

# Protocol #0808-942

## Recombinant DNA Advisory Committee (RAC) 115th Meeting

December 3, 2008

### Presenters:

- Study PI: Barbara A. Murphy MD  
Vanderbilt Ingram Cancer Center, Nashville TN
- Stephen T. Sonis DMD DMSc  
Brigham and Women's Hospital, Dana Farber/Harvard Cancer Center, Boston MA
- Bernard Coulie MD PhD  
Vice President R&D, ActoGeniX NV, Belgium
- Lothar Steidler PhD  
Senior Director Technology Development, ActoGeniX NV, Belgium

# Oral Mucositis (OM)

- Painful, common, and potentially debilitating toxicity of drug and radiation treatment used for cancer treatment
- Described by patients as most bothersome adverse event associated with their cancer treatment
- Incidence of ulcerative OM (WHO grade >1) in induction CT cycle 1 for head/neck cancer is 30-40%. Risk of OM increases up to 70% in subsequent treatment cycles (probably under-reported)
- Associated with increased morbidity, mortality, analgesic use, hospital stay, need for parenteral nutrition, and total charges
- Prevention/treatment options are sparse and largely ineffective, and mainly based on palliative rinses, barrier devices and pain control
- Only approved medication for OM is palifermin (Kepivance®, KGF-1): limited to OM associated with conditioning regimens for hematopoietic stem cell transplantation (4% of all OM cases)

# Rationale for local trefoil factor 1 (TFF1) in OM

- TFF1 peptide is constituent of human saliva and serum 
- TFF1 has wound-healing properties and plays a role in protecting and healing mucosal tissues
- Its potential as an intervention for oral mucositis has been shown in validated animal models 
- Topical application of the related peptide TFF3 has been reported to reduced the incidence of OM in patients receiving chemotherapy for colorectal cancer (Barker et al. 2008)
- *L. lactis* delivery of TFF1 (AG013) is expected to provide high efficacy in a cost-effective way through mucosal targeting and continuous release of TFF1 for several hours after rinsing event

# Summary Overview of AG013

- Product: AG013 is formulated freeze dried sAGX0085, the engineered *Lactococcus lactis* strain derived from *L. lactis* MG1363, expressing hTFF1 
- Concept: Local oral delivery of hTFF1 by genetically modified living food-grade bacteria *Lactococcus lactis* (non-colonizing gr<sup>+</sup> food bacteria)
- Pharmacology: sAGX0085 at 10<sup>8</sup> and 10<sup>10</sup> CFU bid sustained application or 10<sup>10</sup> qd and tid rinsing is effective in reducing severity and course of OM in animal model 
- PK: Living sAGX0085 bacteria as well as hTFF1 are quantifiable in the hamster cheek pouch up to 24 hrs after administration. No living bacteria nor hTFF1 detected beyond the mucosal compartment, nor in the systemic circulation 
- Toxicology of AG013: No significant treatment-related changes in body weight, food/water consumption, hematology, urinalysis, clinical chemistry, organ weights, ophthalmoscopy, and histopathology (2 species) 

# Summary Overview of AG013

- Clinical development:



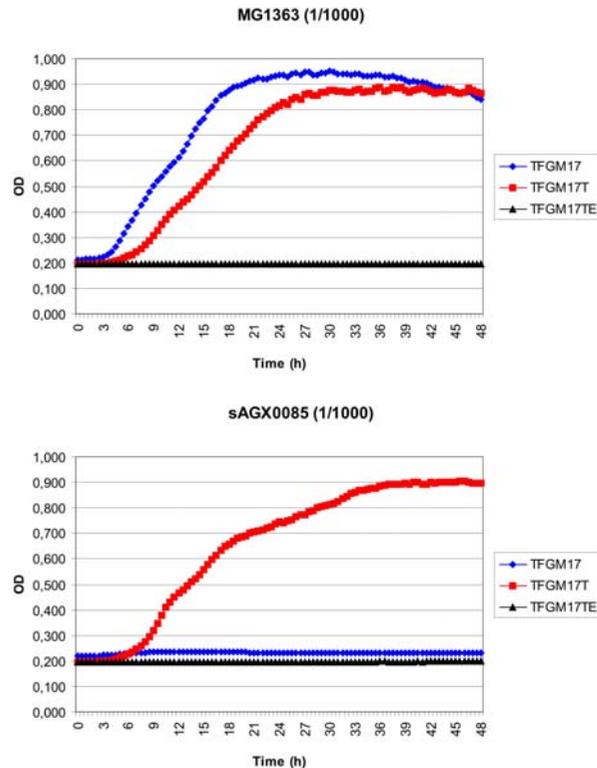
- Phase 1b, multicenter, single-blinded, placebo-controlled, sequential dose escalation study to assess primarily safety of topically applied AG013 in subjects receiving induction chemotherapy for the treatment of cancers of the head and neck
- Exploratory efficacy data will also be collected
- IND filing February 2009
- Pre-IND meeting with CBER/FDA on October 23rd, 2008

# Responses to Questions from RAC reviewers

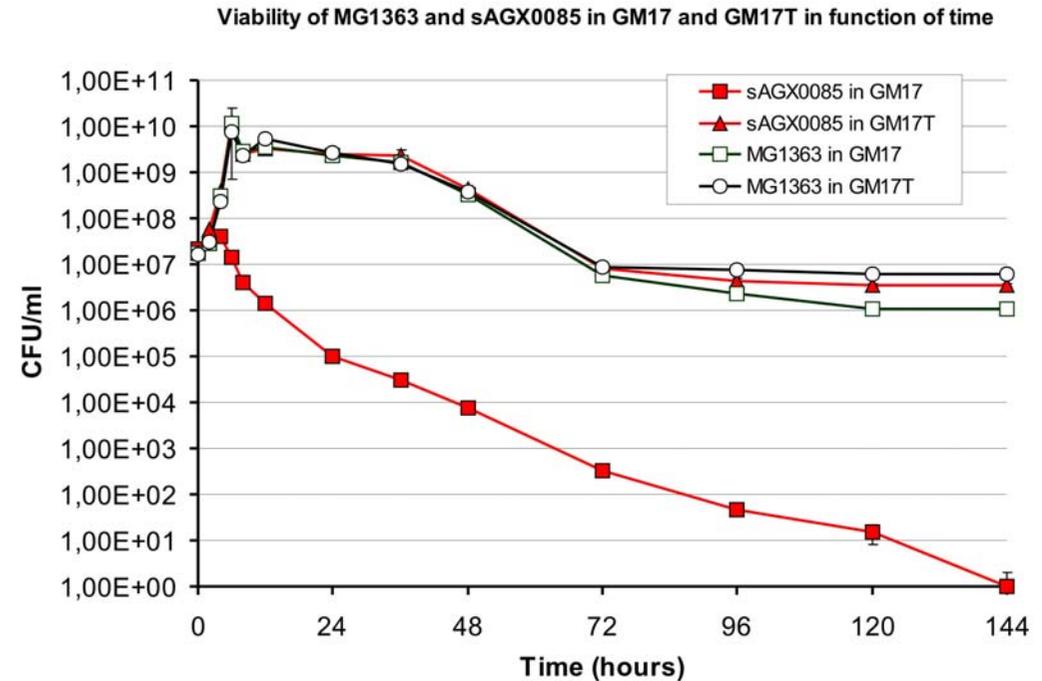
- sAGX0085 characteristics: auxotrophy, lateral gene transfer (Dr. L. Steidler)
- Safety: (Dr. B. Coulie)
  - Review of available safety data
  - Safety assessment in neutropenia and mucositis
  - Prior use of genetically modified *L. lactis* in humans
- Clinical protocol (Dr. B. Murphy, PI)
- Informed Consent Form (Dr. B. Murphy)

# sAGX0085: in vitro auxotrophy

## Growth kinetics of MG1363 vs. sAGX0085



## Survival of sAGX0085 requires thymidine



The loss of thyA- through a suppressor mutation is unlikely as thyA has been removed during the construction of sAGX0085

# sAGX0085: in vivo auxotrophy

- No data on in vivo auxotrophy available
- In vivo auxotrophy confirmed of highly comparable engineered *L. lactis* Thy12 strain:

<i>L. lactis</i> strain	Thy12	Thy12 + thymidine	MG1363
<i>L. lactis</i> genotype	<i>thyA</i> <sup>-</sup>	<i>thyA</i> <sup>-</sup>	<i>thyA</i> <sup>-</sup>
Dosage	6.12 x 10 <sup>10</sup> CFU	6.90 x 10 <sup>10</sup> CFU	8.22 x 10 <sup>10</sup> CFU
Total ileum	2.94 x 10 <sup>8</sup> CFU	8.05 x 10 <sup>8</sup> CFU	3.64 x 10 <sup>8</sup> CFU
Total weight ileum samples	123 g	132 g	109 g
Ileum CFU vs dosage	0,48%	1,17%	0,44%
Total feces	1.25 x 10 <sup>7</sup> CFU	3.10 x 10 <sup>8</sup> CFU	2.69 x 10 <sup>8</sup> CFU
Total wt feces samples	1,153 g	1,630 g	571 g
Feces CFU vs dosage	<b>0,02%</b>	<b>0,45%</b>	<b>0,33%</b>

# sAGX0085: lack of thyA lateral gene transfer

## “Mixed colony” co-culture

1. solid agar PCA: supports “donor” growth.
2. solid agar PCAT: supports “donor” + sAGX0085(CmR, EmR) growth.
3. solid agar PCATCE: supports sAGX0085(CmR, EmR) growth.
4. solid agar PCACE: supports “*thyA*+” sAGX0085(CmR, EmR) growth.

