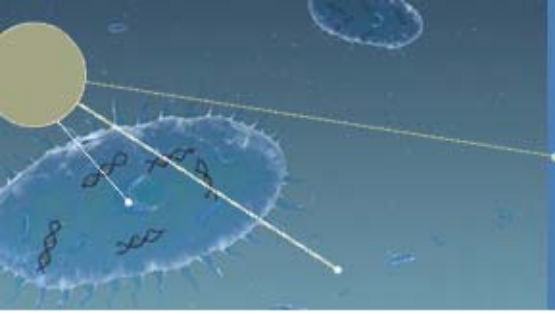
Phase I Protocol: CEQ508 in FAP
CEQ508.FAP.01

PI: Gideon Steinbach, MD, PhD, Seattle, WA

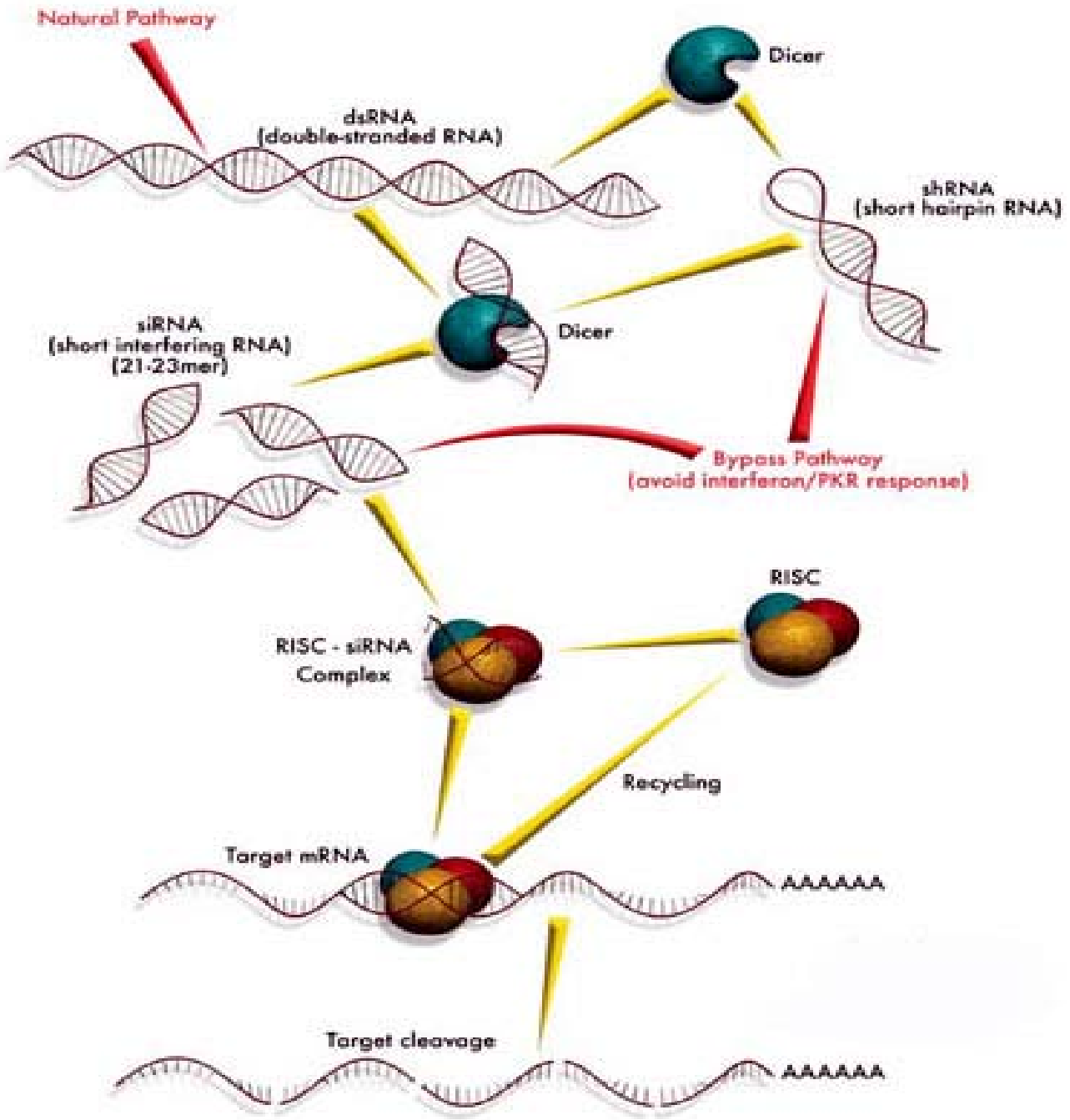
Patrice Courvalin, MD, Institut Pasteur, Paris
Johannes Fruehauf MD, Cequent Pharmaceuticals
Alison Silva, Cequent Pharmaceuticals



Background

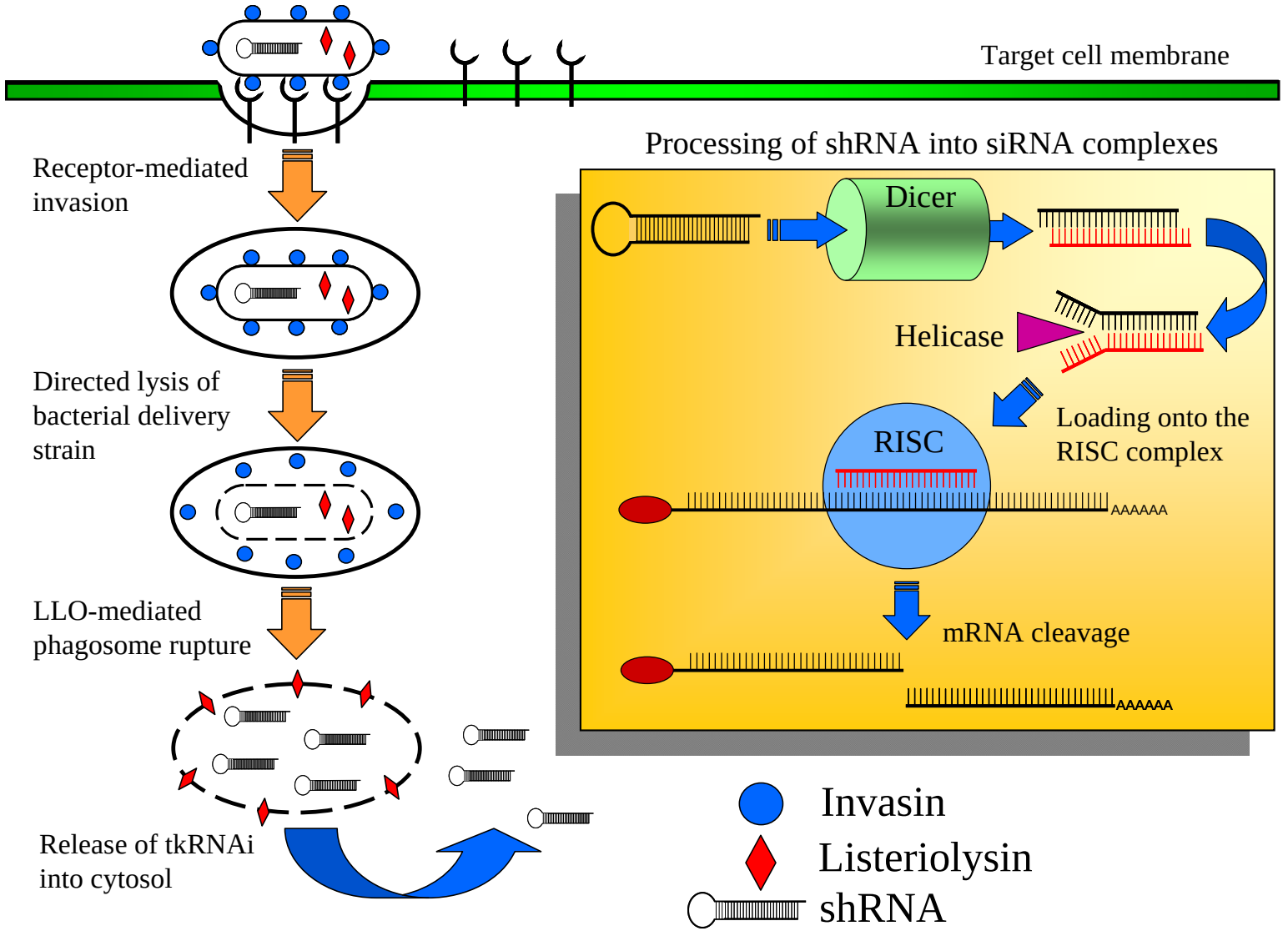
Introduction to CEQ508 and transkingdom RNAi

