

**Protocol #1107-1117**  
**A Phase I/II Safety, Pharmacokinetic,  
and Pharmacodynamic Study of APS001F  
with Flucytosine and Maltose  
for the Treatment of  
Advanced and/or Metastatic Solid Tumors**

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Presenter: Barry Anderson, M.D., Ph.D. Theradex, Inc.

Sponsor: Anaeropharma Science, Inc.

September 14, 2011

# Outline

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- **Strategy**
- **Preclinical Studies**
  - **Proof of Principle (POP) and Efficacy Studies**
  - **Review of Toxicity Studies**
- **Proposed Clinical Study**

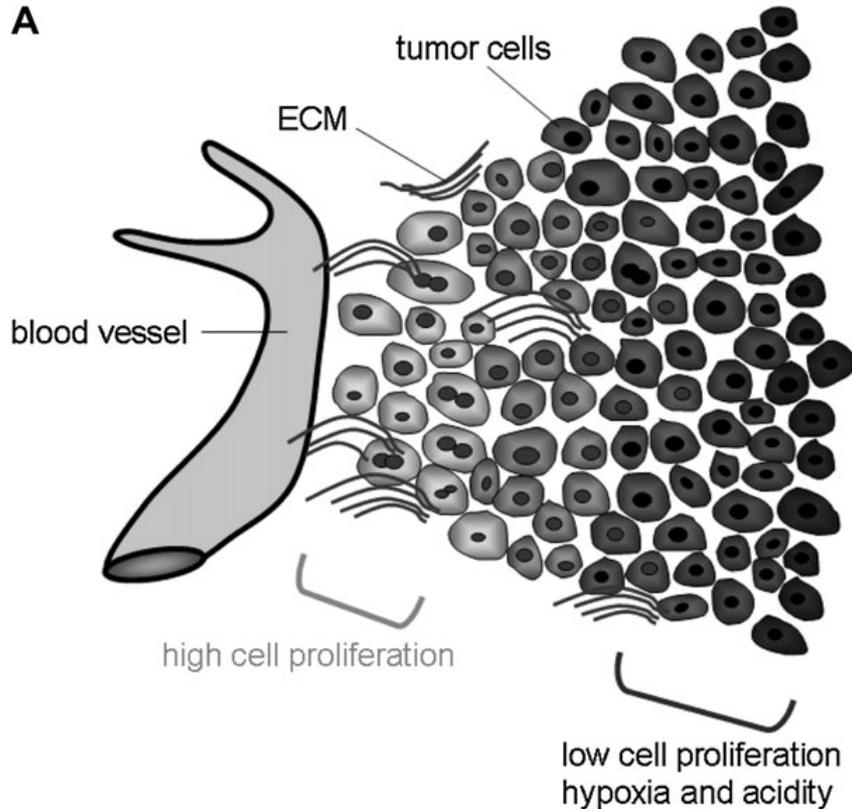
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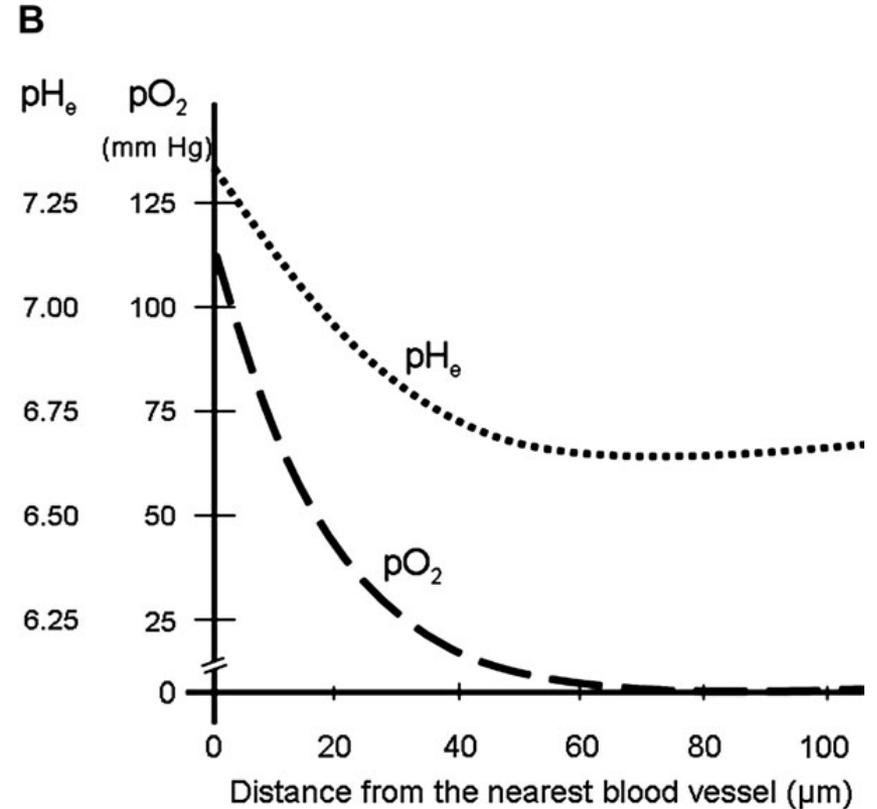
The tumor is a hypoxic region.

The tumor microenvironment in relation to blood vessels.



**A ) Diagrammatic representation of tumor cells and the extracellular matrix (ECM) surrounding a capillary.**

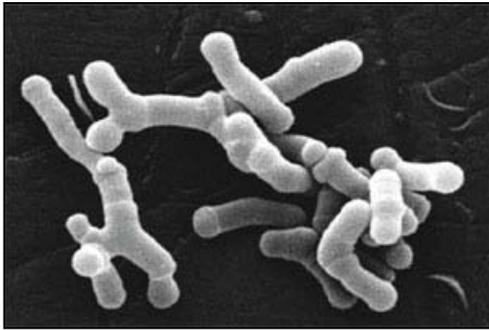
**(J Natl Cancer Inst 2007;99: 1441 – 54)**



**B ) Schematic representation of the gradient of oxygen concentration (  $pO_2$  : dashed line ) and of pH ( dotted line ) in relation to the nearest tumor blood vessel. The relationship of  $pO_2$  and pH with distance from the nearest blood vessel is similar to that reported by Vaupel (Semin Radiat Oncol 2004 ; 14 : 198 – 206 . ).**