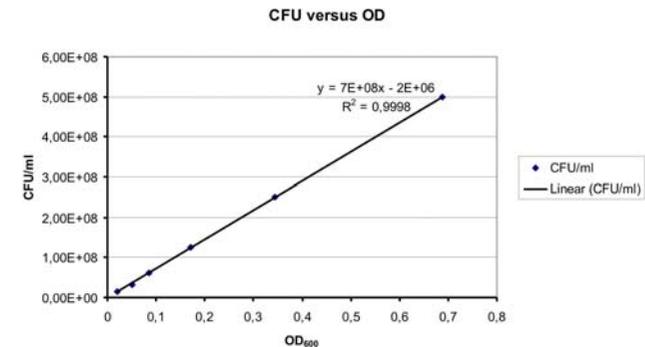
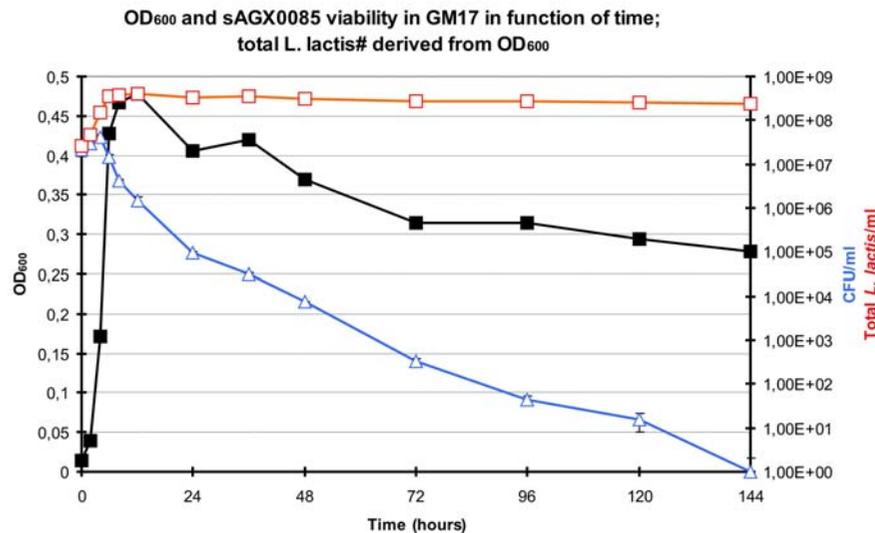
strains	PCA	PCAT	PCATCE	PCACE
sAGX0085(CmR, EmR) + <i>L. lactis</i> MG1363	∞	∞	>2,000	0
sAGX0085(CmR, EmR) + <i>L. lactis</i> IL1403	∞	∞	>2,000	0
sAGX0085(CmR, EmR) + <i>Lactobacillus acidophilus</i>	139	68	0	0
sAGX0085(CmR, EmR) + <i>Lactobacillus plantarum</i>	>2,000	∞	1	0
sAGX0085(CmR, EmR) + <i>Streptococcus mutans</i>	>2,000	>2,000	>1,000	0
sAGX0085(CmR, EmR) + <i>Escherichia coli</i> DH5a	∞	∞	121	0
sAGX0085(CmR, EmR) + <i>Escherichia coli</i> O157:H7	∞	∞	>1,000	0
sAGX0085(CmR, EmR) + <i>Salmonella enterica</i>	∞	∞	36	0

**No colonies indicate no transfer of *thyA*<sup>+</sup> phenotype to sAGX0085**

# CFU of co-cultures of the indicated strains, obtained on solid agar PCA, PCAT, PCATCE and PCACE plates following incubation for 48 hour at 30°C

# sAGX0085: risk for release of naked intact DNA

- Lateral transfer of TFF1 expression cassette by integration of naked DNA into rapidly growing bacteria or viruses is highly unlikely:
  - Thymineless death precedes bacterial cell lysis



Kinetics of OD<sub>600</sub> and viable cell count of sAGX0085 in GM17

- sAGX0085 bacteria do not colonize the oral cavity and are only present for relatively short time in presence of other bacteria or viruses
- Stable integration of TFF1 expression cassette is mechanistically unlikely because it requires the competency for the uptake of DNA, stable integration before degradation, and clonal expansion of the TFF1 expression cassette in the recipient

# Available Safety Data

## L. Lactis

- No reported evidence that neutropenia predisposes to *L. lactis* infection
  - 21 reported cases of *L. lactis* infection
    - Very low incidence despite very high, daily exposure through dairy products
    - All reported cases cured by commonly used antibiotic therapy
    - Approximately half had underlying conditions or predisposing events
- Although documented incidence of *L. lactis* infection is extremely low given the high exposure in dairy foods, in the proposed study subjects will be monitored very closely including for any signs and symptoms of local or systemic infection.



# Available Safety Data

## AG013, sAGX0085

- 14 day GLP toxicity studies in rat and dog (cross-reactive with hTFF1):
  - No treatment-related abnormal findings and no detectable levels of *L. lactis* bacteria or hTFF1 beyond the mucosal compartment
- PK studies in hamster mucositis model:
  - No bacteremia or measurable serum levels of hTFF1
- Thymidine rescue in serum or in the inflammatory exudate is highly unlikely:
  - Documented thymidine serum concentrations ( $10^{-7}$  M) are sufficiently low to ensure “thymineless death” (“thymineless death” occurs at  $10^{-5}$  M of thymidine)
  - PK experiments in mucositis model: no systemic exposure of *L. lactis* or survival/colonization in oral cavity



# Safety assessment in neutropenia and mucositis

- **Ongoing safety assessment of AG013 topically applied in the cheek pouch of hamster model with concomitant mucositis and neutropenia**

Group	Animals	Radiation Day 0	5FU Days 9, 11	Dose tid with rinse Days 15-16	Sac 4 hours post final dose	Sac 48 hours post final dose
1	10♂	40 Gy	60 mg/kg	Placebo	5♂	5♂
2	10♂	40 Gy	60 mg/kg	AG013 $1.3 \times 10^{10}$ CFU	5♂	5♂



## Assessments

- **QC of treatment:**
  - Plating / Elisa of left cheek pouch
- **Evaluation of bacteremia:**
  - Rectal temperature (measured daily and at necropsy)
  - White blood cell / Neutrophil granulocyte count
  - Plating of blood
- **Evaluation of tissue distribution:** collection of liver, spleen, kidney, gonads, draining lymph nodes and brain for plating and DNA preparation

# Safety assessment in neutropenia and mucositis

## Dose calculations versus the intended doses in patients

- Total dose per kg body weight administered in the neutropenic mucositis hamster model is 40x higher compared to the highest dose that will be administered to patients
- Total dose per oral mucosal surface area administered in the neutropenic mucositis hamster model equals the mid dose that will be administered to patients

hamster cheek pouch	surface = 17 cm <sup>2</sup> ; body weight = 80 gram				
	treatment frequency	dosage/admin	total dose CFU	total dose/cm <sup>2</sup>	total dose/kg
sAGX0085 culture	tid	1 x 10 <sup>10</sup> CFU	3 x 10 <sup>10</sup>	1.7 x 10 <sup>9</sup> CFU	3.75 x 10 <sup>11</sup>
AG013 DP	tid	1.3 x 10 <sup>9</sup> CFU	3.9 x 10 <sup>9</sup>	2.3 x 10 <sup>8</sup> CFU	4.88 x 10 <sup>10</sup>
<b>AG013 DP (neutropenia)</b>	<b>tid</b>	<b>1.3 x 10<sup>10</sup> CFU</b>	<b>3.9 x 10<sup>10</sup></b>	<b>2.3 x 10<sup>9</sup> CFU</b>	<b>4.88 x 10<sup>11</sup></b>

human mouth	surface = 215 cm <sup>2</sup> ; body weight = 70 kg				
	treatment frequency	dosage/admin	total dose CFU	total dose/cm <sup>2</sup>	total dose/kg
AG013	1	2 x 10 <sup>11</sup> CFU	2 x 10 <sup>11</sup>	9.3 x 10 <sup>8</sup> CFU	2.85 x 10 <sup>9</sup>
AG013	3	2 x 10 <sup>11</sup> CFU	6 x 10 <sup>11</sup>	2.8 x 10 <sup>9</sup> CFU	8.6 x 10 <sup>9</sup>
AG013	6	2 x 10 <sup>11</sup> CFU	1.2 x 10 <sup>12</sup>	5.6 x 10 <sup>9</sup> CFU	1.7 x 10 <sup>10</sup>

