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Probiotics for Liver Disease

Hani El-NEZAMI
Hong Kong

Organised by: ISAPP INTERNATIONAL SCIENTIFIC ASSOCIATION OF PROBIOTICS AND PREBIOTICS™

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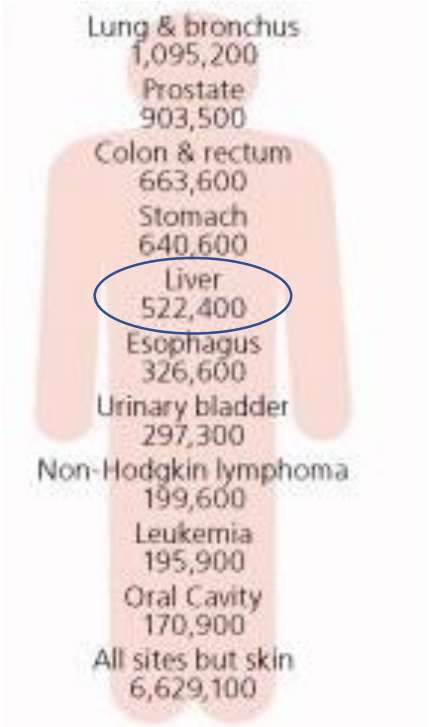
Probiotics and Liver Diseases

Hani El-Nezami, PhD
Professor Gut Health and Food Safety

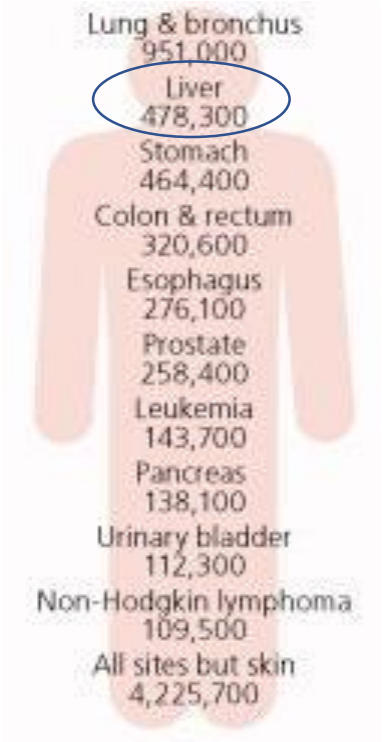
School of Medicine, University of Eastern Finland
&
School of Biological Sciences, The University of Hong Kong

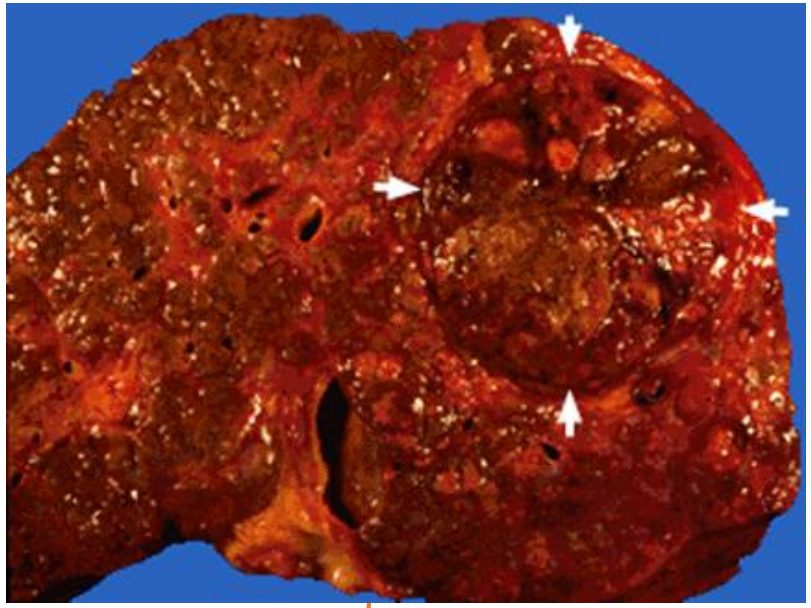
Hepatocellular carcinoma (HCC)

Estimated new cases



Estimated new death





(El–Serag and Rudolph 2007)

- Current Treatment:
  - Hepatic resection and liver transplantation
    - limited by the underlying cirrhosis and poor hepatic reserve commonly associated with patients with HCC
  - Local ablation treatment (PEI, RFA)
    - only to patients who meet stringent specific criteria with tumors localized to the liver.
  - Chemotherapy
    - response rates are low due to the highly chemotherapy-resistant nature of the disease
- HCC is clearly a disease for which alternative therapeutic modalities must be developed.

- The number of HCC related deaths almost equals the no. of cases being diagnosed each year

- The 5-year survival rate is below 9%.

