981693 Development Plans

- Scale-up manufacturing complete for tox/Phase I
- Formulation development
- IND-directed toxicology completed
- Continue to evaluate molecular target profile
- Strengthen IP through analoging and MOA studies
- IND filing 4Q07

ENMD-981693 Summary

- Oral, Aurora kinase-angiogenesis inhibitor
- Unique combination of target activities
  - Antiangiogenic, cell cycle, antiproliferative
- Proprietary heterocyclic compound with tractable chemistry
- Well-tolerated
- Excellent efficacy in multiple xenograft models

Acknowledgements

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