# Available safety data from prior use of genetically modified *L. lactis* in humans

## **Thy12 (*L. lactis* secreting IL-10) Phase 1**

- 7d treatment in 10 patients with moderate to severe Crohn's disease on concomitant immunosuppressive therapy
- Good safety and tolerability
- Environmental containment

## **AG011 (*L. lactis* secreting IL-10) Phase 2**

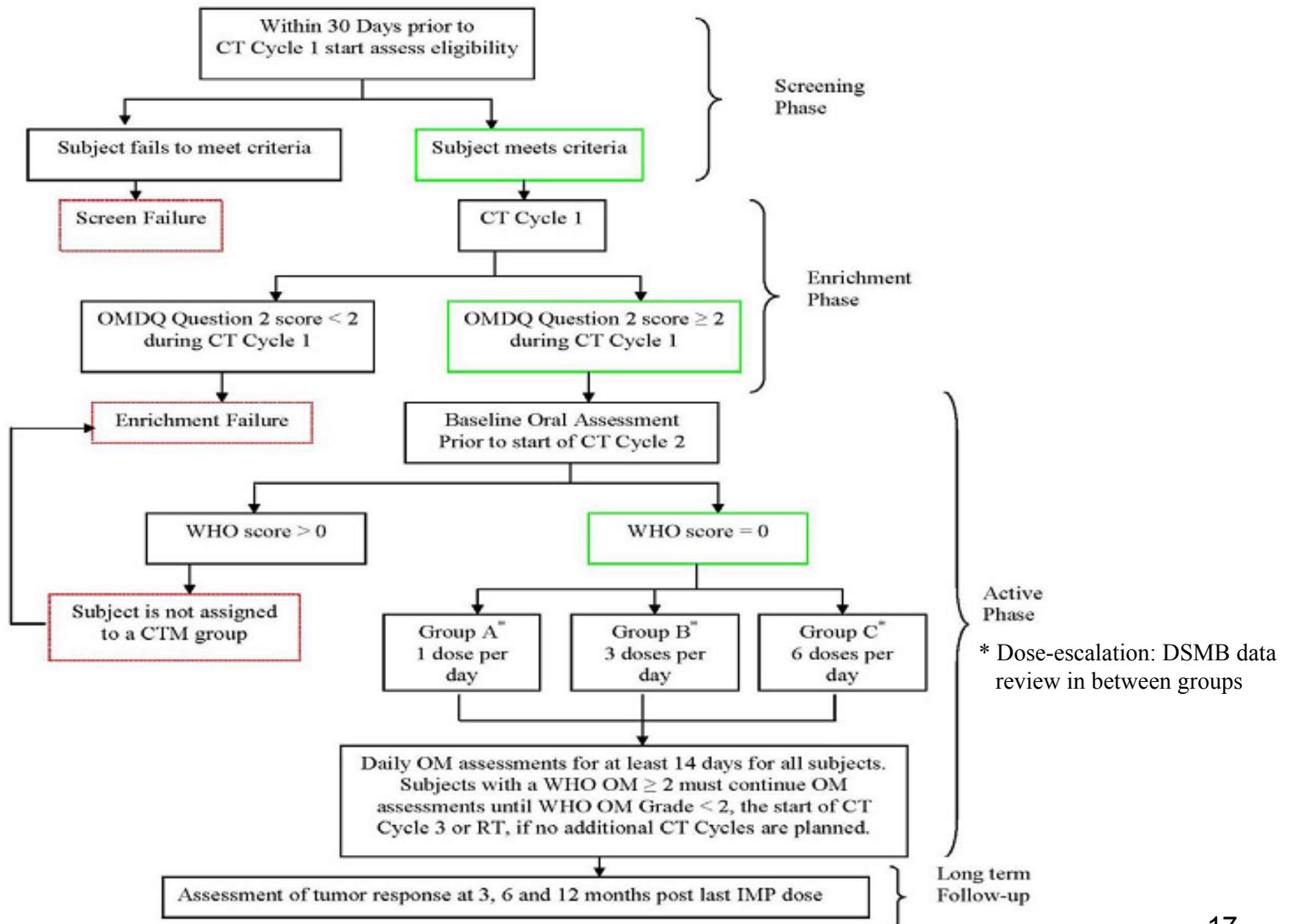
- RAC protocol# **0804-917** (113th meeting, June 23rd 2008)
- Ongoing study in 60 patients with moderate ulcerative colitis: 4 wks treatment, 3 doses and placebo
- Currently 10 patients have completed treatment: safety and tolerability confirmed

# AG013 – Phase 1b study

- A Phase 1b, multi-center, single blinded, placebo-controlled, sequential dose escalation study to assess the safety of topically applied AG013 in subjects receiving induction chemotherapy for the treatment of cancers of the head and neck



<b>Number of patients</b>	21 (15 active/6 placebo)
<b>Number of treatments</b>	4
<b>Treatments</b>	1. Oral rinsing dose frequency (1/day) 2. Oral rinsing dose frequency (3/day) 3. Oral rinsing dose frequency (6/day) and matching placebo (2 placebo to 5 active)
<b>Application</b>	Once to 6 times daily rinsing (outpatient setting)
<b>Duration of the administration</b>	14 days (in 2nd CT cycle)



# Study design

## **Primary objective is evaluation of safety and tolerability**

- Protocol has the appropriate cancer treatment regimen for assessing safety and tolerability of AG013
- No inclusion of patients undergoing 5-7 wk radiation therapy for head/neck cancer
  - More complex AE pattern which may hamper correct safety assessment
  - Only 14 day documented exposure in toxicity studies

## **Assessment of effect on mucositis is exploratory endpoint**

- Patients are receiving highly mucotoxic combination CT regimen (cisplatin, fluorouracil with or without docetaxel)
- Risk for developing ulcerative mucositis in cycle 1 is at least 30%
- Enrichment in the treatment phase (CT cycle 2) by only including patients with documented mucositis during CT cycle 1
- Proposed design successfully applied in Phase 3 study investigating effect of experimental agent on prevention of mucositis (Peterson et al., 2007)



# Concomitant use: xerostomia products

- Xerostomia is not an AE of induction CT for HNC
- No product approved for the treatment of xerostomia in patients undergoing postoperative radiation treatment for head and neck cancer is approved as a mucositis intervention in any patient population
- Current mucositis guidelines (MASCC/NCCN, Cochrane) do not advise the use of amifostine or sialogogues in the prevention or treatment of mucositis
- Drugs used for treatment of xerostomia will not be allowable per protocol (eg. amifostine, pilocarpine, etc.)



# Other protocol considerations

## Effect of chemotherapy regimen on production of TFF1 by *L. lactis*

- No interaction is expected:
  - Mode of action of chemotherapeutic agents is not affecting *L. lactis* metabolism
  - Chemotherapeutic agents will only be given 3-4 days at start of cycle, AG013 will be administered during entire cycle (14 days)

# Informed Consent form adaptations

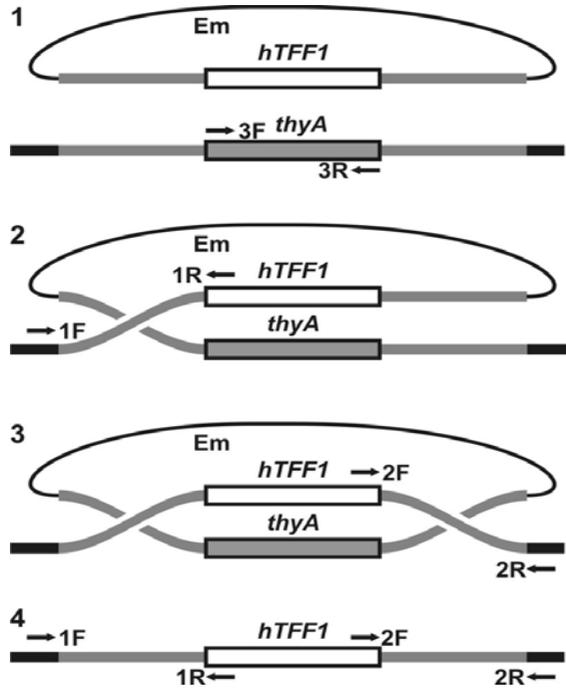
- Volume and purpose of blood samples will be clarified
- Approximate time needed to complete the questionnaire will be added
- Informed Consent form will specify that it is unlikely that the research participant will benefit from this treatment
- The risk for bacteremia will be added to the risk section of the Informed Consent form