- CEQ508 is a live attenuated *E.coli* expressing and delivering shRNA to silence β-catenin expression
- The underlying technology is termed “transkingdom RNA Interference” (*tk*RNAi)
- *tk*RNAi uses nonpathogenic bacteria to deliver RNA interference to mucosal tissues
- *tk*RNAi bacteria are administered orally to treat epithelial cells in the gastrointestinal mucosa



RNA Interference (RNAi)

Schematic of *transkingdom* RNAi System

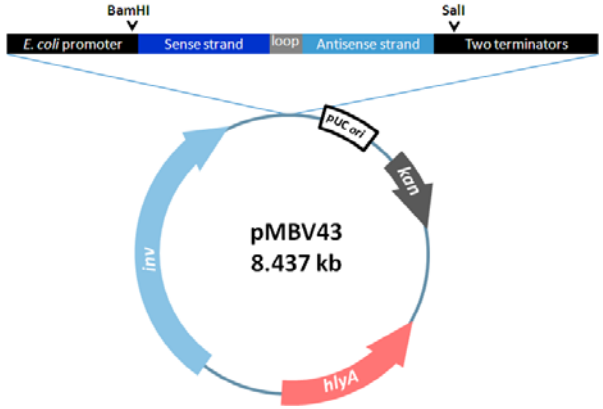


Components of CEQ508

E. coli K-12 MM294 — Harvard, 1969

- Deletion of *dapA* and *rnc*
- Expression of invasin, listerilysin O, shRNA and Kanamycin resistance.

E. coli K-12 CEQ508 — Cequent, 2008



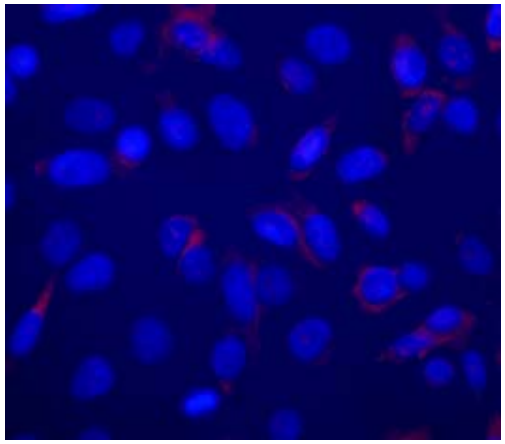
- **Deletion of *dapA* (diaminopimelic acid [Dap] auxotrophy) and *rnc* (RNase III) genes.**
  - Dap, required for crosslinking of peptidoglycan. mutants lyse in the absence of Dap.
  - Δ*rnc* mutation causes slow growth, defects in gene expression, defects in growth at high or low temperatures and stationery phase, further attenuating CEQ508

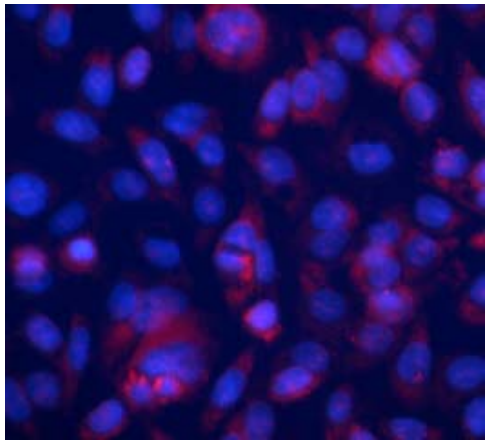
- **Plasmid pMBV43 causes expression of invasin, listeriolysin O, shRNA and Kanamycin resistance.**
  - High level of invasin and shRNA expression causes slow growth

