

ENMD-2076, an Oral Aurora A and Angiogenesis Kinase Inhibitor

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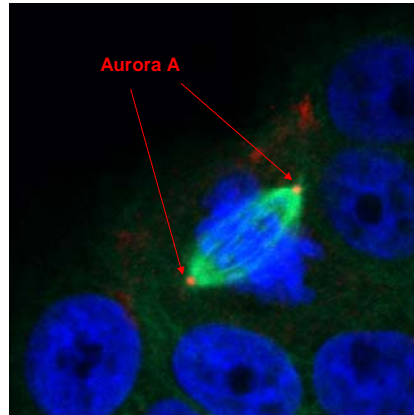
Disclosure Information: AACR New Drugs on the Horizon 2 Mark R. Bray, Ph.D.

I have the following financial relationships to disclose:
Employee of: EntreMed, Inc.

I will not discuss off label use and/or investigational use in
my presentation.

Aurora Kinases Contribute to Oncogenesis and Tumor Progression

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



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Rationale for Aurora Kinase Inhibitor Program

- **Strong interest and internal expertise in Aurora/survivin control of mitotic pathways**
 - *Tak W. Mak, J. Exp. Med. 2004 199(3): 399-410*
- **Medicinal chemistry expertise in kinase inhibitors, heterocycles**
- **Evolving target, no marketed products**

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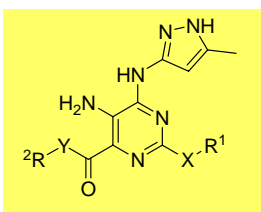
MedChem Approaches to Aurora Kinase Inhibitor Design

- Incorporate existing SAR and structure-based approaches
- Retain key inhibitor-enzyme interactions (multiple H-bonds and π - π interactions)
- Create structurally distinct, synthetically tractable, low MW heterocycles
- **GOALS:** Oral, acceptable safety profile, broad single agent activity

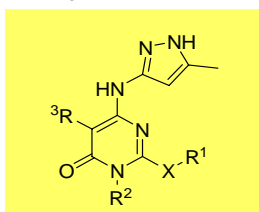
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Three Main Lead Series Established in Aurora Kinase Inhibitor Program

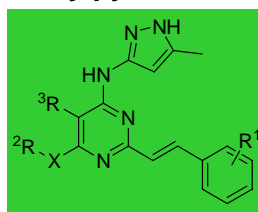
Aminopyrimidines



Pyrimidones



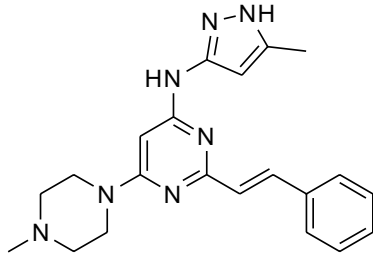
Vinylpyrimidines



- Multiple lead series established
 - > 300 analogs prepared in initial screening
 - 40 Compounds with AurA IC₅₀ < 100 nM & HCT116 GI₅₀ < 1 μ M
 - Vinylpyrimidine R¹, R³ = H clearly best molecule in efficacy screen
- Strong IP position: Four U.S. and International patent applications

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ENMD-2076 is an Aurora and Angiogenic Kinase Inhibitor



L-(+)-Tartaric acid

ENMD-2076

- Aurora A IC₅₀ 14 nM
Aurora B IC₅₀ 290 nM
- Kinase inhibition profile includes multiple angiogenic kinases
 - VEGFR, FGFR, PDGFR
- Activity through both iv and oral routes
 - Good oral bioavailability
- Tumor regression in multiple models

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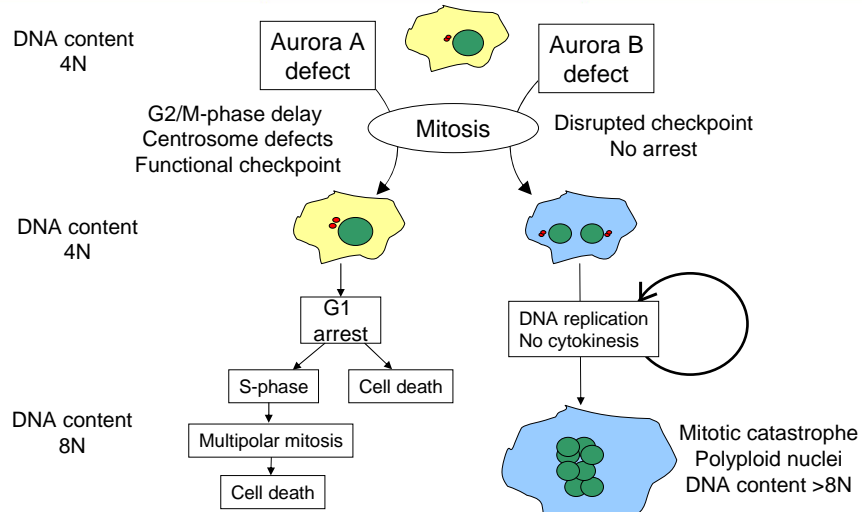
ENMD-2076 Inhibitory Activity in Cell-Free and Cellular Assays *in vitro*