**Back up slides**

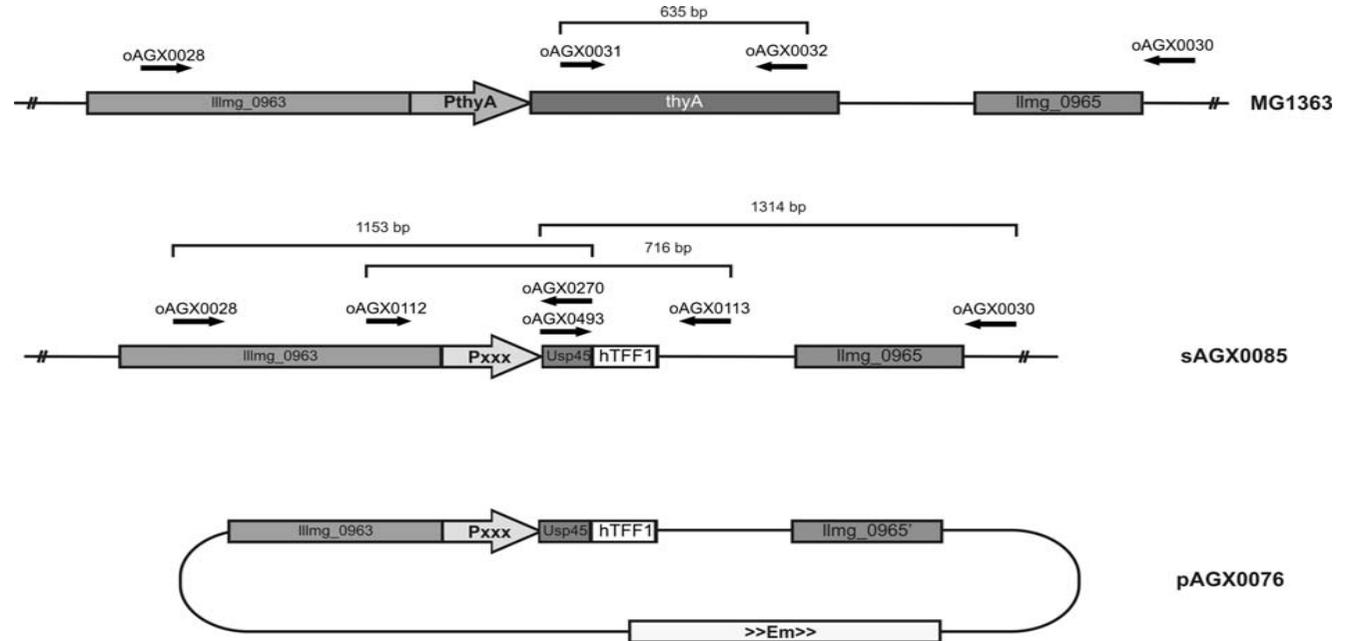
# AG013

## Strain description (sAGX0085)

# sAGX0085: construction and identity



Construction of sAGX0085



Identity of sAGX0085

- *hTFF1* gene expression locally in the mouth
- There is no gene transfer to somatic cells

# AG013: strain description

- sAGX0085 is the **engineered** *Lactococcus lactis* strain derived from *L. lactis* **MG1363**
- In MG1363, the **thyA expression** cassette [P thyA>>thyA] is replaced by a **TFF1 expression** cassette [(pspp)<sup>#</sup>>>SSusp45>>TFF1], in which the promoter from the MG1363 (pspp) gene precedes a fusion between the lactococcal secretion leader (SSusp45) and TFF1
- The **coding sequence of TFF1** is synthetic and optimized for *L. lactis*. The protein sequence is identical to natural TFF1

# proprietary structural protein promoter

# AG013: genetic structure

- The **DNA sequence** of the [(pspp)>>SSusp45>>**TFF1**] expression cassette present in sAGX0085, as determined on a PCR fragment derived from sAGX0085, is **identical to the predicted** sequence. The successful removal of [P thyA>>thyA] has been documented by DNA analysis
- Complete DNA sequencing of the sAGX0085 genome has been finalized and revealed > **99.995% sequence homology** to the predicted sAGX0085 sequence

# AG013: genetic stability

- Analysis of the genetic stability of *L. lactis* strain sAGX0085 was performed after a minimum of **100 generations of growth**, obtained by repeated sequential dilution and growth to saturation. Genetic stability was also analyzed by **DNA sequence** determination of the [pspp>>SSusp45>>**TFF1**] expression cassette of *L. lactis* strain sAGX0085
- The genetic stability was analyzed by four parameters:
  - **Inability** of sAGX0085 to **grow in thymidine-deficient medium**
  - Unchanged **TFF1 secretion** by sAGX0085
  - **PCR** analysis of the **modified thyA locus** of sAGX0085
  - **DNA sequence** verification of the [pspp>>SSusp45>>**TFF1**] expression cassette of *L. lactis* strain sAGX0085
- **For all of these parameters, genetic stability was absolute**

# AG013: sensitivity to antibiotics

AG013 is **sensitive** to following **antibiotics**:

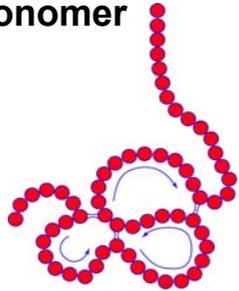
- Penicillin G (penicillins)
- Ampicillin (penicillin)
- Amoxicillin + clavulanic acid (penicillin + beta lactamase inhibitor)
- Gentamicin (aminoglycoside)
- Chloramphenicol (phenicol)
- Tetracycline (tetracycline)
- Erythromycin (macrolide)
- Bacitracin (polypeptide)
- Vancomycin (glycopeptide)
- Levofloxacin (3rd generation fluoroquinolones)
- Nitrofurantoin (nitrofuranes)
- Cefepime (3rd generation cephalosporin)
- Imipenem (carbapenem)
- Linezolid (oxazolidinones)

# Rationale for AG013 in Oral Mucositis

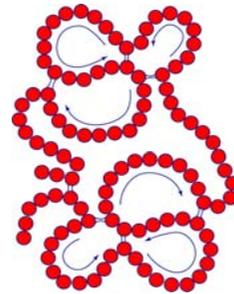
## The Family of Trefoil Factors

**TFF1**

Monomer



**TFF2**



**Trefoil peptides:**

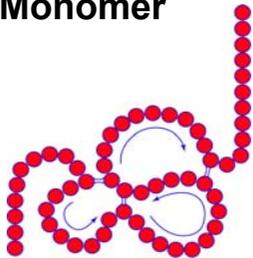
- TFF1 and TFF3 have 1 trefoil domain

- TFF2 has 2 trefoil domains

- TFF1 and 3 exist both as dimers and monomers

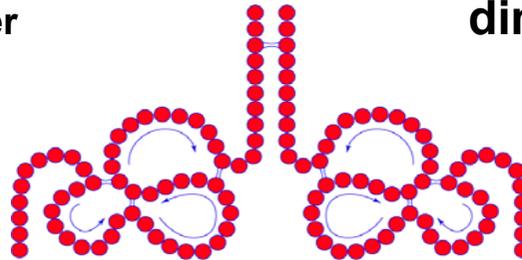
**TFF3**

Monomer



**TFF3**

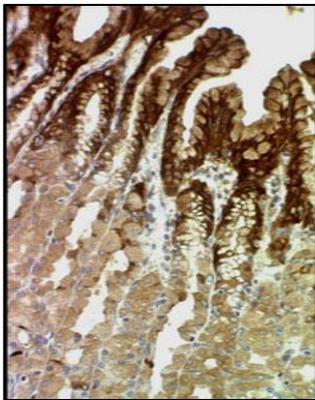
Dimer



# The Family of Trefoil Factors

## TFF1

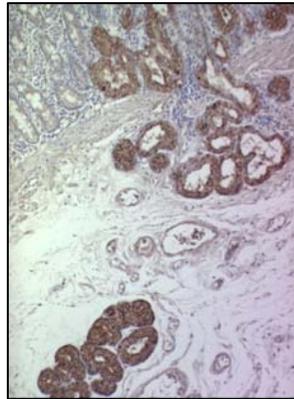
- ✘ Mainly restricted to the surface epithelium of the stomach
- ✘ Other sites of expression: goblet cells in the conjunctiva, ductus deferens, salivary glands