*tk*RNAi Delivers shRNA into the Cytoplasm of Eukaryotic Cells

Fluorescence in situ hybridization (ISH) – SW480 cells

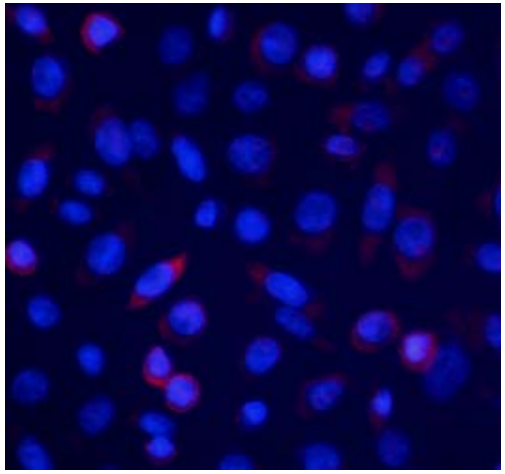
untreated

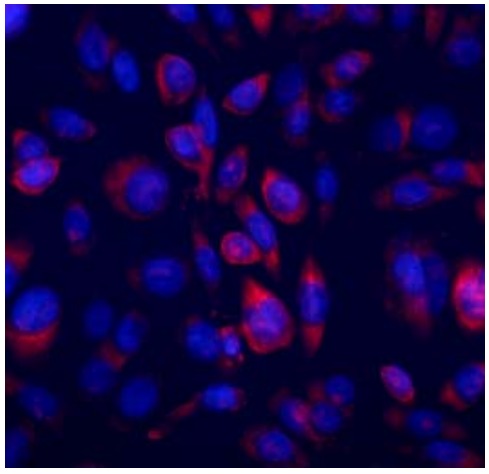




transfected w
20 nM siRNA
Against CTNNB1

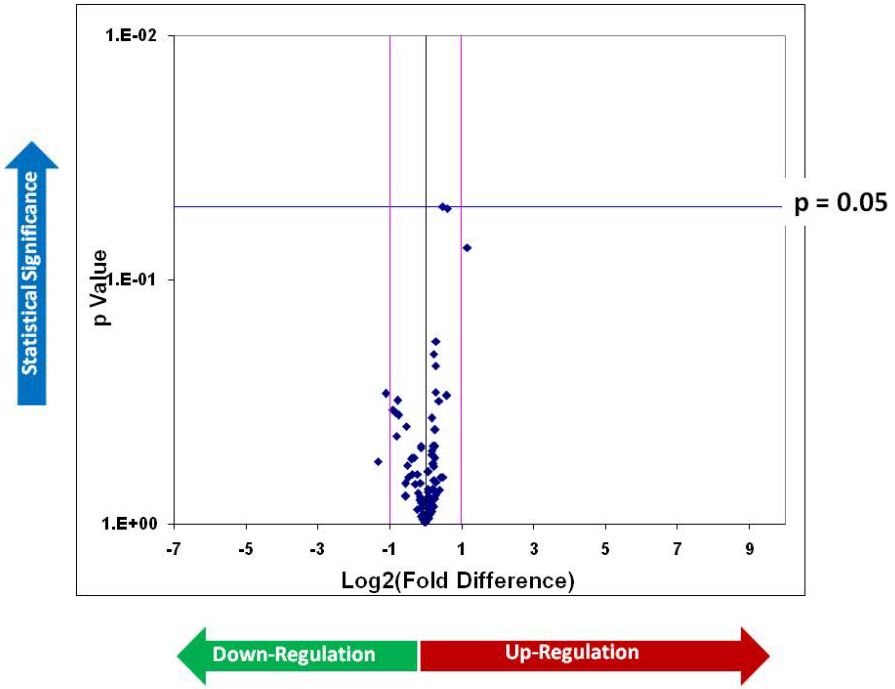
treated w
E. coli only
(MOI 500)





treated w
E. coli expressing shRNA
against CTNNB1

Cytokine/Chemokine "footprint" of *tk*RNAi *in vivo*



Results:

1.Oral treatment with CEQ601 for 11 days (10^9 cfu/d) induced only minor changes of cytokine gene expression in colon samples of mice compared to mice treated with PBS alone

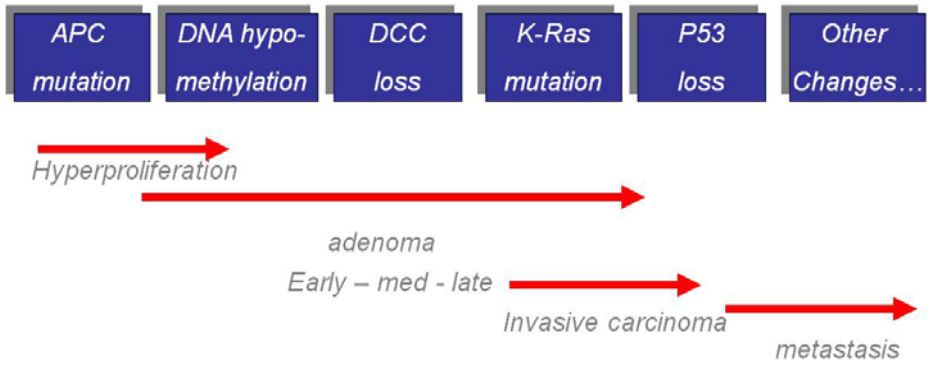
2.No gene was found to be significantly up- or downregulated by more than 2-fold.

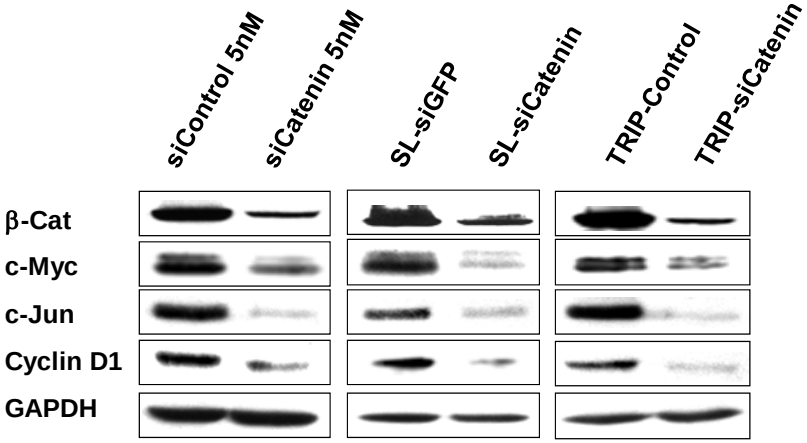
3.Cells in the gastrointestinal tract are constantly exposed to bacterial particles leading to desensitization

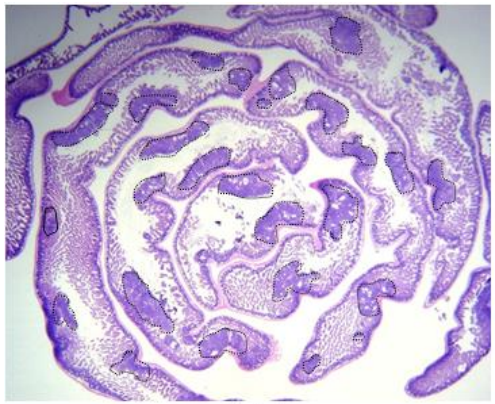
Conclusions:

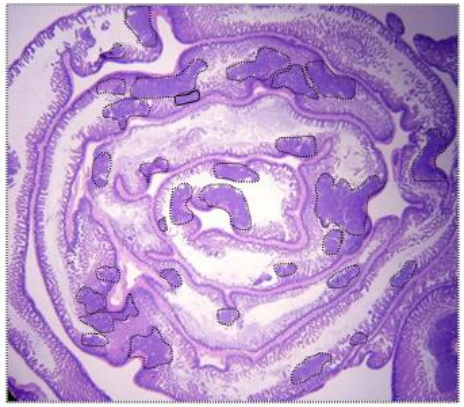
Treatment with *tk*RNAi *in vivo* does not lead to significant non-specific effects on gene expression of cytokines/chemokines

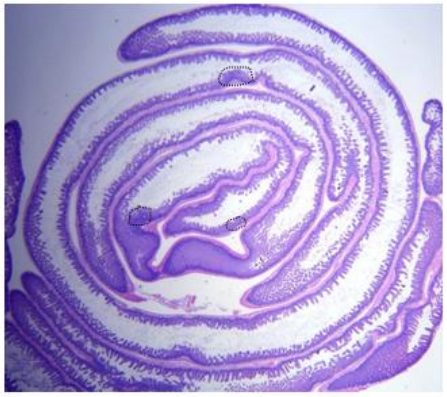
Pharmacology:
Inhibition of β-catenin Prevents Polyps in APC^{min} Mice





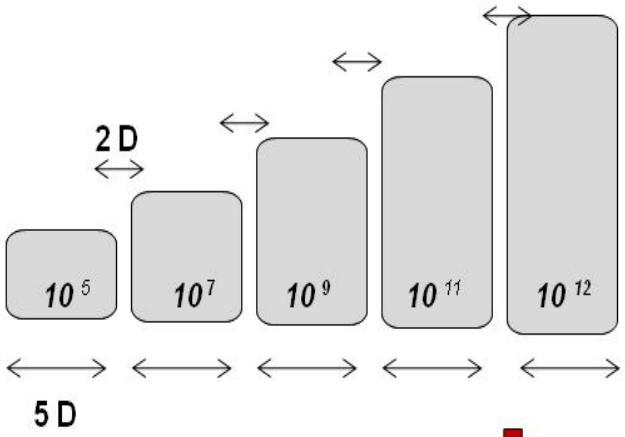

Control


SL-siGFP


SL-siCATENIN

*tk*RNAi NHP Study: Safety and Efficacy

CEQ501.01:
2 animals



CEQ501.02:
22 animals
(6/group; 4 controls)