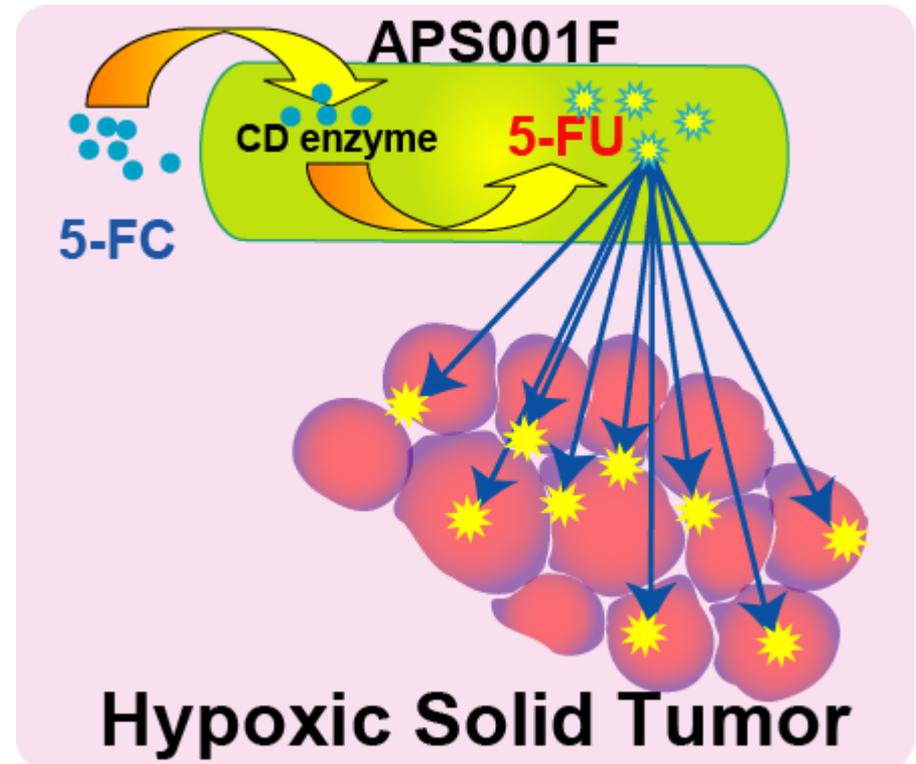
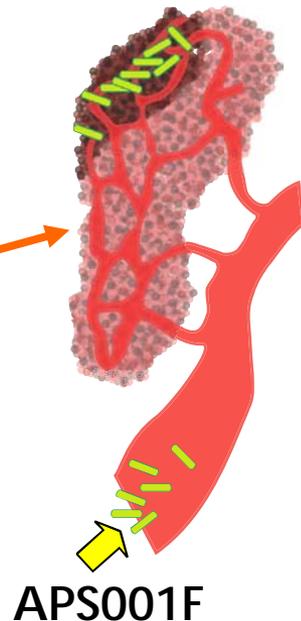
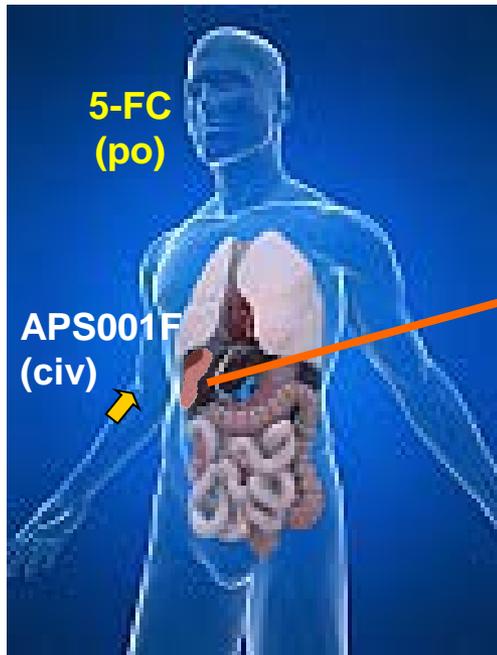
# Proposed Therapeutic Strategy for Solid Tumors

## -APS00F, CD Expressing *B. longum*, targets tumor hypoxia



### Features of APS001F, CD Expressing *B. longum*

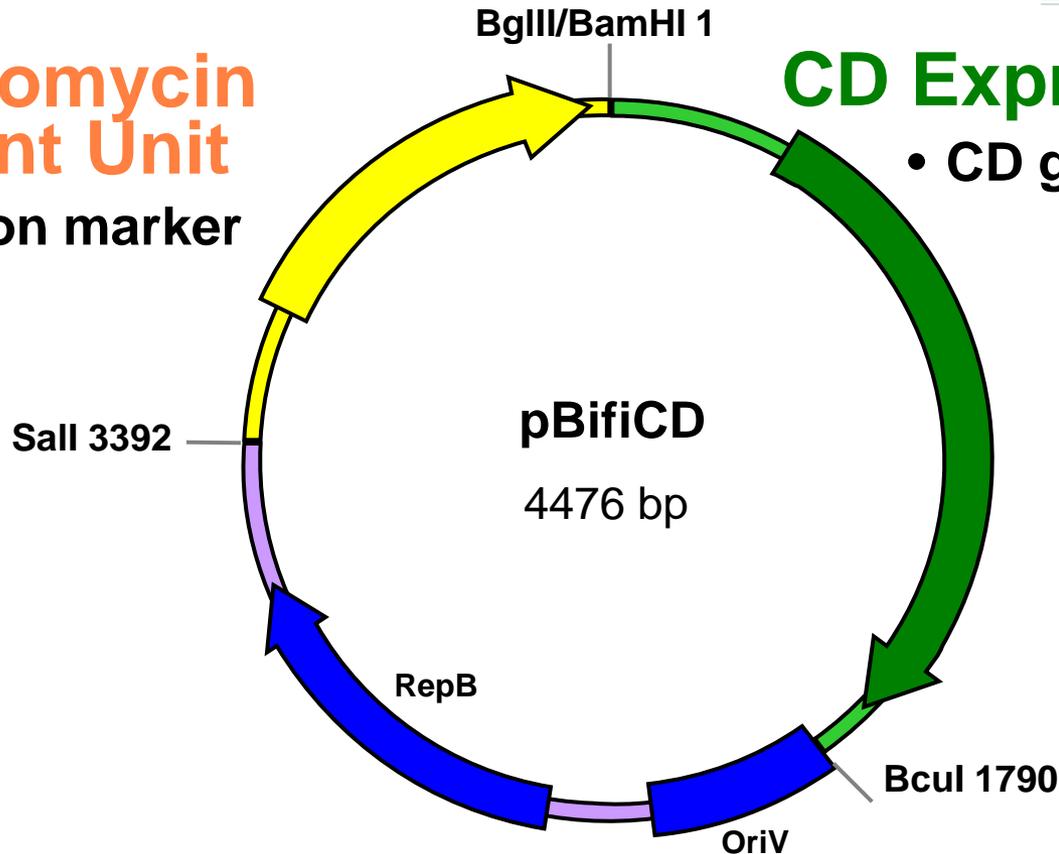
- Derived from flora in human intestine
- Obligate anaerobes
- Engineered with *E. coli* CD gene in plasmid
- Selective colonized in hypoxic solid tumors



# Plasmid Diagram

## Spectinomycin Resistant Unit

- Selection marker



## CD Expression Unit

- CD gene form *E. coli*

## Plasmid Replication Unit

- Originated from *B. longum*
- Works only in Bifidobacteria
- Extra sequences have been removed to prevent unexpected gene expression, which has been recommended by FDA