	IC ₅₀ nM Recombinant Protein	Cellular IC ₅₀
Flt3	3	20 nM
AurA	14	130nM
Src	20	100 nM
KDR/VEGFR2	36	80 nM
FGFR1	93	600 nM
cKit	120	40 nM
FAK/PTK2	55	1-5 μM
PDGFRα	56	1-5 μM
Abl (T315I)	81	25 μM
Abl	295	> 25 μM

All *in vitro* data was generated using the free base of ENMD-2076

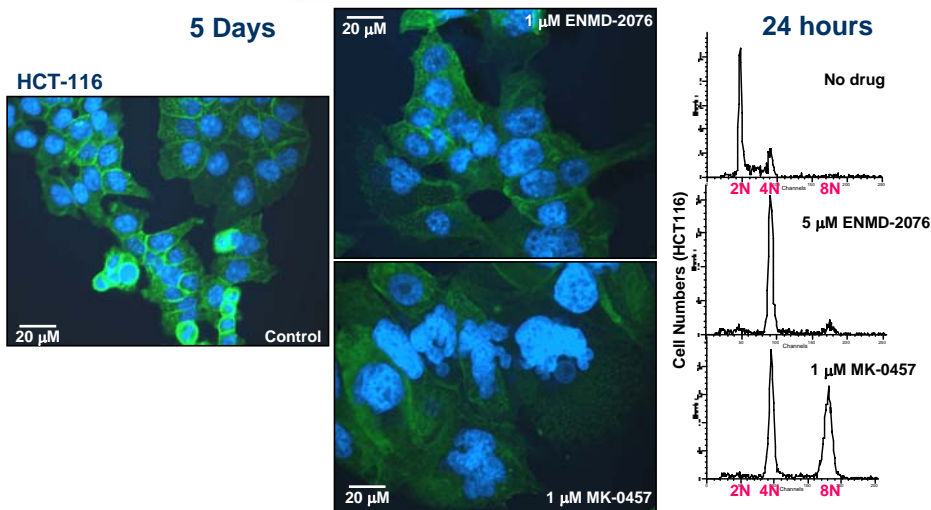
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Cellular Consequences of Aurora Kinase Inhibition are Isoform Specific



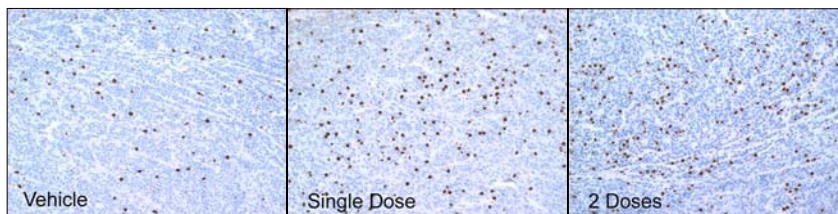
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ENMD-2076 Induces Cell Cycle Arrest at G2/M (4N) Consistent with Inhibition of Aurora A

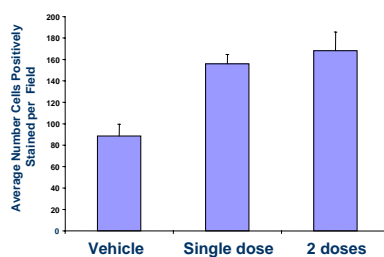


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ENMD-2076 Increases Proportion of Phospho-Histone H3 Positive Cells *in vivo*