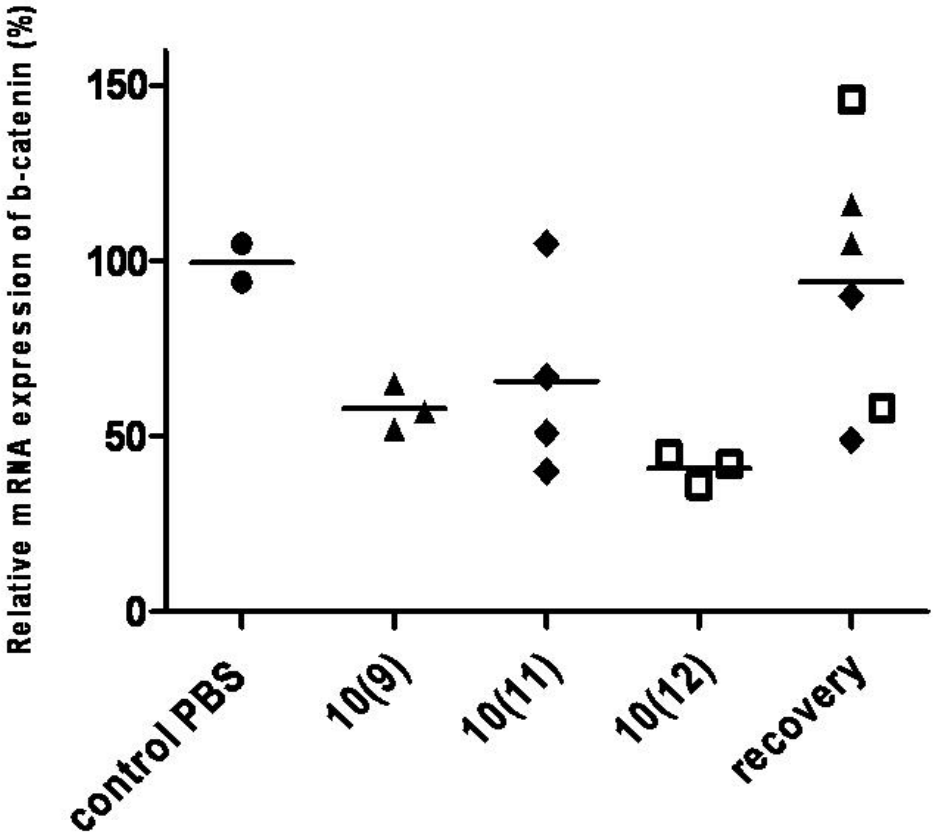
D29 D50

- Low Dose (10^9) Terminal Necropsy (n=4)
- 10^9 Recovery Necropsy (n=2)
- Mid Dose (10^{11}) Terminal Necropsy (n=4)
- 10^{11} Recovery Necropsy (n=2)
- High Dose (10^{12}) Terminal Necropsy (n=4)
- 10^{12} Recovery Necropsy (n=2)

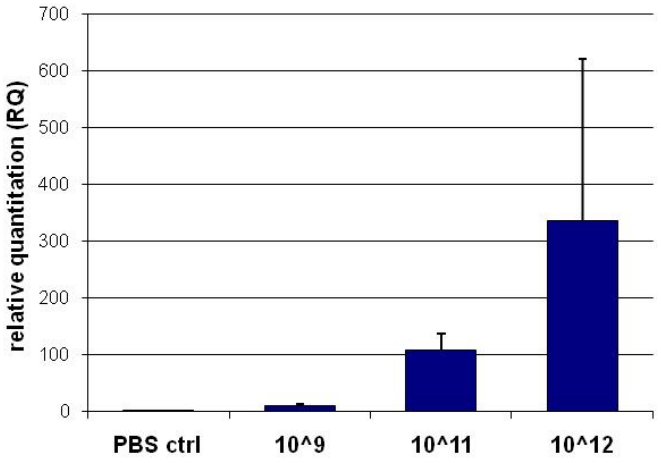
- ✓ Food consumption
- ✓ Body weights
- ✓ Electrocardiography
- ✓ Blood pressure
- ✓ Rectal temperatures
- ✓ Ophthalmology
- ✓ Urinalysis
- ✓ Hematology
- ✓ Coagulation
- ✓ Serum chemistry

→ *All Normal*

*tk*RNAi NHP Study: Efficacy on β-catenin

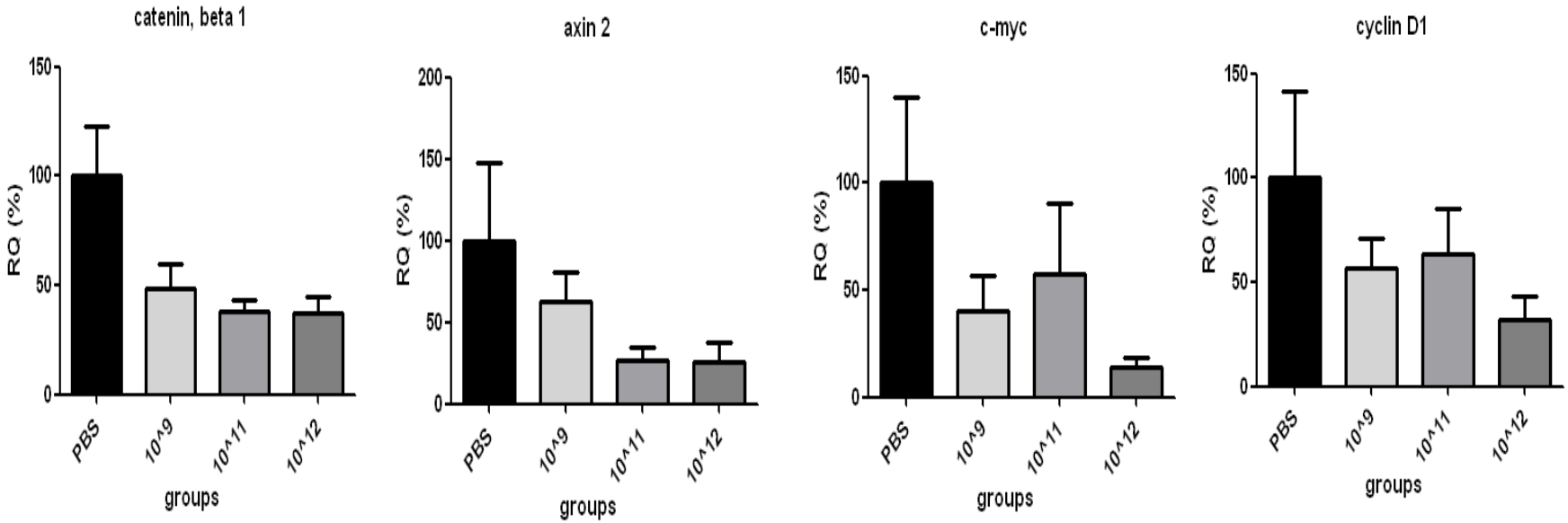


Changes of β-catenin levels in GI mucosa



Recovery of shRNA from GI mucosa

*tk*RNAi NHP Study: Effects of β-catenin Silencing on Downstream Targets



***tk*RNAi down-regulates downstream β–catenin target genes associated with colon cancer: c-myc, c-jun and Cyclin D1**

CEQ508 Toxicology Program:
Preclinical Work

1. **GLP Toxicology study (CEQ508.01):** All in life and necropsy findings appear normal with no therapeutic article associated toxicology observed.