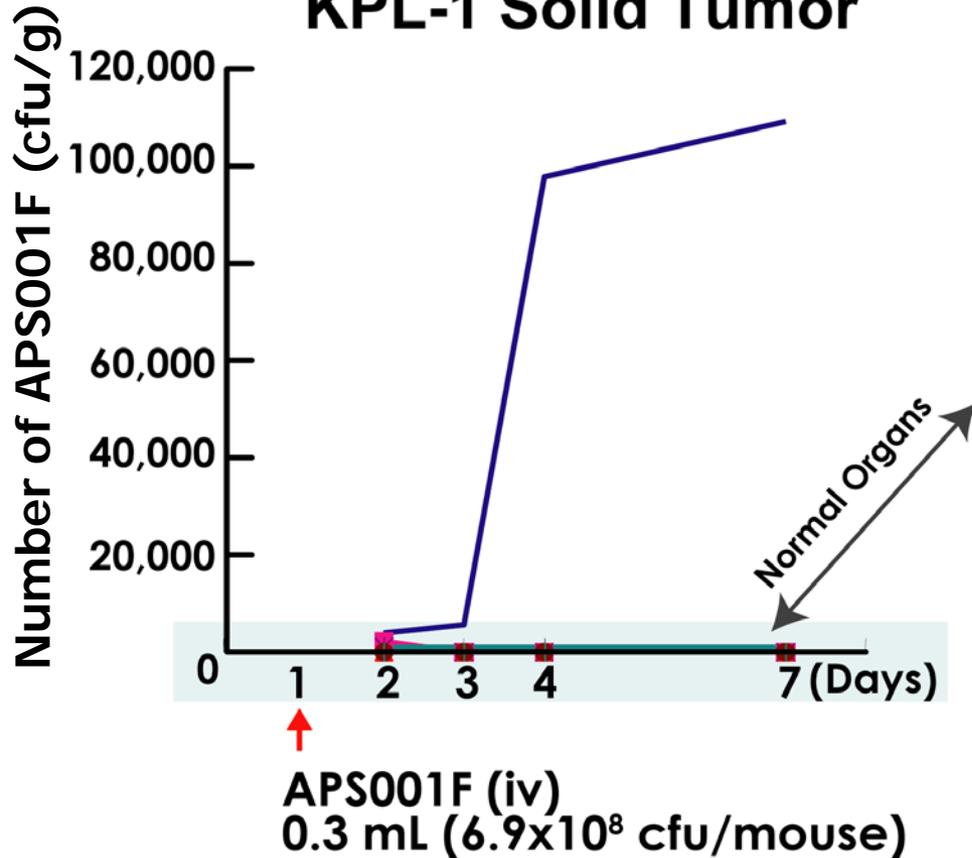
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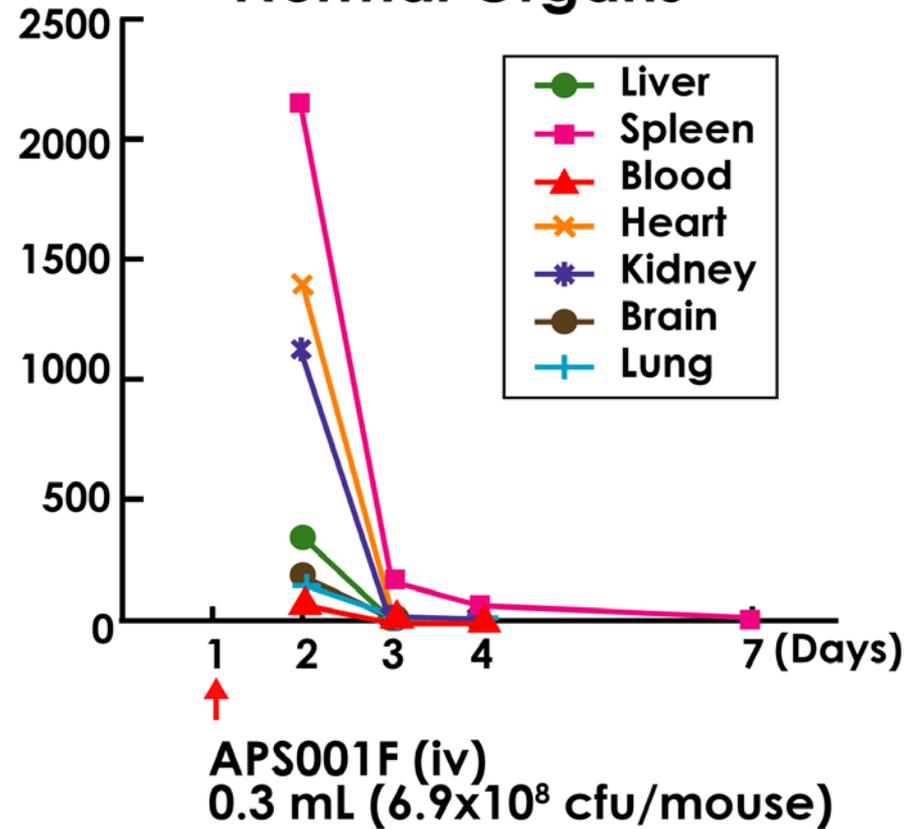
- Strategy
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  - **Proof of Principle (POP) and Efficacy Studies**
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# POP Study; Selective Localization of APS001F in KPL-1 Human Breast Cancer, Clearance from Blood + Normal Organs (Nude Mice)