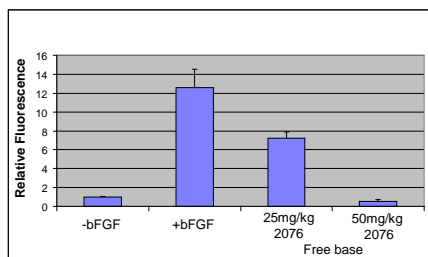
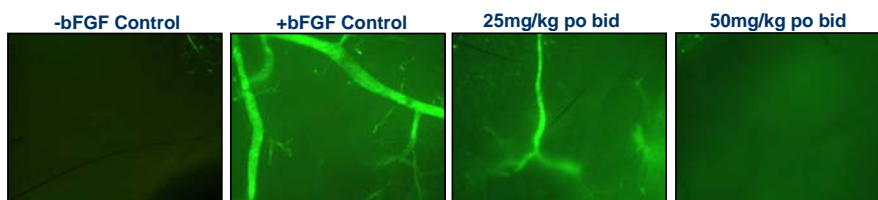


- 1A9 ovarian cell line xenograft
- 5 hours after single 150 mg/kg (free base) treatment or two treatments 24 hours apart



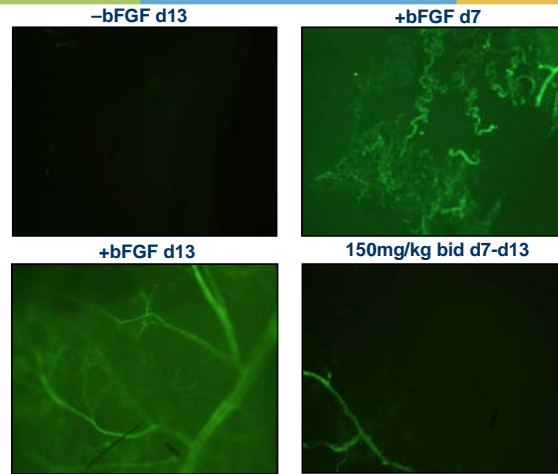
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ENMD-2076 Inhibits Angiogenesis in Matrigel *in vivo*



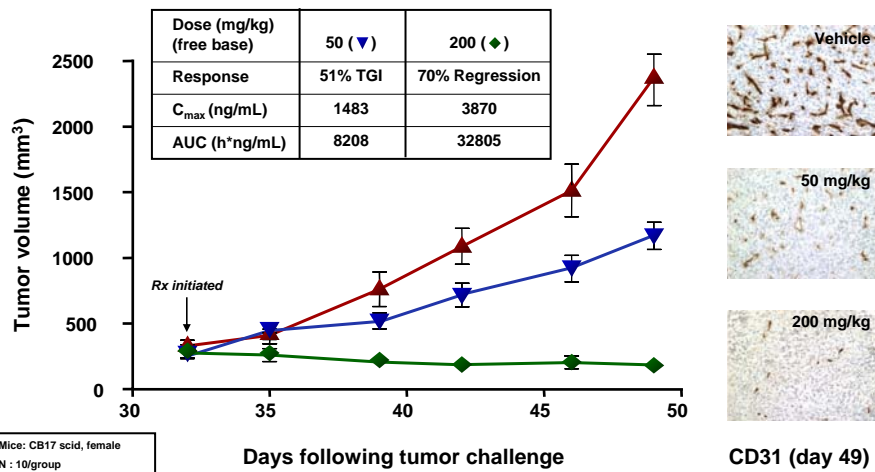
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ENMD-2076 Regresses Newly Formed Vessels Grown in Matrigel *in vivo*



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Impact of ENMD-2076 Treatment on MDA-MB-231 Tumor Progression



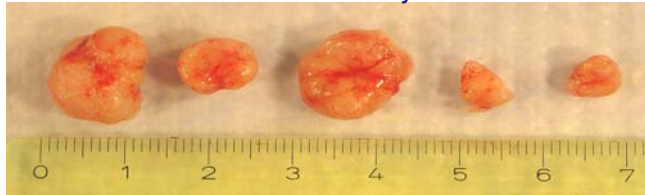
Mice: CB17 scid, female
 N : 10/group
 Tumor type: human BrCA
 Tumor site: mfp, day 0
 Rx initiated: day 32, po, qd

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ENMD-2076 Treated Tumors Show Clear Evidence of Antiangiogenic Effects

HT29 Colon Carcinoma Xenograft

Vehicle Control Day 28



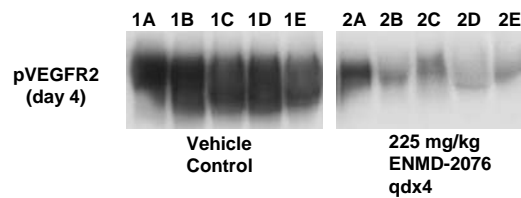
ENMD-2076 po, qd (200 mg/kg) Day 28



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Treatment with ENMD-2076 Reduces VEGFR2 Activation in A375 Melanoma Xenograft