2. **Pharmacokinetic Study:** Transcribed hairpin can be detected in the ileum after administration of CEQ508.

3. **Biodistribution Study:** CEQ508 given orally (single or multiple treatments) via gavage does not transit out of the GI tract; contrasting tail vein injection RoA found bacteria in all organs with clearance at approximately 4 days post dose.

4. **Clearance Study:** Complete clearance is observed approximately 60 hours post dose. Maximum clearance observed at approximately 20 hours post dose

5. **Cytokine Study:** CEQ508 did not induce an up regulation of the 6 cytokines analyzed from serum samples.

6. **GLP Toxicology study (NHP-CEQ501.02):** Continuous oral dosing of CEQ501 is safe up to 10^{12} cfu/day. CEQ501 significantly suppressed target gene expression in the gastrointestinal tract. mRNA silencing of 40-60% in non disease model primates is equivalent to phenotypically active knockdown observed in the APC^{min} mouse model .

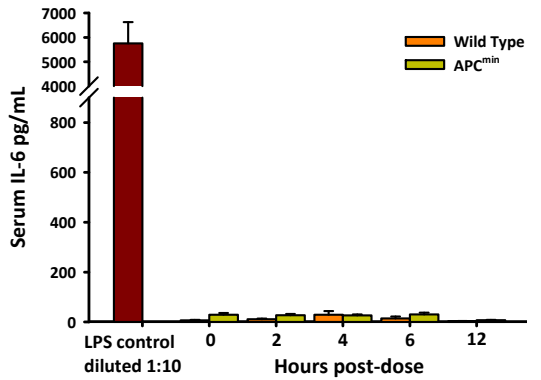
Cequent pharmaceuticals

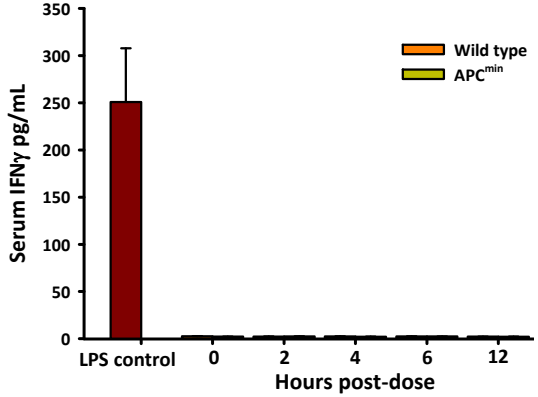
CEQ508 Cytokine Profile:
2nd Study Design

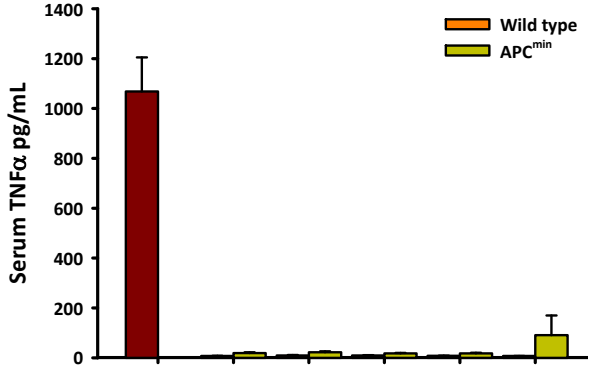
Treatment Group	Daily Dose (cfu)	No. of Treatments	Analysis Point (hrs post last treatment)	No. of Animals (wt+APC^{min})
No treatment	-	0	0	5+9
CEQ508	$5x10^9$	1	2 hrs	6+8
CEQ508	$5x10^9$	1	4 hrs	7+10
CEQ508	$5x10^9$	1	6 hrs	6+10
CEQ508	$5x10^9$	1	12 hrs	5+9

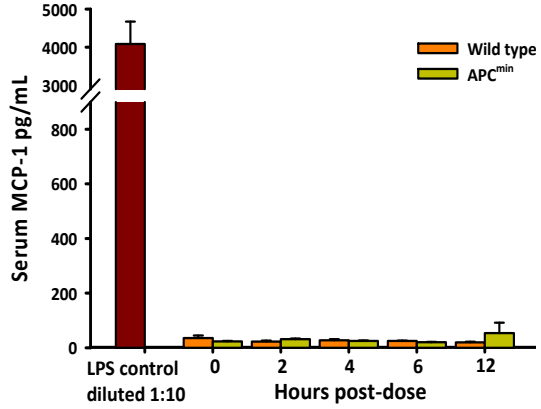
- **Serum cytokines analyzed using FACS-based cytometric bead array (CBA)**
- **LPS injected mice (400ug) were used as a positive control**

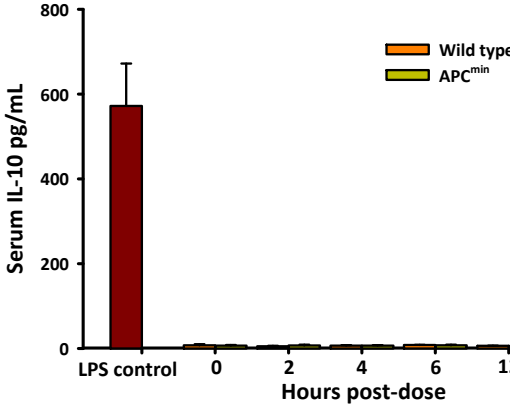
CEQ508 Cytokine Profile:
Results (2nd Study)

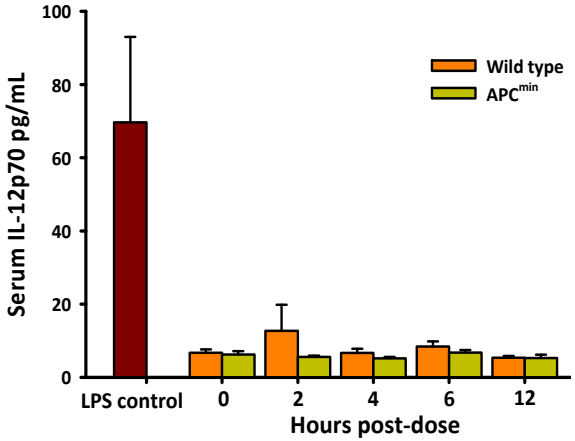




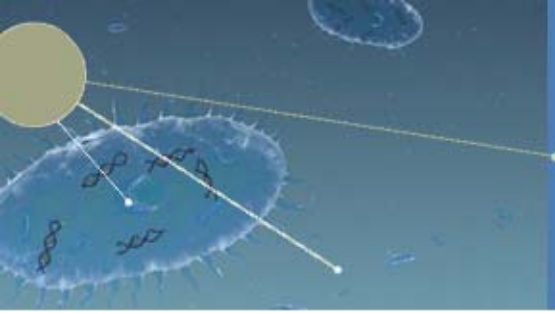






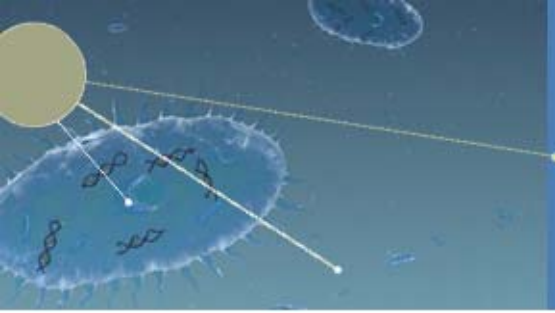


Oral dosing with CEQ508 does not elicit a significant increase in circulating pro-inflammatory cytokine levels in either healthy or APC^{min} polyposis animals. Similar results were found in the NHP toxicology study.