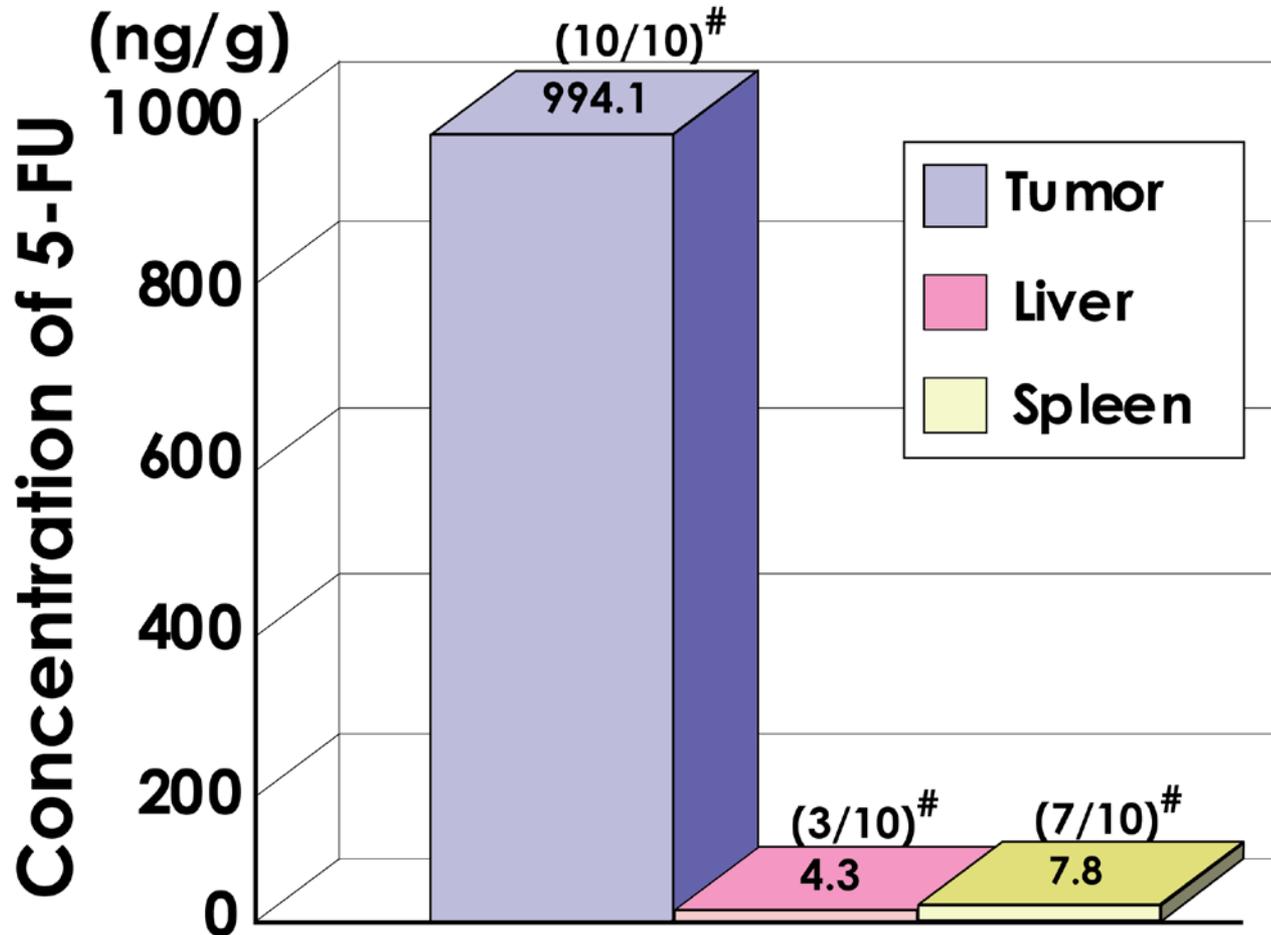
## KPL-1 Solid Tumor



## Normal Organs



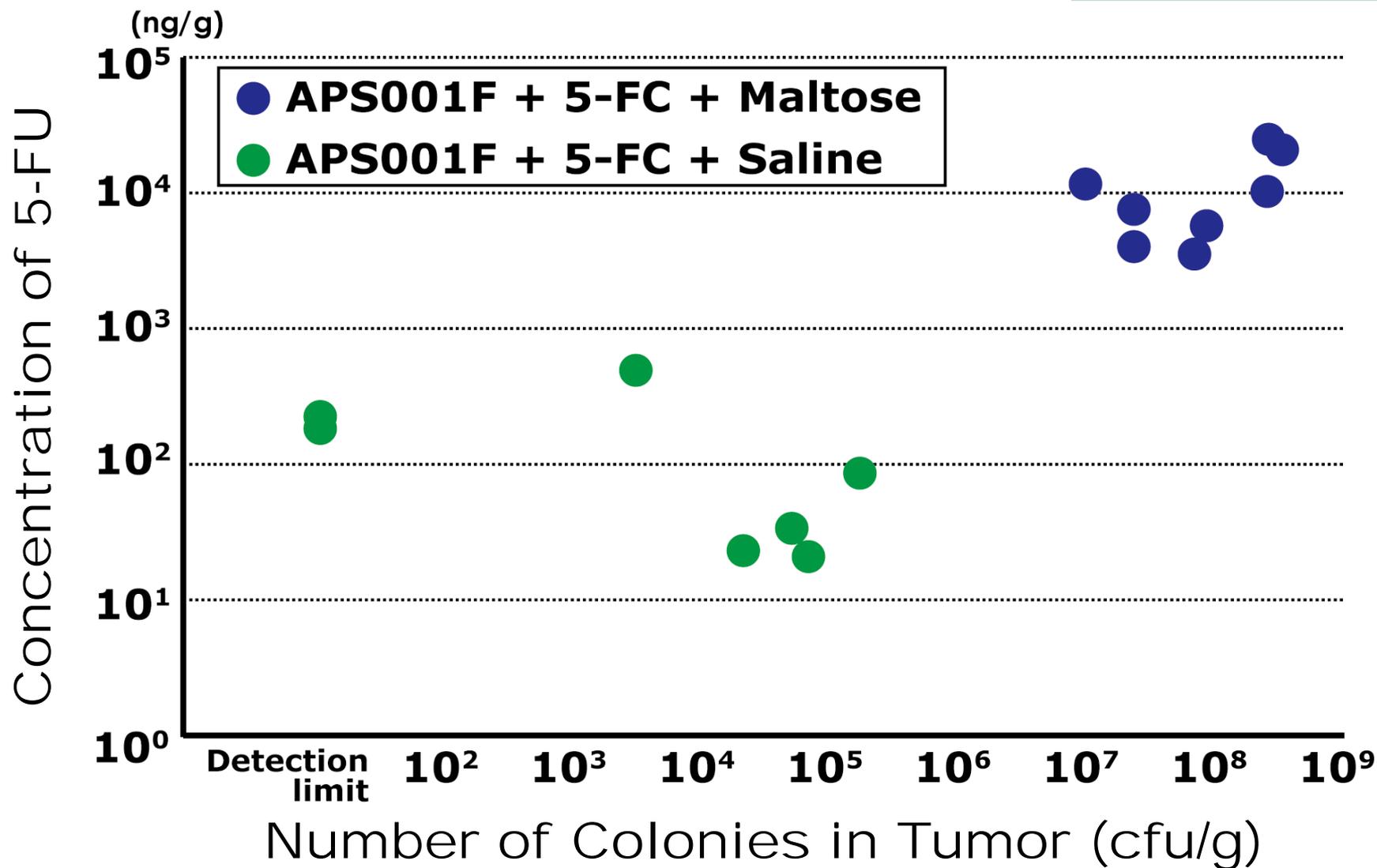
# POP Study; High Level of 5-FU Produced in KPL-1 Cancer, but not in Liver and Spleen (Nude Mice)