Surface epithelium of human stomach

## TFF2

- ✘ Localized only to the stomach and duodenum



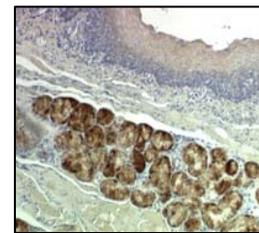
Brunners glands of human duodenum

## TFF3

- ✘ The “general” TFF localized to mucus secreting surfaces
- ✘ In the GI-tract: goblet cells in small intestine and colon, gall bladder, tongue, salivary and esophageal glands



Submandibular glands of human oral cavity



Tongue

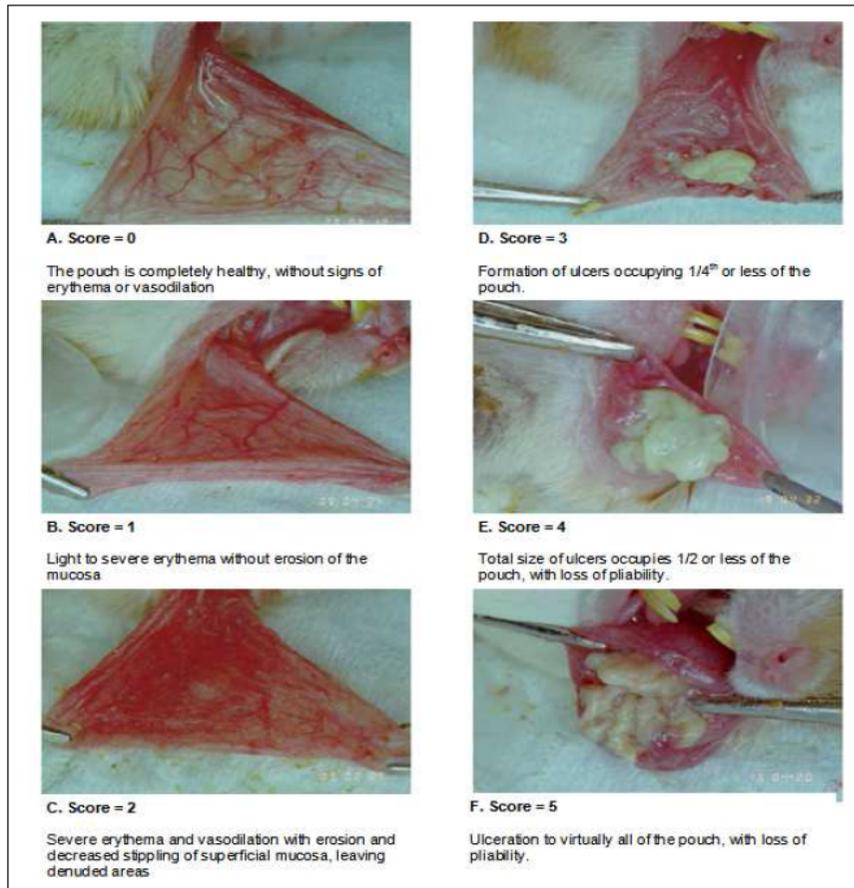
# AG013

## Pharmacology studies

# AG013 Animal Pharmacology

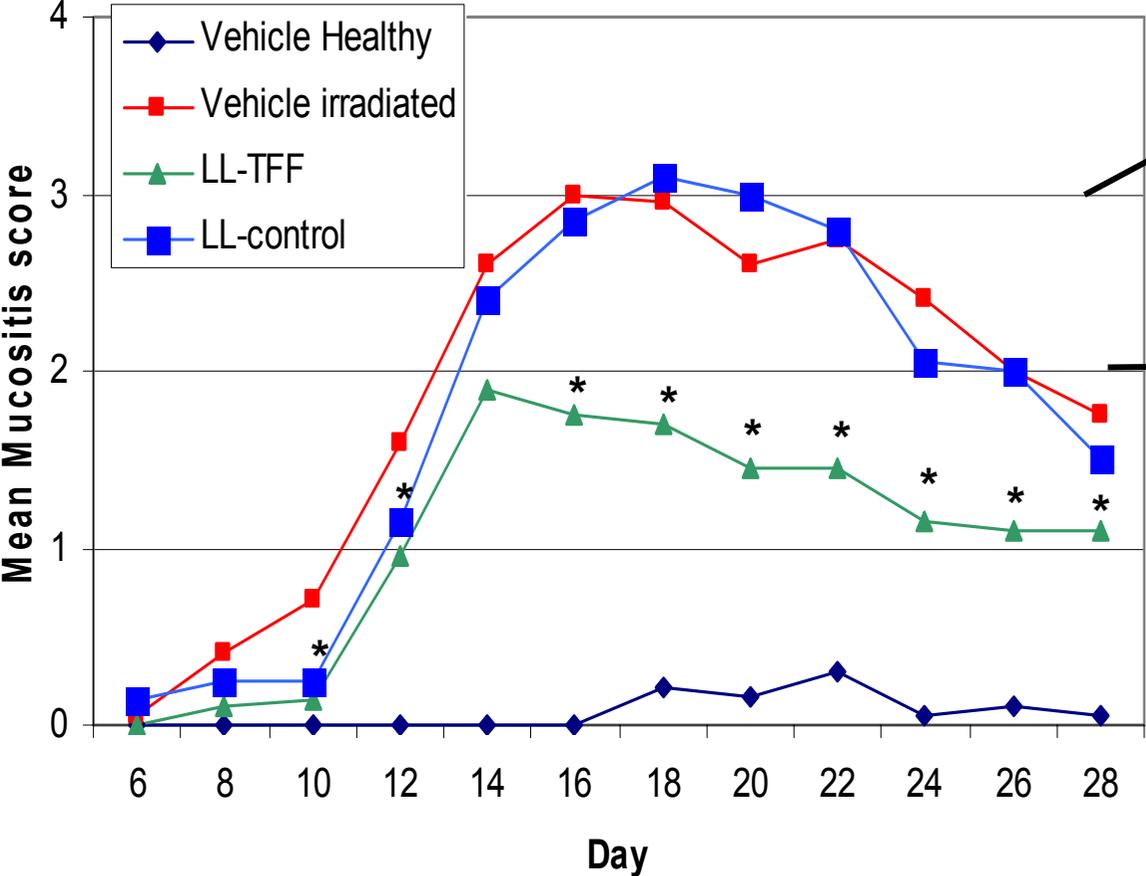
## Hamster mucositis model

One single radiation dose of 40 Gy: max mucositis around day 16



Score	Description	NCI score equivalent
0	Pouch completely normal.	0
1	Light to severe erythema and vasodilation. No erosion of mucosa.	1
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.	1
3	Formation of off-white ulcers in one or more places. Cumulative size of ulcers should equal about 1/4 of the pouch. Severe erythema and vasodilation.	2
4	Cumulative size of ulcers should equal about 1/2 of the pouch. Loss of pliability. Severe erythema and vasodilation.	3
5	Virtually the entire pouch is ulcerated. Loss of pliability, pouch can only partially be extracted from the mouth.	4

# AG013: Preclinical Efficacy Data in Hamster Model of Oral Mucositis



\*: significant improvement



D. Score of 3. Formation of ulcer occupying 1/4 or less of the pouch.



C. Score of 2. Severe erythema and vasodilation with erosion and decreased stippling of superficial mucosa leaving denuded areas.

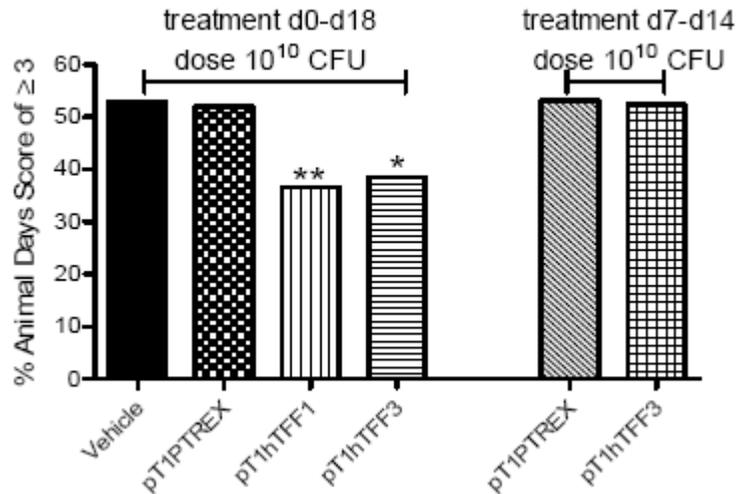


A. Score of 0. The pouch is completely healthy with no erythema or vasodilation.

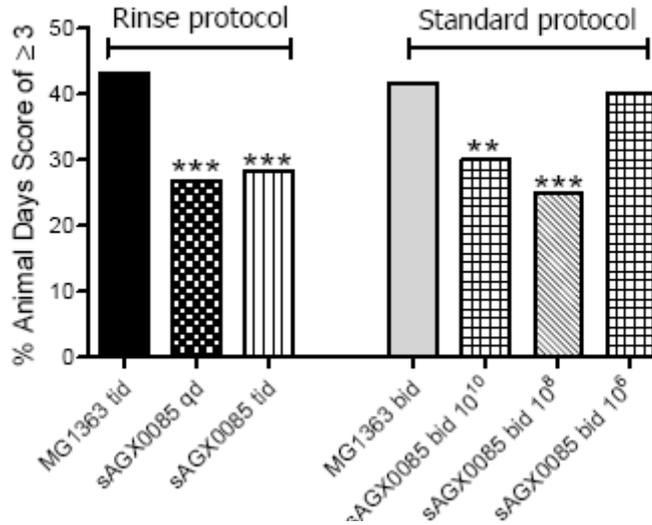
# AG013 Animal Pharmacology

Effect of TFF1/TFF3 on level of clinically significant mucositis, as defined by presentation with open ulcers. Summation of total number of days in which animals from one group exhibit a score of 3 or higher, and expressed as a percentage of the total number of days scored for that group