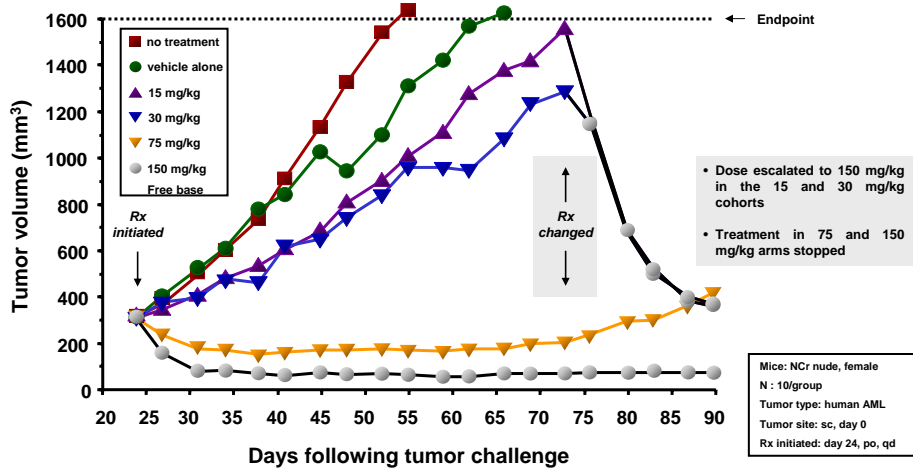
A375 Tumor Extracts (Individual Mice)



- PK-PD correlation ongoing for kinase targets of ENMD-2076
 - Activity demonstrated towards Aur A, FGFR, Src, PDGFR *in vivo* at efficacious doses
 - PET, DC-MRI studies with tumor-bearing animals in progress at UCHSC

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Antitumor Activity of ENMD-2076 on MV4;11 Tumor Growth in Nude Mice



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ENMD-2076 ADMET Studies

- Approximately 90% protein bound
- No significant inhibition at 1 μM against a panel of five P450 enzymes
 - CYP1A2, CYP2A6, CYP2C19, CYP2C9*1, CYP3A4
- Mixed gender Phase I/glucuronide metabolism tested with microsomes from five species
 - Oxidation predominant pathway in all species
 - Low or absent glucuronide conjugation
- Mass distribution, metabolite ID studies in progress

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Supportive IND-Enabling Toxicology Program for ENMD-2076

- Negative for *in vitro* mutagenicity
- Safety Pharmacology
 - Telemetry study in non-anesthetized dogs demonstrated no effects on the cardiovascular or respiratory system
- Toxicology
 - 28-day continuous oral dosing (rats and dogs)
 - Dose-proportional increases in Cmax and AUC
 - Gastrointestinal effects observed in both rats and dogs
 - Reversible hard tissue effects in rats suggestive of kinase and antiangiogenic activities
 - Reversible LFTs and hematological effects in rats

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ENMD-2076 CMC Summary

- Properties
 - Highly water-soluble tartrate salt of low molecular weight active base (MW 375). Free-flowing yellow solid.
- Stability:
 - Good stability of both free base active and tartrate salt. Stable hydrate at room temperature / controlled humidity.
- Synthesis
 - Non-racemic active base produced from readily available starting materials in 6-step synthesis. Most steps 80-90% yield. >10kg scale.
- Formulation
 - First-in-man studies using 'Powder In Capsule' approach. Formulation development underway for capsule or tablet drug product.

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ENMD-2076 Clinical Development Status



- **Phase 1 clinical study initiated March 2008**
 - University of Colorado HSC; additional sites to join shortly
- **Phase 1 design and endpoints**
 - 3 + 3 dose escalation with safety, PK and clinical benefit endpoints
- **PD evaluation of soluble KDR**
- **Cardiovascular monitoring**
- **Goal is to determine the MTD in solid tumor patients**
- **Additional studies in hematological cancers planned**

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ENMD-2076 Summary

- **Orally active, multi-kinase inhibitor**
- **Target activities include:**
 - Antiangiogenesis, cell cycle inhibition
- **Excellent efficacy in multiple xenograft models**
- **Well-tolerated**
- **Proprietary heterocyclic compound with tractable chemistry**
- **Phase I trial in progress**

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Acknowledgements

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