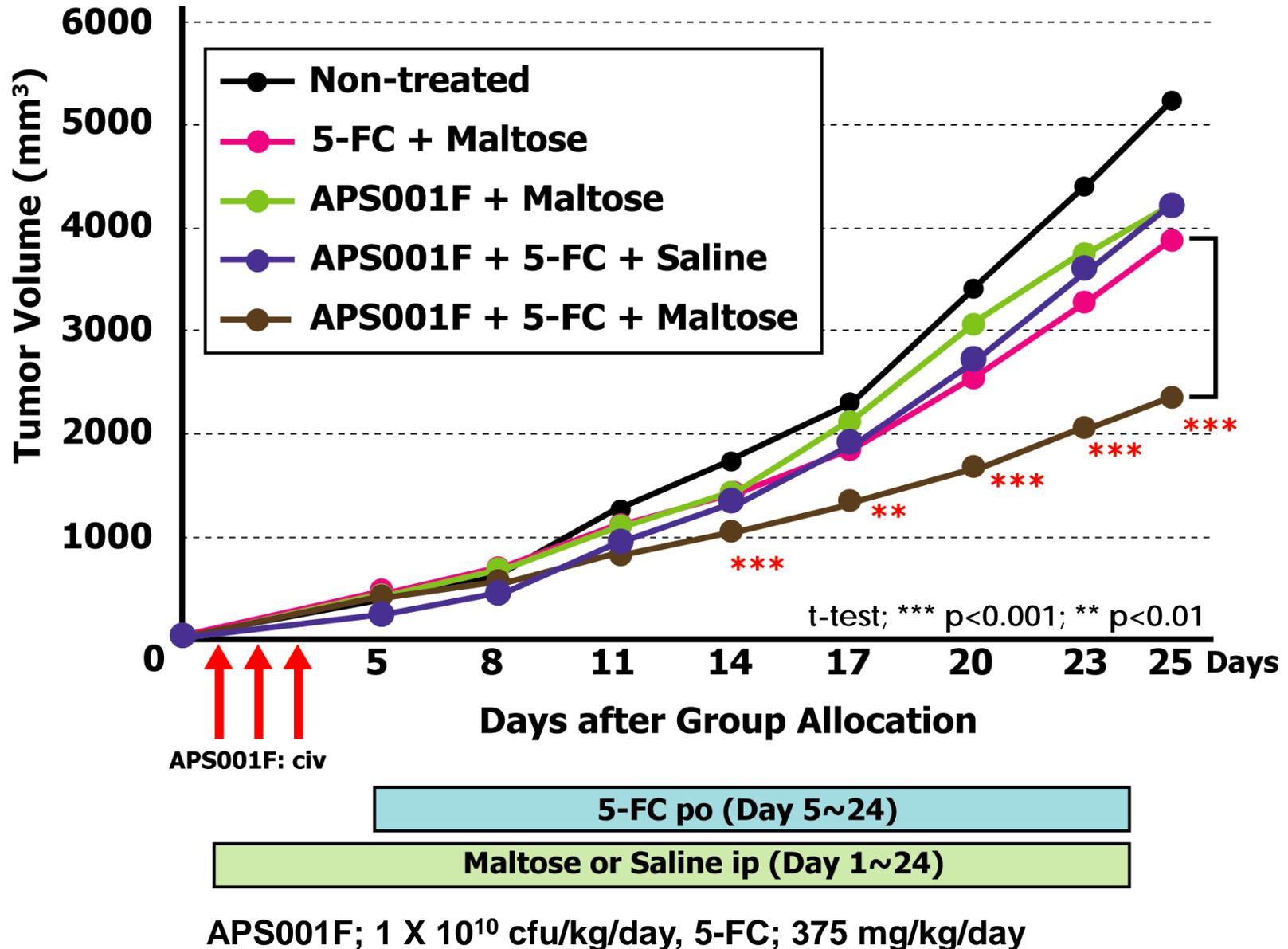
**APS001 F (n=10)**

# Incidence on Day 8 (5-FU detected mice / total mice)

# POP Study; Positive Correlation between 5-FU Production and Number of APS001F in KPL-1 Cancer (Nude Mice)



# Anti-tumor Efficacy in MKN45 Human Gastric Cancer (Nude Rats)



# Minimum Homing Dose in Intra tumoral colonization of APS001F

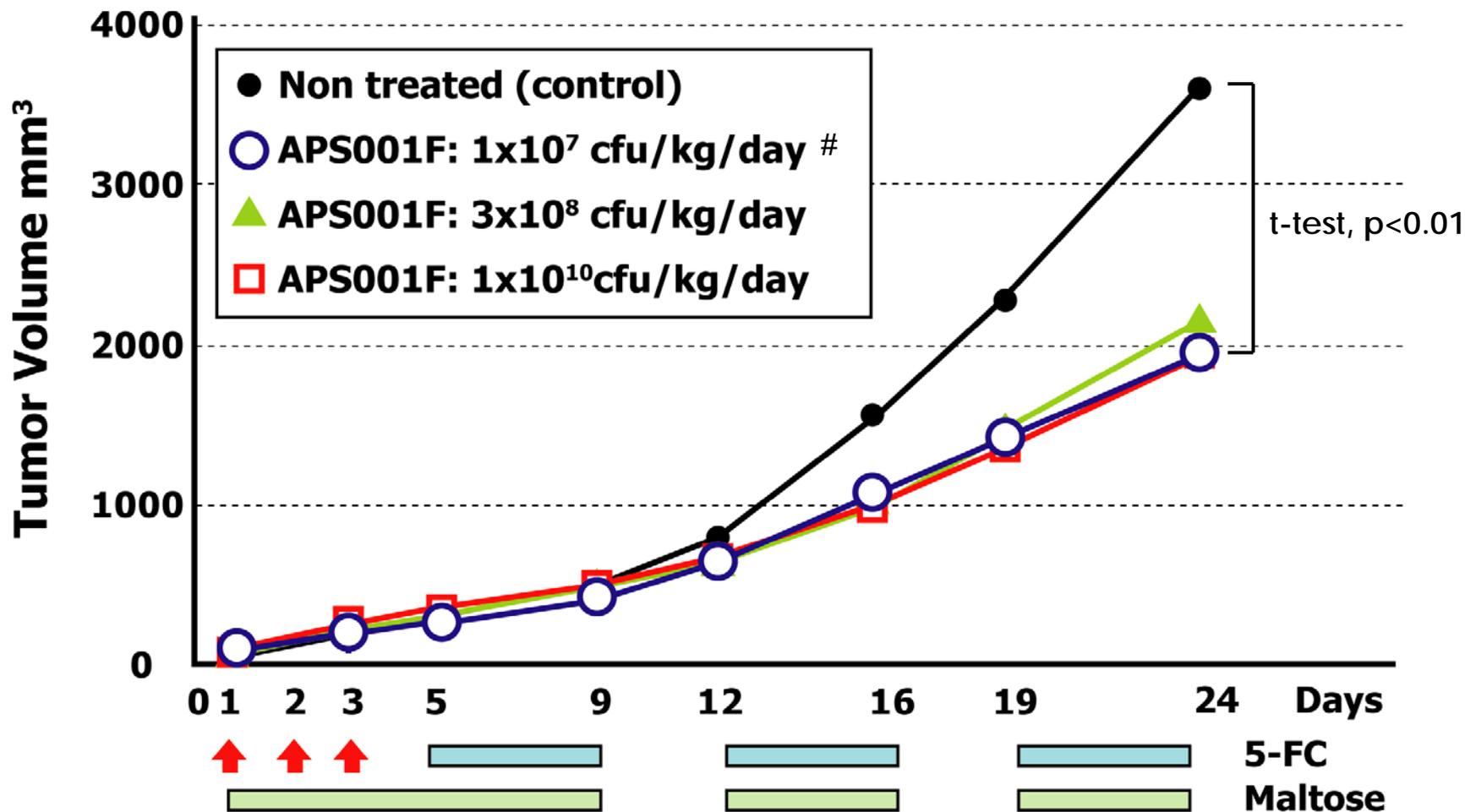
## 3 Days administration (infusion) schedule of APS001F

Dosage of APS001F (cfu/kg/day)	The Rate* of Intra Tumor Colonization of APS001F
$3.3 \times 10^7$	5 / 6
$1.0 \times 10^7$	5 / 6
$3.3 \times 10^6$	1 / 6
$1.0 \times 10^6$	0 / 6
$3.3 \times 10^5$	0 / 6

(\* ) The Number of Rats Detected APS001F in Tumor/the Number of total Rats

**$1.0 \times 10^7$  cfu/kg/day ( $6.0 \times 10^7$  cfu/m<sup>2</sup>/day) was determined as minimum homing dose .**

# Anti-tumor Efficacy at Minimum Homing Dose of APS001F in MKN45 Cancer (Nude Rats)



# The lowest dose is equivalent to “the minimum dose for tumor homing” required to colonize in the tumor of almost all animals (5 of 6 animals).

# Summary of Proof of Principle Studies

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**APS001F has been demonstrated to:**

- **Be rapidly cleared from normal tissues and blood**
- **Selectively localize and grow in tumors**
- **Convert 5-FC to 5-FU in tumors**
- **Exhibit anti-tumor activity in combination with 5-FC and maltose**
- **Possess enhanced intra-tumoral colonization in combination with maltose, which was associated with enhanced anti-tumor efficacy**

# Outline

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- Strategy
- **Preclinical Studies**
  - Proof of Principle (POP) and Efficacy Studies
  - **Review of Toxicity Studies**
- Proposed Clinical Study

# General Toxicity Studies

**Repeat dose toxicity studies of APS001F combined with 5-FC and maltose:**

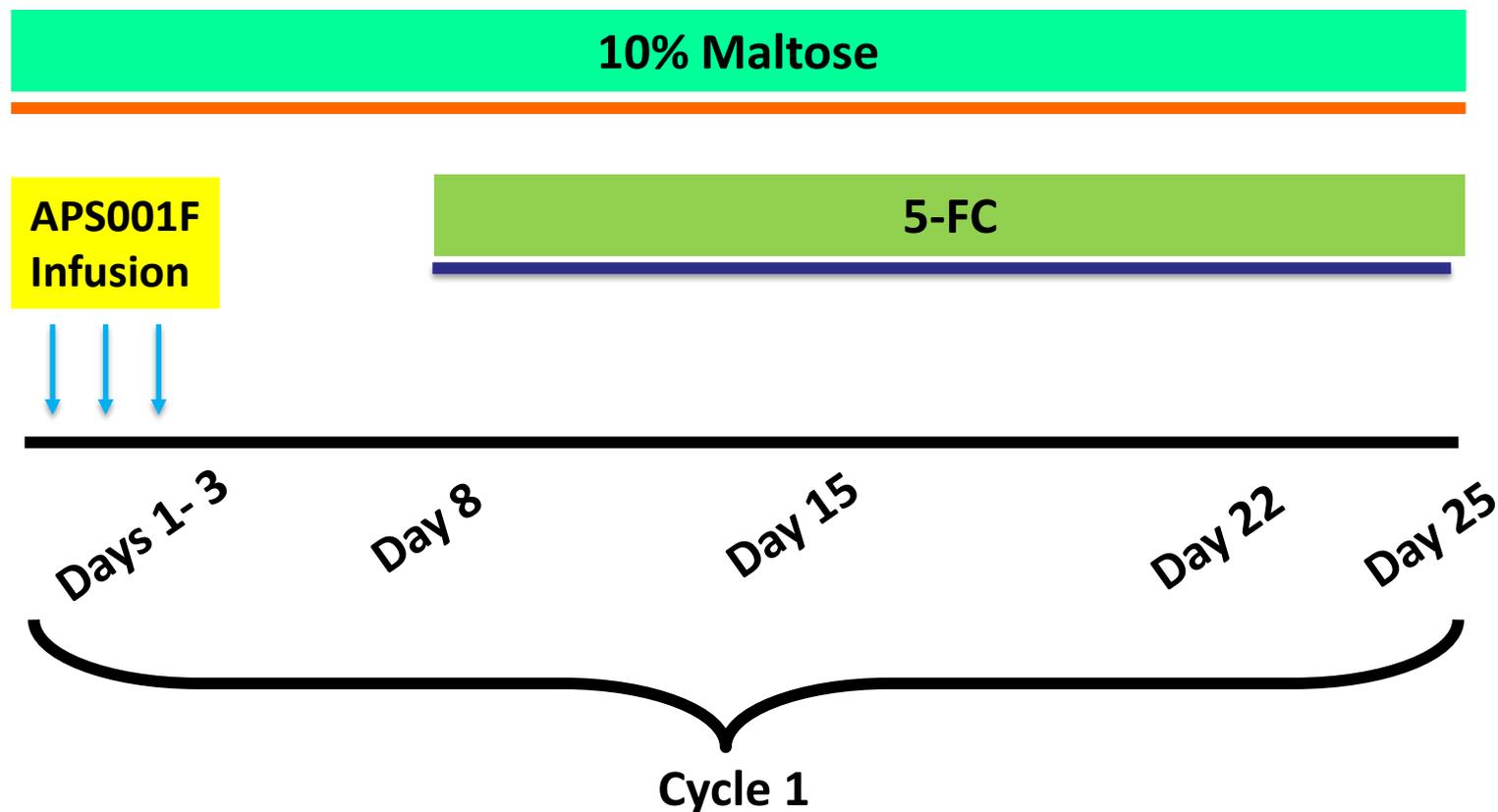
**Study:** 4-week (1 cycle) repeat dose studies (non-GLP)  
8-week (2 cycle) repeat dose studies (GLP)

**Animal:** 4-week (1 cycle); SD rats, beagle dogs  
8-week (2 cycle); SD rats, beagle dogs,  
cynomolgus monkeys

**Schedule:** mimicking the administration schedule of the proposed clinical trial.

Cycle	APS001F	5-FC	Maltose	Duration	
1 <sup>st</sup> cycle	Days 1-3	Days 8-25	Days 1-25	4-week	8-week
2 <sup>nd</sup> cycle	Days 29-31	Days 36-53	Days 29-53		