Microbiology/Genetic Safety Considerations

Patrice Courvalin MD, Institut Pasteur



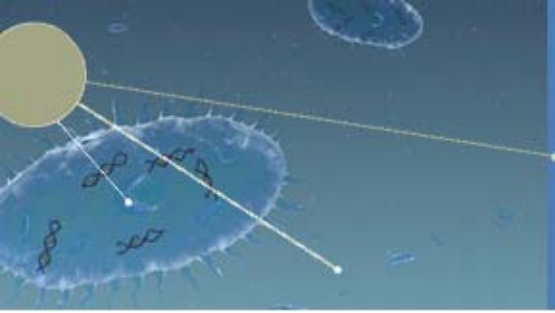
Horizontal Gene Transfer Outline

- Bacterial host (CEQ221)
- Plasmid (pMBV43)

- Transfer : donor or recipient
  plasmid
  chromosome

- Heterologous gene expression

- Stabilization of incoming DNA



Horizontal Gene Transfer
E.coli CEQ508

***E.coli* CEQ221(derivative of MM294)**

- *ΔdapA*
- *Δrnc*
- *thiA*
- no mobile genetic element
- does not colonize the human GI tract

Horizontal Gene Transfer
pMBV43

Transfer:

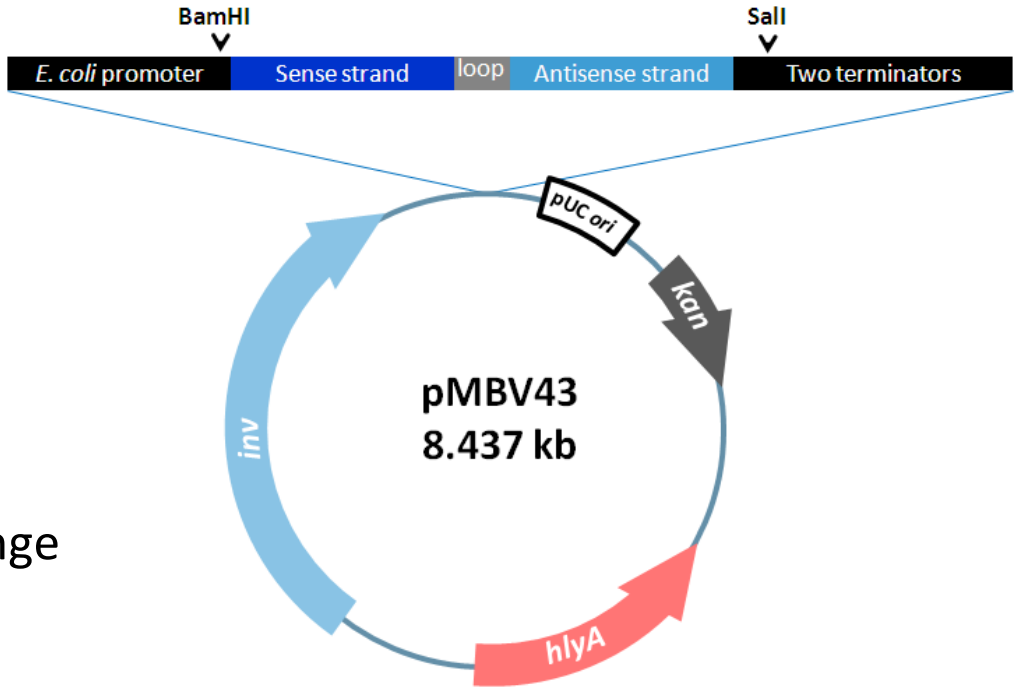
1. Conjugation
   - not conjugative
   - not mobilizable
2. Transformation
   - plasmid DNA release
   - naturally competent species
3. Transduction
   - phages with narrow host range
   - headful mechanism

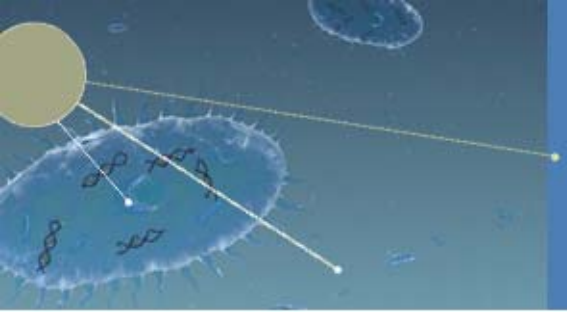
Heterologous expression :

- *inv*
- *hlyA*
- *kan*

Stabilization:

- Narrow host range ColE1 *ori*





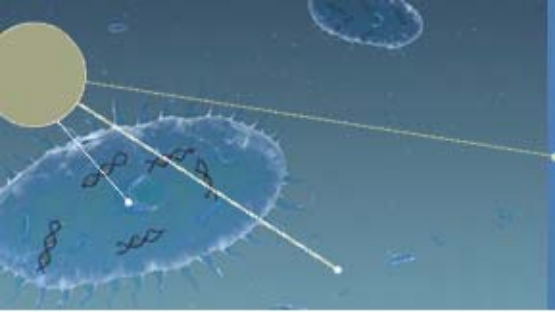
Horizontal Gene Transfer
Chromosome

CEQ508 as a donor:

- no gene of interest
- no selective advantage
- stabilization by recombination

CEQ508 as a recipient:

- *hsdr17*$^{-}$
- *recA*$^{+}$
- stabilization by recombination
- low fitness
- multiple independent events (except for plasmid)



CEQ508 Program:

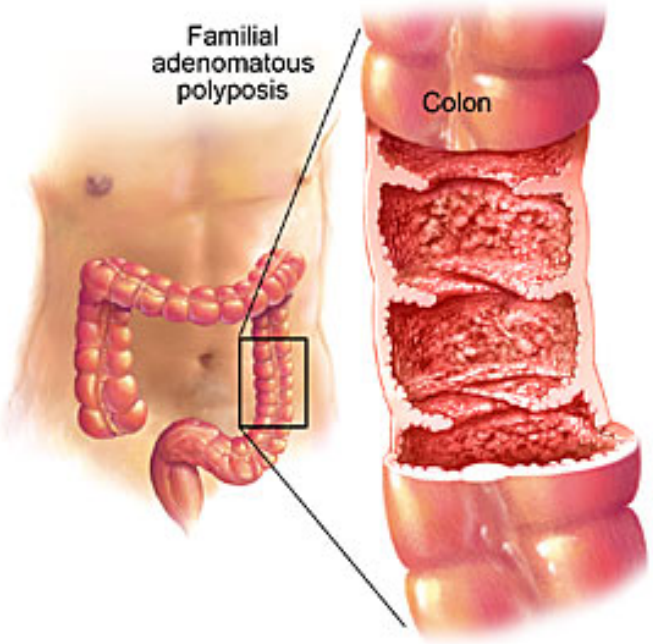
Clinical Review

PI: Gideon Steinbach MD, PhD

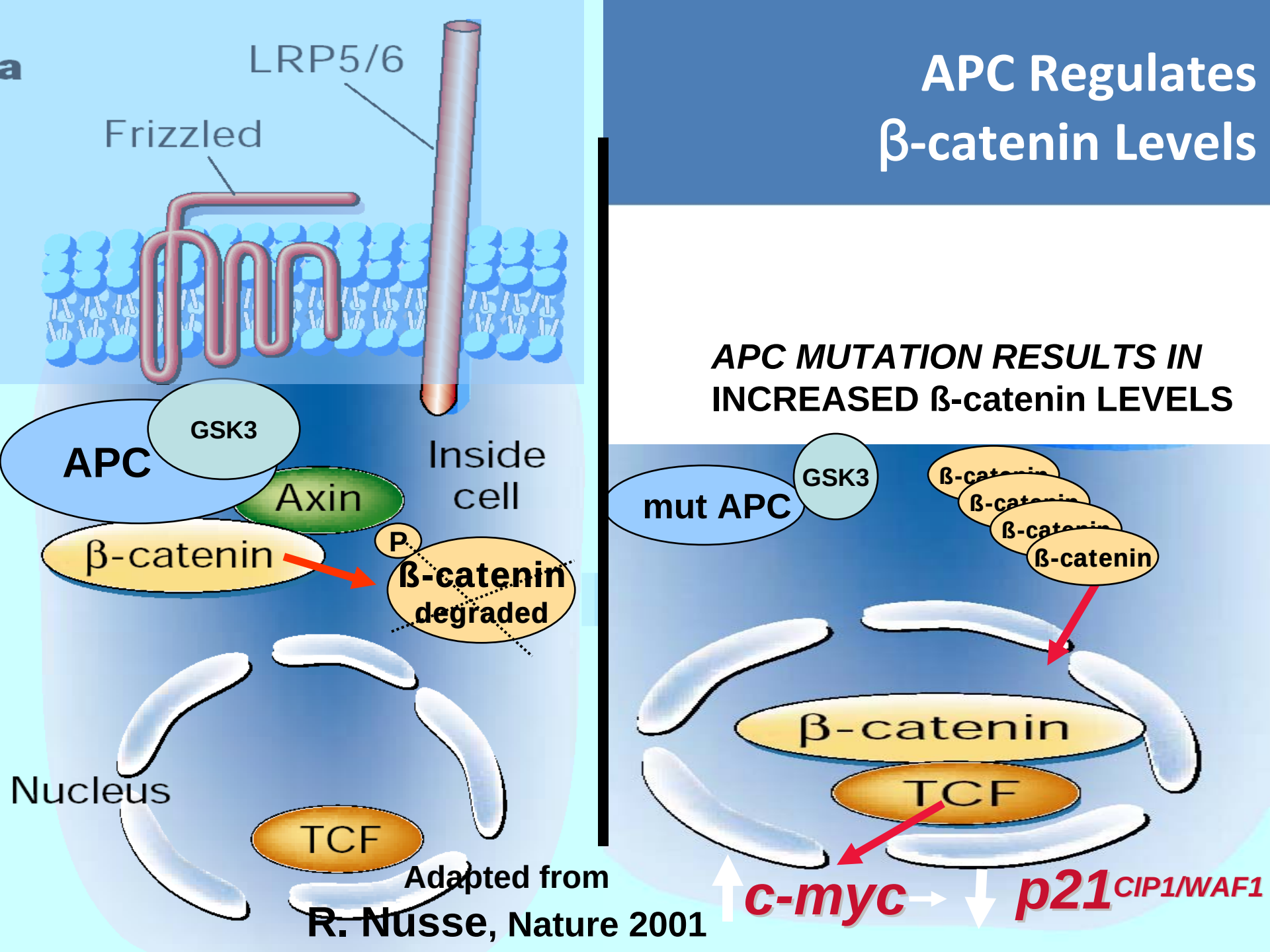
Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) - Inherited form of colon cancer

- Rare hereditary disease – 30,000 Pts US; 46,000 EU
  - Mutation in adenomatous polyposis coli (APC) gene
  - Dysregulation & accumulation of β-catenin
- FAP results in the formation of multiple colon polyps



APC Regulates β-catenin Levels



Adapted from
R. Nusse, Nature 2001

Familial Adenomatous Polyposis: Classical

CLASSICAL FAMILIAL ADENOMATOUS POLYPOSIS (FAP)

- Hundreds-thousands of adenomas
- Colorectal Cancer by age 40-50
  (Onset >age 20)

Treatment:

- Proctocolectomy by age 19
- Colectomy considered in selected patients
- Risk of Rectal Cancer in patients with retained rectum
  - yearly surveillance proctoscopy & polypectomy is required
- Risk of Ileal Pouch Cancer
  - after proctocolectomy (IPAA)
- Adjunct medical treatment
  - does not replace surgery and polypectomy

Familial Adenomatous Polyposis: Attenuated

ATTENUATED FAMILIAL ADENOMATOUS POLYPOSIS (AFAP)

- < 100 COLON ADENOMAS (range 0 - >1000)
- Later onset of adenomas and CRC
  - CRC mean age > 50 yr, uncommon < 30 yr
- Right side predominance, usual rectal sparing
- Phenotype variable
- Lifetime Risk of CRC > 60%; not predictable based on polyp #

Treatment

- Based on polyp number and size
- Polypectomy: if few recurrent polyps
  - If all polyps can be fully/safely excised
- Colectomy: rectal sparing with ileo-rectal anastomosis
  - If many recurrent polyps
- Colectomy considered in all patients with adenomas at age 40-50

Genetics

- *APC* gene mutations: 5' to codon 168, (exons 1 -4); 3' to codon 1580, alternatively spliced region of exon 9

Familial Adenomatous Polyposis: Duodenal Cancer

DUODENAL CANCER

- Duodenal adenomas in > 90 %
- Median age of onset < 38 yr
- Duodenal Cancer Risk: 5 –10%
- Mean age > 55 yr, rare under age 30

Surveillance

- Duodenoscopy at 25 - 30 yr
- Intervals: every 1 – 5 years
    - dependent on stage and age
    - every 6 months in advanced disease

Phase I Outline

Phase I Study Site: Seattle Cancer Care Alliance

- Clinical Oncology and Hematopoietic Stem Cell Transplant facility for the Fred Hutchinson Cancer Research Center and the University of Washington Hospitals.

- Site of the Cancer Prevention Program and Cancer High Risk Clinics for the University of Washington Hospitals.