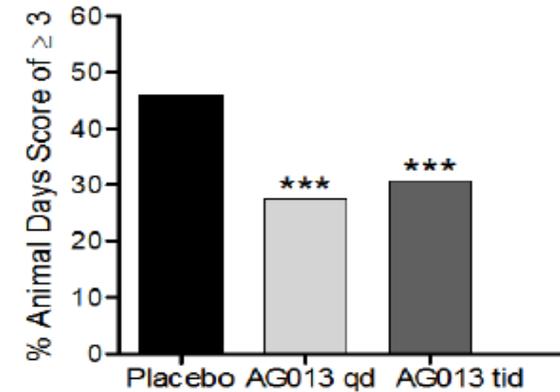
## Plasmid expression of TFF1/TFF3



## Clinical strain (sAGX0085)



## Formulated Drug Product



# AG013

## Pharmacokinetic studies

# Pharmacokinetic studies in hamster

Study (Status) [Reference]	Number of Animals	Strain / Dose (CFU/inoculum)	Live Bacteria (10 <sup>5</sup> CFU / tissue)						Soluble hTFF1 (pg / tissue)					
			0	1H	2H	4H	6H	24H	0	1H	2H	4H	6H	24H
PK Study 1 (completed) [SICAL08005]	18 healthy hamsters (n=3/tp)	5 x 10 <sup>9</sup> CFU/dose sAGX0085 Em+Cm+ cultures	1330 ± 251	425 ± 61	463 ± 117	166 ± 138	401 ± 197	4.5 ± 0.6	48 ± 7	ND	148 ± 113	26 ± 2	59 ± 55	59 ± 13
PK Study 2 (completed) [SICAL08005]	18 healthy hamsters (n=3/tp)	1 x 10 <sup>10</sup> CFU/dose sAGX0085 Em+Cm+ drug product	1560 ± 725	722 ± 260	639 ± 324	610 ± 351	252 ± 181	6.6 ± 4.6	257 ± 46	144 ± 3	154 ± 11	111 ± 16	88 ± 12	35 ± 4
PK Study 3 (ongoing) [SICAL08012]	15 radiated hamsters (n=3/tp)	5 x 10 <sup>9</sup> CFU/dose sAGX0085 Em+Cm+ cultures	13 ± 5	14 ± 10	25 ± 4	18 ± 14	17 ± 9		41 ± 5	66 ± 18	73 ± 21	24 ± 2	46 ± 5	
PK Study 4 (ongoing) [SICAL08012]	15 radiated hamsters (n=3/tp)	1 x 10 <sup>10</sup> CFU/dose sAGX0085 Em+Cm+ drug product	29 ± 15	28 ± 17	5.8 ± 1	5.5 ± 2	7 ± 5		39 ± 18	36	41 ± 17	ND	ND	

Total number of CFU and human TFF1 detected topically in the left cheek pouch of hamsters at different time-points after a single dose of sAGX0085Em+Cm+ cultures and drug product

# AG013

## Toxicology studies

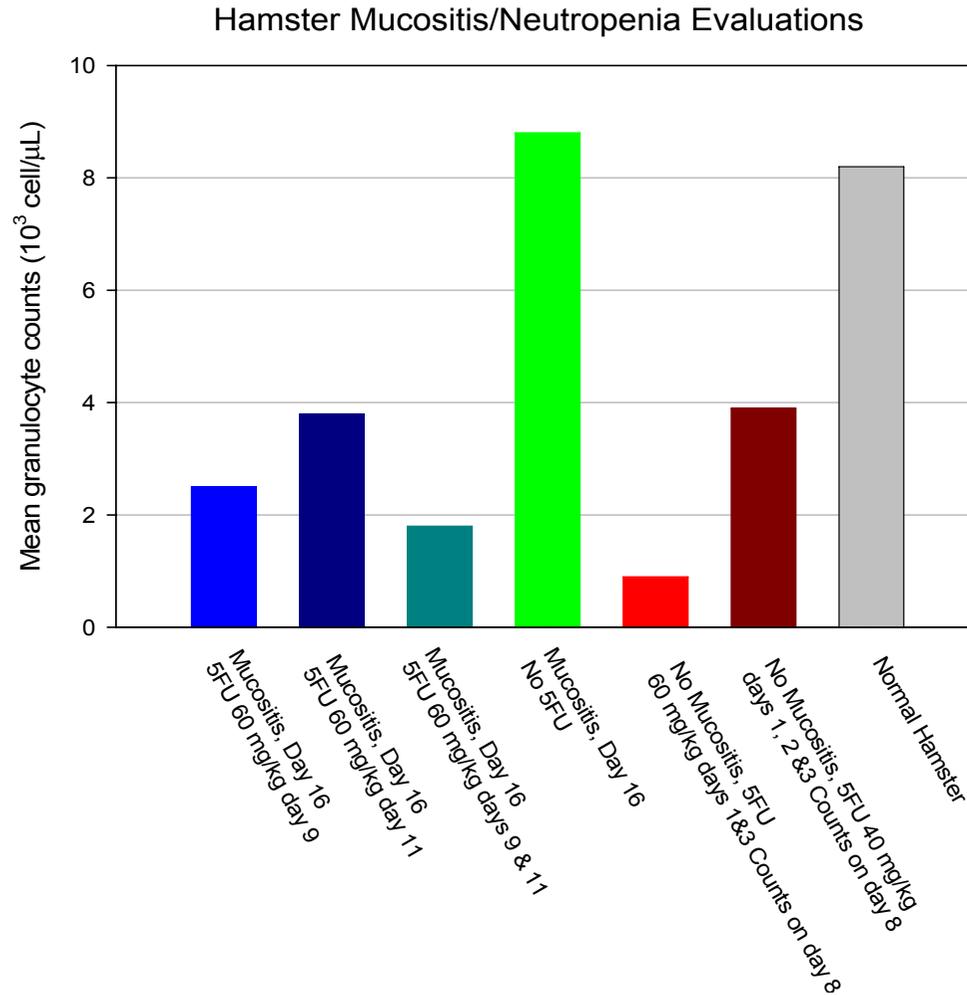
# Rat toxicology study

- Subacute (**14-day**) **oral** (gavage) toxicity study with AG013 in rats
- 4 groups of 10 males and 10 females each  
one vehicle control group and 3 test groups receiving **different doses** ( $1.6 \times 10^{10}$ ,  $1.6 \times 10^{11}$ ,  $1.6 \times 10^{12}$  CFU daily).
- **No treatment-related toxic effects** on clinical signs, body and organ weight, food and water consumption, ophthalmoscopic examination, hematology, clinical chemistry, renal concentration test, urinalysis, faeces analysis and histopathology
- **NOAEL of AG013 set at the highest dose tested:** daily dose of  **$1.6 \times 10^{12}$  CFU/kg BW**
- NOAEL is approximately **90-fold greater** than the **maximum** proposed total daily clinical dose ( **$1.7 \times 10^{10}$  CFU/kg BW**)

# Dog toxicology study

- Subacute (**14-day**) **oral** (gavage) toxicity study with AG013 in beagle dogs
- 4 groups of 4 males and 4 females each  
one vehicle control group and 3 test groups receiving **different doses** ( $8 \times 10^9$ ,  $8 \times 10^{10}$ ,  $8 \times 10^{11}$  CFU daily).
- **No treatment-related toxic effects** on clinical signs, body and organ weight, food consumption, ophthalmoscopic examination, ECG, hematology, clinical chemistry, urinalysis and histopathology
- **NOAEL of AG013 set at the highest dose tested:** daily dose of  **$8 \times 10^{11}$  CFU/ kg BW**
- NOAEL is approximately **45-fold greater** than the **maximum** proposed total daily clinical dose ( **$1.7 \times 10^{10}$  CFU/kg BW**)