# Treatment Outline of the Animal Toxicity Studies



Repeat for cycle 2

# 8-Week Toxicity in Dogs

## Major changes observed in the 8-week toxicity study in dogs

- No appreciable change
- +  $\Delta$  33-66% / Body Temp  $\geq$  40C
- ++  $\Delta \geq$  67%

- \* Observed on Day 4
- \*\* Observed on Day 4 & Day 32
- \*\*\* Observed during Day 1 to 3 and Day 29 to 31

Treatment	No of Animals (male/female)	Vomiting	↑Temp	↓Platelets	↓Lymphocyte (Day 54)	↓Reticulocyte (Day 54)	Death
Control	5/5	-	-	-	-	-	-
5FC + Maltose	3/3	-	-	-	+	+	-
APS 3x 10 <sup>7</sup> 5FC+Maltose	3/3	-	-	-	+	+	-
APS 1x 10 <sup>9</sup> 5FC+Maltose	3/3	-	+***	+*	+	++	1 Male (Day 53)
APS 3x10 <sup>10</sup> 5FC+Maltose	5/5	+***	+***	+**	+	+	1 Male (Day 42)

After recovery period (Day 74) , no appreciable change was observed.

# Cytokine response (Dogs)

## IL-6 (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Days 3 - 4		Day 29				Days 31 - 32	
			Pre	2 h	2 h	24 h	Pre		2 h		2 h	24 h
III	3 x 10 <sup>7</sup> cfu/m <sup>2</sup>	3M	—	—	—	—	— (2)	118.6 (1)	—		—	—
		3F	—	—	—	—	— (2)	74 (1)	— (2)	50 (1)	—	—
V	3 x 10 <sup>10</sup> cfu/m <sup>2</sup>	5M	—	22751	649	—	—		17918		364	—
		5F	—	17912	428	—	—		20397		543	—

## TNF-α (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Days 3 - 4		Day 29				Days 31 - 32	
			Pre	2 h	2 h	24 h	Pre		2 h		2 h	24 h
III	3 x 10 <sup>7</sup> cfu/m <sup>2</sup>	3M	—	—	—	—	—		—		—	—
		3F	—	—	—	—	—		—		—	—
V	3 x 10 <sup>10</sup> cfu/m <sup>2</sup>	5M	—	949	— (1)	18(4)	—		— (1)	205(4)	—(3)	20(2)
		5F	—	458	— (3)	34(2)	—		3309		— (3)	8(2)

## IFN-γ (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Days 3 - 4		Day 29				Days 31 - 32	
			Pre	2 h	2 h	24 h	Pre		2 h		2 h	24 h
III	3 x 10 <sup>7</sup> cfu/m <sup>2</sup>	3M	—	—	—	—	—		—		—	—
		3F	—	—	—	—	—		—		—	—
V	3 x 10 <sup>10</sup> cfu/m <sup>2</sup>	5M	—	1093	—	—	—		— (3)	314(2)	—	—
		5F	—	389	— (4)	73(1)	—		— (2)	384(3)	—	—

# 8-Week (2 Cycle) Toxicity in Dogs

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- **The two cycles of the combination treatment of APS001F ( $3 \times 10^7$  cfu/m<sup>2</sup>/day) with 5-FC and maltose were well tolerated.**
- **Transient fever and decreased platelet counts occurred at mid- to high-APS dose levels. Transient vomiting occurred at high-APS dose levels.**
- **A severe decrease in reticulocyte and lymphocyte count was observed at the end of the 2nd -cycle of the treatment at all APS dose levels (5-FC effect).**
- **5-FC related toxicities appear to be prolonged, but resolved by Day 74.**
- **$3 \times 10^7$  cfu/m<sup>2</sup>/day of APS001F did not exhibit any appreciable toxicity, and this dose was determined to be the no adverse effect level (NOAEL).**
- **Cause of death for the animals treated with test articles: Mainly general prostration due to 5-FC toxicities.**

# 8-Week Toxicity in Monkeys

## Major changes observed in the 8-week toxicity study in monkeys

- No appreciable change
- +  $\Delta$  33-66% / Body Temp  $\geq$  40C
- ++  $\Delta \geq$  67%
- \* Observed on Day 4 & Day 32, recovered after dosing
- \*\* Observed during Day 29 to 31 (not measured in 1<sup>st</sup> cycle)

Treatment	No of Animals (male/ female)	Vomiting	↑Temp	↓Platelets	Death Observed
Control	3/2	-	-	-	1 Male (Day 29)
5FC + Maltose	2/1	-	-	-	-
APS 3x 10 <sup>8</sup> 5FC+Maltose	2/1	-	-	-	-
APS 1x 10 <sup>10</sup> 5FC+Maltose	2/1	-	+**	-	-
APS 3x10 <sup>11</sup> 5FC+Maltose	3/2	-	+**	+*	2 Male (D31, 35) 1 Female (D33)
Maltose	2/1	-	-	-	-
APS 3x10 <sup>11</sup> +Maltose	2/1	-	+**	+*	1 Male (D35)

After recovery period (Day 74) , no appreciable change was observed.

# Cytokine response (Monkeys)

## IL-6 (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Day 3		Day 4		Day 29		Days 31-32	
			Pre	2 h	Pre	2h	Pre	2h	Pre	2 h	Pre	2h
III	3 x 10 <sup>8</sup> cfu/m <sup>2</sup>	2M	—	—	—	—	—	—	—	—	—	—
		1F	—	—	—	—	—	—	—	—	—	—
V	3 x 10 <sup>11</sup> cfu/m <sup>2</sup>	3M	—	2186	4.3	398	—	—	—	2586	—	952
		2F	—	1522	—	585	—	—	—	1420	—	910

## TNF-α (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Day 3		Day 4		Day 29		Days 31-32	
			Pre	2 h	Pre	2h	Pre	2h	Pre	2 h	Pre	2h
III	3 x 10 <sup>8</sup> cfu/m <sup>2</sup>	2M	—	—	—	—	—	—	—	—	—	—
		1F	—	—	—	—	—	—	—	—	—	—
V	3 x 10 <sup>11</sup> cfu/m <sup>2</sup>	3M	—	104929	—	2459	—	—	—	477429	—	37991
		2F	—	131365	—	10770	—	—	—	166804	—	26761

## IFN-γ (pg/mL)

Group	Dose (/day)	No /sex	Day 1		Day 3		Day 4		Day 29		Days 31-32	
			Pre	2 h	Pre	2h	Pre	2h	Pre	2 h	Pre	2h
III	3 x 10 <sup>8</sup> cfu/m <sup>2</sup>	2M	—	—	—	—	—	—	—	—	—	—
		1F	—	—	—	—	—	—	—	—	—	—
V	3 x 10 <sup>11</sup> cfu/m <sup>2</sup>	3M	—	145.8	—	19.1	—	—	19.3(1)	327.9	15.0(1)	52.4
		2F	—	191.5	—	35.7	—	—	—	187.4	—	84.4

# 8-Week (2 Cycle) Toxicity in Monkeys

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- **The two cycles of the combination treatment of APS001F ( $1 \times 10^{10}$  cfu/m<sup>2</sup>/day) with 5-FC and maltose were well tolerated.**
- **Transient decrease in platelet counts occurred at high-APS dose level.**
- **Cause of death for animals treated with high dose of APS001F ( $3 \times 10^{11}$  cfu/m<sup>2</sup>/day) with (2 male and 1 female) or without (1 male) 5-FC and maltose : Renal failure (thrombus in glomerulus and necrosis of tubular epithelium in kidneys)**
- **These renal histopathology findings were observed only in animals that died. No evidence of renal failure was observed in surviving animals.**

# Summary of Toxicity Studies

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- The toxicity of APS001F, mainly observed as a decreased platelet count, fever and vomiting, recovered quickly.
- Dogs were the most sensitive species, and the NOAEL of APS001F in the combination therapy was  $3 \times 10^7$  cfu/m<sup>2</sup>/day for both male and female dogs.
- The NOAEL approximates the APS001F minimum effective tumor homing dose and the dose resulting in a significant antitumor effect ( $6 \times 10^7$  cfu/m<sup>2</sup>/day).
- Death/moribund animals were observed in 8-week (2 cycle) studies at high-APS dose levels, and the cause of death was defined as:
  - Monkeys: Renal failure
  - Dogs: General prostration due to 5-FC toxicities
- The starting dose of APS001F ( $3 \times 10^4$ /cfu/m<sup>2</sup>/day) in our proposed clinical trial is 3 logs lower than the NOAEL, demonstrating a 1,000-fold safety margin.