Phase I Outline

Inclusion Criteria

- 18-65 years of age
- Male and female
- Clinical or genetic diagnosis of FAP
- Pre and post colectomy; no immediate need for colectomy
- Known endoscopic history of polyposis
- Eligible to undergo baseline and endpoint endoscopies
- Ability to be taken off other chronic FAP medication (Sulindac, Aspirin, etc.)
- Informed Consent

Phase I Outline

Exclusion Criteria

- Inability to return for scheduled treatment and assessments
- Evidence of chronic or intercurrent acute medical disorder
- Significant clinical laboratory and hematology observations
- Pregnancy, Nursing
  - Pregnant and/or nursing women and those attempting to become pregnant
- Antibiotic Use
  - Current or anticipated antibiotic treatment during the study period
  - Antibiotic use within the past 2 weeks
- Significant Active ulcerations or inflammation found at baseline endoscopy

Phase I Outline

Primary Endpoint: General safety of CEQ508

- Clinical observations
- Laboratory evaluation
- Histological examination of biopsy samples

Secondary Endpoints:

- Biomarker changes in biopsy samples
  - Gene expression for β-catenin and downstream genes
- Clearance and shedding in stool samples

Phase I Outline

Screening: Days -30 to -7

- Inclusion assessment
- Lab tests
  - Stool sampling, pregnancy test, cytokine analysis, chemistry, hematology, coagulation
- Informed consent
- Medical history
  - Symptoms questionnaire

Phase I Outline

Active Study Phase: Baseline to Follow-up Examination

- Physical examination and baseline endoscopy w/ biopsies on day 1
- Start daily dosing of CEQ508 (d1-d28)
- Lab tests and stool sampling on days 1, 8, 15, 22, and endpoint (d 26-28)
- Physical examination and endpoint endoscopy w/ biopsies on d 26-28
- Follow-up physical exam to include lab test and stool sampling on day 57

Phase I Outline

Patient Dosing & Monitoring

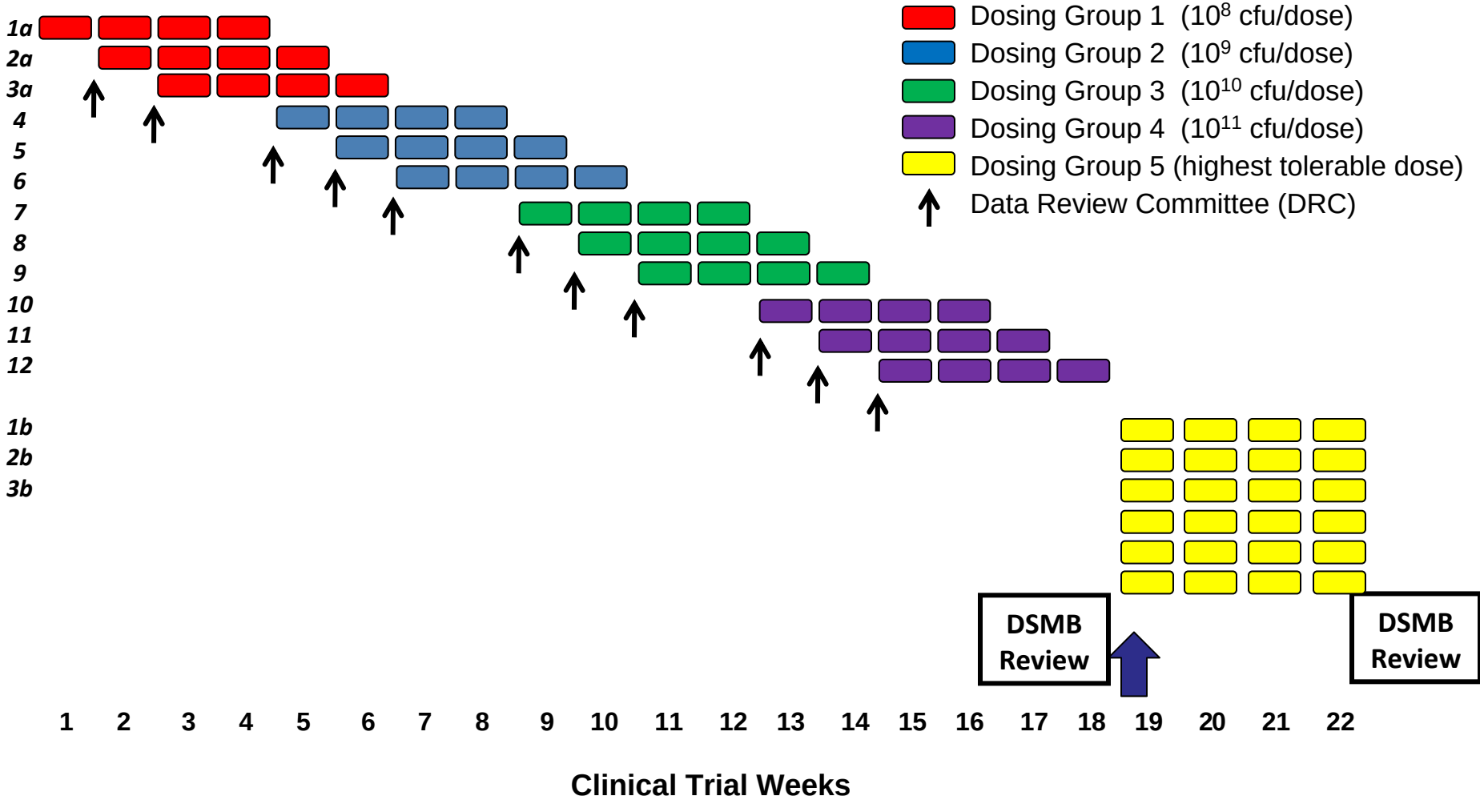
- Daily, at home nursing visit
- Dose administration assistance by nurse
- At home lab draws (days previously specified)
- At home stool sampling (days previously specified)
- Symptoms questionnaire
- Ongoing communication with PI

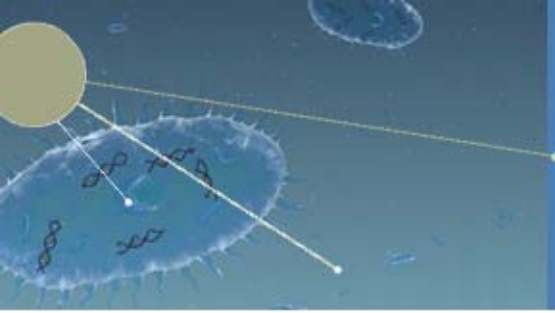
Phase I Outline

Data Safety Monitoring Board

- Protocol Review Process at the SSCA & FHCRC
- IBC
- Scientific Review Committee
- IRB

Dose Escalation Phase & Stable Dose Phase Schedule





Phase I Clinical Protocol Synopsis: CEQ508.FAP.01

Protocol Title:	**A PHASE 1 OPEN-LABEL, ESCALATING-DOSE STUDY, OF THE SAFETY AND TOLERABILITY OF SINGLE DAILY DOSES OF CEQ508, AN RNAi-BASED THERAPY FOR FAMILIAL ADENOMATOUS POLYPOSIS**
Sponsor:	Cequent Pharmaceuticals, Inc
Study Site:	Seattle Cancer Care Alliance, Seattle, WA
Principal Investigator	Gideon Steinbach, MD, PhD
Projected start date:	January 2010
Study Phase:	Phase I
Study Medication:	CEQ508, a *tk*RNAi drug candidate (live *E.coli* carrying RNAi to silence β-catenin)
Objectives:	Primary: The primary study objective is to evaluate establish general safety for orally administered CEQ508 in a daily dosing schedule and to determine the Maximum Tolerated dose (MTD) (of 3 planned does) and/or the Highest Safe Dose. Secondary: •To examine shedding of CEQ508 in the stool of patients during and after daily oral dosing with CEQ508 for 28d, and the four-week recovery period •To examine gene expression changes after oral dosing of CEQ508 in GI mucosa of FAP patients
Patient Population:	• Patients [from the FAP registry at Seattle Cancer Care Alliance] with known FAP and attenuated FAP (AFAP)
Sample size:	• Maximum of 18 patients, • Dose Escalation Phase: 12 patients, at least 4 patients from each sex • Stable Dose Phase: 6 patients, who may be newly enrolled or re-enrolled from the 12 patients who were part of the Dose Escalation Phase (certain criteria apply for re-enrolment, see 3.1.1)