# Hamster model for neutropenia



	Type of infection	Age / sex	Co-morbidity	Most likely source of infection	Site of isolation	Year; [Ref]
1	Endocarditis and appendicular abscess.	41 / M	None reported	Not available	Blood cultures	1955; [1]
2	Endocarditis	21 / M	None reported	Intake of large amounts of a specific sour cream, combined with irritated dental gums.	Gum and blood cultures	1955; [11]
3	Endocarditis	11 / M	Congenital stenosis of the pulmonary artery.	Not available	Blood cultures & drained fluids	1974; [1]
4	Endocarditis	65 / F	Transient ischemic attacks, myocardial infarction and mitral valve disease secondary to rheumatic fever.	Not available	Blood cultures	1990; [9]
5	Necrotizing pneumonitis and emphysema	24 / M	Patient was an HIV-infected intravenous drug addict	Loss of consciousness following a documented heroin overdose, may have permitted aspiration of gastric content (the patient had eaten unpasteurized milk and cheese) into the lungs.	Drained fluids	1990; [18]
6	Septic arthritis of the hip joint	57 / F	None reported	Patient came from a farming community and exclusively drank unpasteurized milk.	Drained fluids	1993; [8]
7	Septicemia	69 / M	Chronic lymphocytic leukemia.	Not available	Blood cultures	1995; [1]
8	Endocarditis	56 / M	Chronic glomerulo-nephritis, mitral valve dysfunction.	Not available	Blood cultures	1996; [1]
9	Gastroenteritis Liver abscess	14 / F	None reported	Not available. No history of recurrent infection or ingestion of unpasteurized milk products.		2000; [7]
10	Cerebellar abscess	45 / F	None reported	Not available. Presumably, the abscess developed after a dental procedure.	Drained fluids	2002; [16]
11	Endocarditis	67 / M	None reported	Patient frequently drank unpasteurized milk	Blood cultures	2002; [19]

	Type of infection	Age / sex	Co-morbidity	Most likely source of infection	Site of isolation	Year; [Ref]
12	Peritonitis	67 / M	Nephroangiosclerosis, continuous ambulatory peritoneal dialysis (CAPD)	Contamination via the patient's own hands, after consuming homemade yogurt	Drained fluids	2003; [6]
13	Thrombophlebitis in lower limb. Pulmonary embolism. Fever and edema.	39 / M	Patient was HIV-infected	Not available	Blood cultures	2004; [1]
14	Liver abscess	79 / F	None reported	Not available. The patient had no history of recurrent infection or ingestion of raw milk products.	Blood cultures, drained fluids	2004; [20]
15	Portal vein thrombosis, liver abscess	26 / M	None reported	Not available.	Drained fluids	2005; [14]
16	Deep neck infection and maxillofacial abscess	68 / M	Patient was treated with surgery and radiotherapy for maxillobuccal cancer	The patient regularly drank unpasteurized milk. A mucosal defect at the retromolar trigone likely facilitated infection.	Drained fluids	2005; [15]
17	Upper canaliculitis in the right eye	80 / F	Cataract and pre-proliferative diabetic retinopathy.	Not available. The patient had dental caries under treatment.	Drained fluids	2006; [4]
18	Purulent pleurisy	66 / M	Liver cirrhosis, potential myeloma.	Ingestion of unpasteurized milk, yoghurt or cheese	Drained fluids	2006; [2]
19	Peritonitis	46 / F	Chronic renal failure secondary to diabetic nephropathy, CAPD.	Contamination via the patient's own hands, after consuming homemade yogurt	Drained fluids	2006; [3]
20	Endocarditis	55 / M	Atrial myxoma. Idiopathic arterial hypertension.	Infection was presumed to be related to dental procedures, in the presence of preexisting cardiac lesions.	Blood cultures	2006; [5]
21	Liver abscess	62 / M	None reported	Patient had ingested a bone which ruptured the gut mucosa, allowing bacteria from unpasteurized yoghurt to enter portal circulation, leading to the formation of an abscess.	Drained fluids/pus	2006; [13]

# AG013

## Phase 1b study

# Protocol

A Phase 1b, multi-center, single blinded, placebo-controlled, sequential dose escalation study to assess the safety of topically applied AG013 in subjects receiving induction chemotherapy for the treatment of cancers of the head and neck

Draft protocol September 30, 2008

# Study objectives

- Primary Objective
  - Evaluate safety and tolerability
- Secondary Objective
  - Evaluate the PK profile
- Exploratory Objective
  - Determine any difference in the extent and severity of OM observed among the three total daily dose levels

# Inclusion Criteria

- Signed **Informed Consent Form**
- Males or females **18 years or older**
- **Recently diagnosed** with pathologically-confirmed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx or larynx
- Planned to receive **at least two cycles of induction chemotherapy** of the same regimen consisting of cisplatin/fluorouracil (PF) or cisplatin/fluorouracil/docetaxel (PFT).  
The planned CT **cycles must be of the same length** and must be a **minimum of 14 days** in length
- Karnofsky performance scale  $\geq$  **60%**

# Inclusion Criteria (Cont'd)

- ✘ Screening **laboratory** assessments:
  - Hemoglobin  $\geq$  10g/dl
  - White blood count  $\geq$  3500 cells/mm<sup>3</sup>
  - Absolute neutrophil counts  $\geq$  1500 cells/ mm<sup>3</sup>
  - Direct bilirubin  $\leq$  2x upper limit of normal
  - Serum creatinine  $\leq$  2 mg/dl
  - Serum pregnancy test: negative for females of childbearing potential
  
- ✘ Subjects of childbearing potential: use **effective contraceptive methods** control during study participation and for 30 days following the last treatment with IMP
  
- ✘ Documented **mouth pain during CT Cycle 1** (i.e., OMDQ question 2 score of  $\geq$  2 during CT Cycle 1) – To be assessed at the Baseline Visit

# Exclusion Criteria

- Head and neck tumors of the **lips, sinuses, salivary glands, or unknown primary** tumor
- **Prior radiation** to the head and neck
- **Chemotherapy within 21 days** prior to study start
- Presence of **active infectious disease** excluding oral candidiasis
- **Current** use of **antibiotics** or antibiotic use **within 7 days** prior to start of IMP administration - To be assessed at the Baseline Visit
- Current **dependence on alcohol**; recovered alcoholics may be included
- Presence of **OM (WHO > 0)** - To be assessed at Screening and at the Baseline Visit

# Exclusion Criteria (Cont'd)

- **Chronic immunosuppression**
- Seropositive for **HIV** or **hepatitis B or C**
- **Use of investigational agent** within **30 days** of signing informed consent
- **Pregnant or nursing** female subjects
- Known **sensitivity** to any investigational agent
- Inability to give informed consent or comply with study requirements
- **Unwilling** or **unable** to complete subject diary
- Any other **clinical condition**, psychiatric condition or prior therapy that, in the opinion of the Investigator, would make the subject unsuitable for the study or unable to comply with follow-up visits

# Study design

- Phase Ib
- US - Multicenter
- Single blinded (blinded for the patient)
- Treatment duration **14 days**
- Sequential escalation of exposure
- 3 dose levels

	AG013	Placebo	Dosing frequency	Final concentration	Total daily dose
Group 1	5 subjects	2 subjects	Once daily	$2 \times 10^{11}$ CFU/15 ml rinse	$2 \times 10^{11}$ CFU/day
Group 2	5 subjects	2 subjects	3 times daily	$2 \times 10^{11}$ CFU/15 ml rinse	$6 \times 10^{11}$ CFU/day
Group 3	5 subjects	2 subjects	6 times daily	$2 \times 10^{11}$ CFU/15 ml rinse	$1.2 \times 10^{12}$ CFU/day

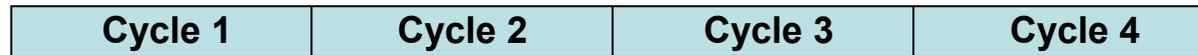
# Study design

<b>Screening Phase</b>	Prior to CT Cycle 1
<b>Enrichment Phase</b>	During CT Cycle 1
<b>Active Phase</b>	During CT Cycle 2 Patient will receive IMP during 14 days
<b>End of Study</b>	14 days after End of Treatment
<b>Long Term Follow-up</b>	3, 6 and 12 months (post IMP use) tumor status assessment