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**PI: John J. Nemunaitis, M.D.**  
Mary Crowley Cancer Research Center,  
Dallas, TX

# Proposed Protocol Dose Levels- 3 Steps

Step 1 -Dose escalation without maltose

Step 2 -Dose escalation with maltose

Step 3 –Expansion Cohort at Recommended Phase II Dose

Dose Level	Dose of APS001F (cells*/m <sup>2</sup> /day)	Increment	Dose of 5-FC (mg/kg/day)	Dose of 10% Maltose (mL/day)	# of patients*
Level 1	3 x 10 <sup>4</sup>	10	120	No	1
Level 2	3 x 10 <sup>5</sup>	10	120	No	1
Level 3	3 x 10 <sup>6</sup>	3.3	120	No	1
Level 4	1 x 10 <sup>7</sup>	3	120	No	3 or 6
Level 5	3 x 10 <sup>7</sup>	3.3	120	No	3 or 6
Level 6	3 x 10 <sup>7</sup>	1	120	500	3 or 6
Level 7	1 x 10 <sup>8</sup>	3	120	500	3 or 6
Level 8	3 x 10 <sup>8</sup>	3.3	120	500	3 or 6
Level 9	1 x 10 <sup>9</sup>	3	120	500	3 or 6
Level 10	3 x 10 <sup>9</sup>	3.3	120	500	3 or 6
Level 11	1 x 10 <sup>10</sup>	3	120	500	3 or 6
Level 12	3 x 10 <sup>10</sup>		120	500	3 or 6
Expansion cohort	MTD or OCD		120	500	30

NOAEL in Dogs

Minimum Homing Dose

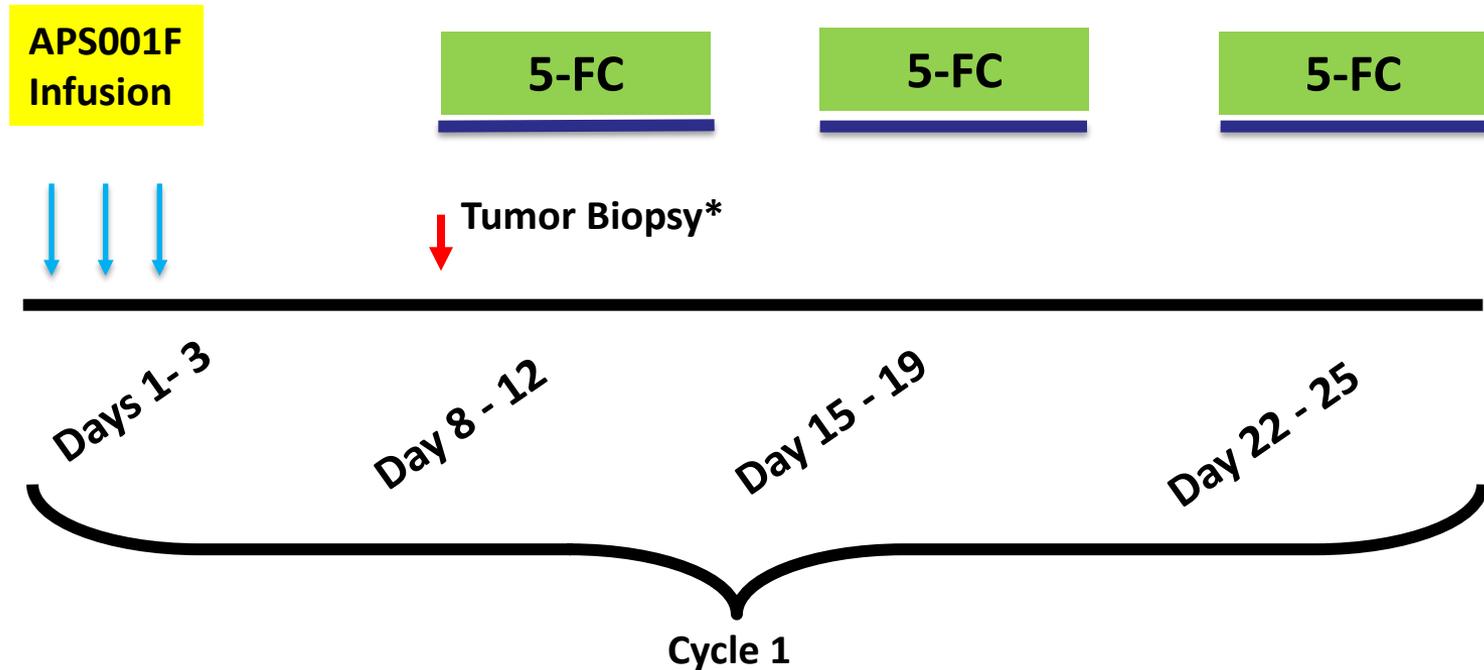


**Optimal Colonization Dose (OCD)**- confirmed colonies of APS001F in tumor tissue of ≥ 4/6 patients

\*a total of 6 patients to be enrolled into the MTD or OCD level

# Dose Escalation- APS001F + 5-FC

( $3 \times 10^4$  cells/m<sup>2</sup>/day to  $3 \times 10^7$  cells/m<sup>2</sup>/day)

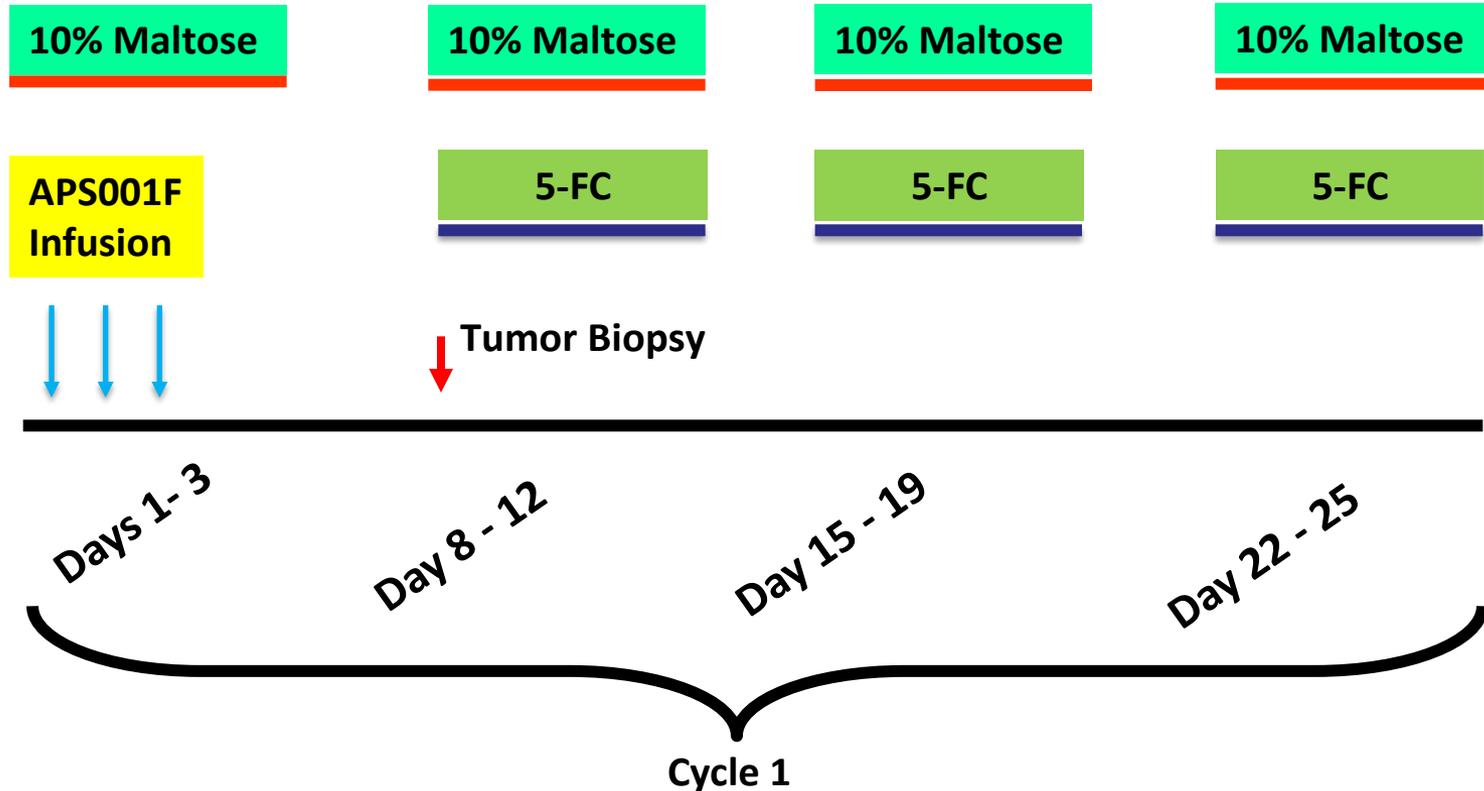


<b>Treatment Outline</b>	Dose escalation trial- up to 30 patients for up to 2 cycles
<b>Primary Endpoint</b>	Safety and tolerability; determine MTD / OCD and RD
<b>Secondary Endpoint</b>	Verify colonization of APS001F in tumor, and tumor response by RECIST criteria

\* only at  $3 \times 10^7$  cells/m<sup>2</sup>/day

# Dose Escalation-APS001F+ Maltose + 5-FC

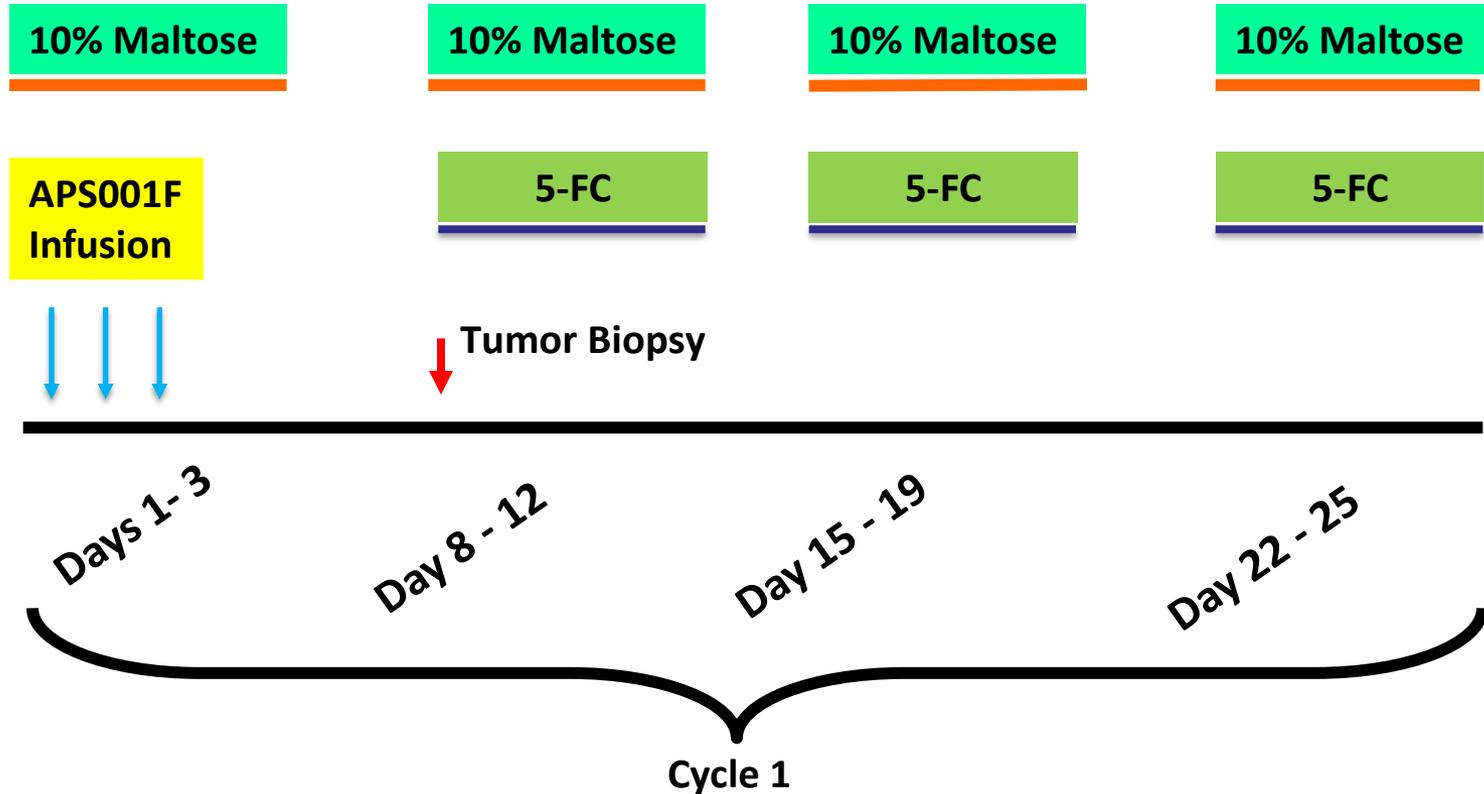
( $3 \times 10^7$  cells/m<sup>2</sup>/day to MTD / OCD)



<b>Treatment Outline</b>	Dose escalation trial- up to 30 patients for up to 2 cycles
<b>Primary Endpoint</b>	Safety and tolerability; determine MTD / OCD and RD
<b>Secondary Endpoint</b>	Verify colonization of APS001F in tumor, and tumor response by RECIST criteria

# Expansion Cohort- APS001F + Maltose + 5-FC

(at MTD or OCD)



<b>Treatment Outline</b>	30 patients at RD for up to 2 cycles
<b>Primary Endpoints</b>	Safety and tolerability of APS001F
	Verify colonization of APS001F in tumor Tumor response by RECIST criteria

# Protocol Entry Criteria

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- **Patients with measurable or evaluable solid tumors.**
- **No longer considered responsive to available treatments**
- **Adequate organ/hematologic function**
- **Negative HIV 1, No chronic active hepatitis B and C; no bacterial syndrome**
- **No artificial implant that cannot be easily removed.**
- **IRB approved consent**

# Amendment of exclusion criteria

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**The following exclusion criteria will be added:**

- **Bicuspid aortic valve**
- **Patients with foramen ovale**
- **Prior history of bacterial endocarditis**
- **Patients with any pleural effusion or ascites**
- **Patients with neutropenia or leucopenia**
- **Patients with any existing thrombus  
(either arterial or venous)**
- **Patients with permanent pacemakers, AICDs, LVADs, or other intravascular cardiac device**
- **Patients with baseline respiratory insufficiency severe enough to require supplemental oxygen**

# Known risks and side effects related to APS001F

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- The consent form will be amended to read:

*“Additionally, since APS001F is a bacteria infused in the vein, there is a risk that an unexpected infectious complication could occur following the bacterial infusion. A severe bacterial infection in the blood or other organs (such as the lungs, liver, kidneys, etc.) could develop that would require intensive care treatment in an attempt to reverse the infection’s harmful consequences. Such an infection may or may not be controlled by antibiotic treatment and could result in significant side effects or death of the patient.”*

# Summary

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- **Preclinical studies demonstrate the concept that the genetically modified *Bifidobacterium* APS001F localizes to solid tumors and generates intratumoral 5-FU (when combined with 5-FC and maltose).**
- **The minimum APS001F dose required for tumor colonization is approximately the same as the NOAEL in dogs.**
- **Anti-tumor effects were demonstrated at the minimal tumor colonization APS001F dose levels.**
- **Fatal events in dogs studies occurred only at APS001F dose levels  $\geq$  33,000-fold higher than the human trial starting dose.**
- **Adverse events at lower APS001F dose levels were transient**
- **Human trial will initiate at 1000-fold lower dose of APS001F than NOAEL in dogs.**