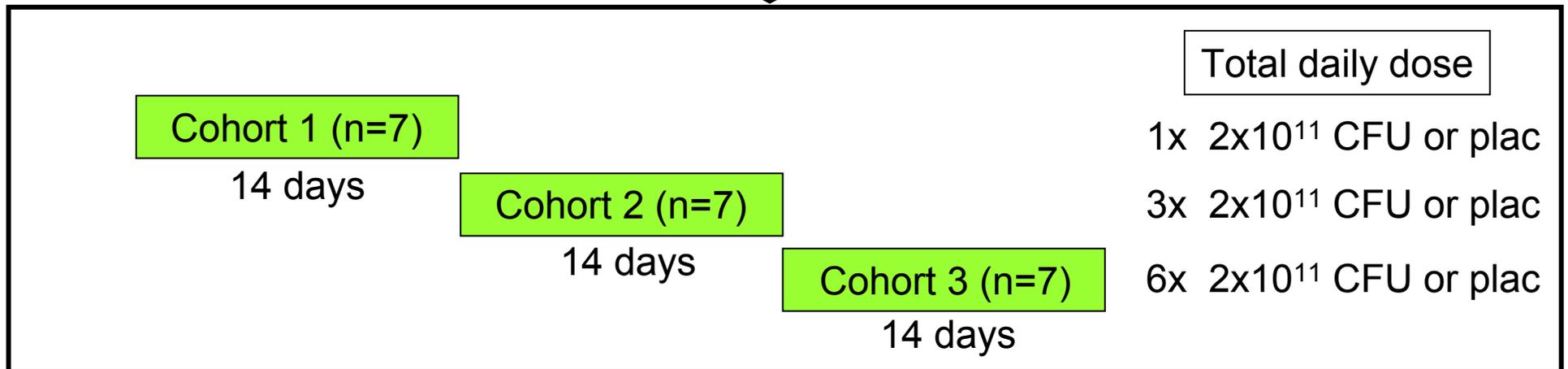
# AG013 – Phase 1b study design

## Dose frequency escalation

Chemotherapy induction phase



Patients with **documented mucositis in cycle 1** will be included in the study during **cycle 2**. For inclusion, mucositis needs to be healed at **start of cycle 2**



# Study procedures

## Screening phase

- **within 30 days** prior to CT Cycle 1 start
- subjects will be **assessed for eligibility**
- subjects must be scheduled to receive **at least two cycles of induction CT** of the same regimen, consisting of **cisplatin (P) and fluorouracil (F) with or without docetaxel (T)**
- all cycles are expected to be of the **same length** and not shorter than **14 days**
- any subjects who do not meet the eligibility criteria following the screening phase will be considered **screen failures**

# Study procedures (Cont'd)

## Enrichment phase

- subjects will complete the **Oral Mucositis Daily Questionnaire (OMDQ)** to determine eligibility
- in order to be eligible in CT Cycle 2: subjects must have **documented mouth pain**, at least once, during CT Cycle 1 as determined by an **OMDQ question 2 score  $\geq 2$**
- if the subject does not have mouth pain during CT Cycle 1, s/he is considered an **enrichment failure**
- if a subject experiences mouth pain, a **Baseline Visit** will occur on the first day of CT Cycle 2
- a subject must have a **World Health Organization (WHO) score equal to 0** and meet **all other eligibility criteria at the Baseline Visit** to be eligible to receive IMP and enter the active phase of the study.

# Study procedures (Cont'd)

## Active phase

- subjects will **rinse** with study medication (1, 3 or 6 times daily) for **14 days**
- **first dose** to be taken after the completion of the Baseline Visit and **prior to the first infusion of CT Cycle 2**
- the first dose must be **witnessed** on 2 consecutive days
- **daily** evaluation for AEs and for the presence and severity of OM:  
presence and severity of OM will be examined by **trained observers** using the **WHO OM toxicity scale**
- **daily visits** for at least 14 days  
If at the end of the 14 days a subject still has ulcerative mucositis (WHO  $\geq 2$ ), **daily oral assessments will continue beyond Day 14** until the lesions resolve, CT Cycle 3 begins or radiation therapy begins if no additional CT cycles are planned
- subjects also will complete a **daily diary** (containing the OMDQ, questions on IMP taste, consistency and tolerability, concomitant medication use, and a record of AEs)

# Study flow chart

Assessments	Screening Phase	Enrichment Phase		Active Phase				Post Active Phase
	Within 30 Days of CT Cycle 1	First Day of CT Cycle 1	Daily During CT Cycle 1	Baseline (Day 1 of CT in Cycle 2)	Daily during CT Cycle 2	Weekly during CT Cycle 2	End of Study <sup>1</sup>	Follow-Up Period (3, 6, 12 months post CT ± 14 days)
Informed Consent	X							
Inclusion/Exclusion Criteria	X							
Physical Exam <sup>2</sup>	X						X	
Vital Signs and Body Weight	X			X		X	X	
Karnofsky Performance Status	X			X			X	
Medical History/HNC History	X							
Current Medications	X							
Record planned CT Parameters <sup>3</sup>	X							
Dental Exam	X							
Clinical Laboratory Measurements <sup>4</sup>	X			X		X <sup>5</sup>	X	
OMDQ <sup>6</sup>		X	X	X	X		X	
OM Assessments <sup>7</sup>	X	X		X	X		X	
Gastrostomy Tube Use/Placement	X				X		X	
Serum Pregnancy Test	X						X	
IMP Assignment				X				
Review Daily Diary		X		X	X		X	
Caries-prevention				X <sup>8</sup>	X			
Adverse Events <sup>9,10</sup>		X			X		X	
Concomitant Medications		X		X	X		X	
Administer IMP				X <sup>11</sup>	X <sup>12</sup>			
Opiate Analgesia					X		X	
IMP accountability					X		X	
PK samples <sup>13</sup>				X		X <sup>14</sup>	X	
Tumor Status Information								X

# Prohibited concomitant medication

- Amifostine (Ethyol®)
- Antibiotic rinses and troches
- Benzydamine hydrochloride
- Cevimeline hydrochloride (Evoxac®)
- Glutamine (as prophylactic agent for OM)
- GM-CSF (e.g., Leukine®)
- IL-11 (Neumega®)
- Povidone-iodine rinses
- 'Magic mouthwash', 'Miracle mouthwash' or other mouthwash containing the following:
  - Chlorhexidine, Hydrogen peroxide, Diphenhydramine

# Prohibited concomitant medication

- Palifermin (Kepivance®) or other keratinocyte or fibroblast growth factor
- Pilocarpine hydrochloride (Salagen®)
- Steroid rinses
- Sucralfate in suspension form (use of sucralfate tablets is not proscribed)
- Other biologic response modifiers – except hematopoietic growth factors for the management of anemia or myelosuppression
- Other investigational agents

# Primary endpoints: safety

- Incidence of adverse events defined by the NCI CTCAE Version 3.0 criteria
  - Severity of AEs will be designated as mild, moderate, severe, life-threatening or fatal per NCI CTCAE version 3.0.
- Laboratory abnormalities
  - WBC (differential in %), Hb, Ht, platelets, RBC, sodium, glucose, potassium, BUN, creatinine, phosphorus, calcium, alkaline phosphatase, LDH, AST, ALT and albumin
    - ➔ screening, baseline visit (day 1 CT cycle 2), day 14 of CT cycle 2 and end of study visit

# Primary endpoints: safety

- Presence of sAGX0085 in whole blood:
  - Culture at baseline visit, once weekly during treatment period and at end of study
- Vital signs, including temperature, systolic and diastolic blood pressures, heart rate and respiration rate:
  - at baseline visit and once weekly during the treatment period

# Secondary endpoints

- Measurable serum levels of hTFF1
- Measurable oral mucosal and saliva levels of AG013 (both sAGX0085 bacteria and hTFF1)
- Levels of sAGX0085 bacteria in whole blood
  - baseline visit, once weekly during treatment period and at end of study
  - 90' after mouth rinse
  - saliva production will be stimulated with paraffin pellet

# Exploratory endpoints

- Incidence and duration of **severe mucositis** (WHO Grade > 2) within CT Cycle 2
- Incidence and duration of **ulcerative mucositis** (WHO Grade >1) within CT Cycle 2
- Percent of subjects with **OMDQ Question 2 scores  $\geq 2$**
- Incidence and duration of **opioid use**
- Insertion of or need for use of **gastrostomy tube feedings**
- **Unplanned** office or **emergency** room visits

# Patient reported outcomes

- Subjects will personally complete the Oral Mucositis Daily Questionnaire (**OMDQ**).
- The OMDQ will be contained in a subject diary and will be completed **daily** during CT Cycle 1 and daily during CT Cycle 2 (to at least Day 14).
- If a subject continues to have oral assessments beyond Day 14 of CT Cycle 2 the OMDQ should be completed each day the subject has an oral assessment.
- Subjects will also complete the OMDQ **at the end of study** visit.
- As any manipulation of the subject's mouth may influence PRO responses, the OMDQ must be completed **prior to the oral evaluations**.