Chemotherapy drug

Probiotics

Standard treatment involve high cost. A cheaper way to prevent/treat this disease is thus warranted

1. Can probiotics modulate risk factors of liver cancer?

2. Can probiotics modulate liver cancer development and/or progression?

Probiotics and risk factors of liver cancer

(1995-2007)

Risk factors for HCC

- Viral
  - Chronic hepatitis B
  - ***Chronic hepatitis C**
- Preexisting liver disease
  - Cirrhosis
    - -Metabolic liver disease
    - -Alcohol abuse
  - Adenoma
- Environmental
  - ***Aflatoxin**
  - Contraceptives and androgens

The aflatoxins

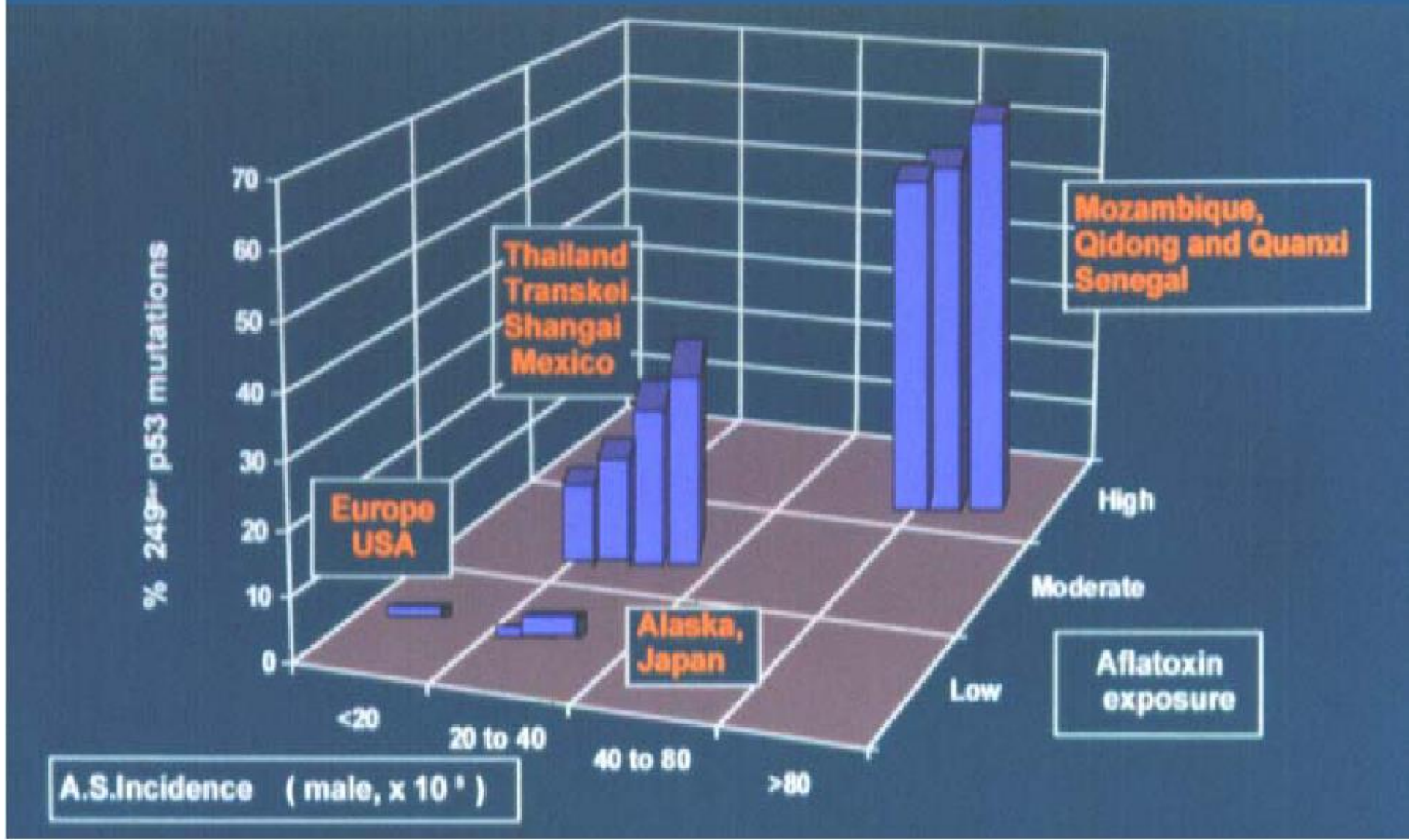
- Turkey "X" Disease
  - Fungal infection by *Aspergillus flavus* and *Aspergillus parasiticus*
  - Primary contamination
    - High energy content foods e.g. grain, nut and soy products
  - Secondary contamination
    - Dairy products, meat & eggs

Commodities in which aflatoxins have been detected

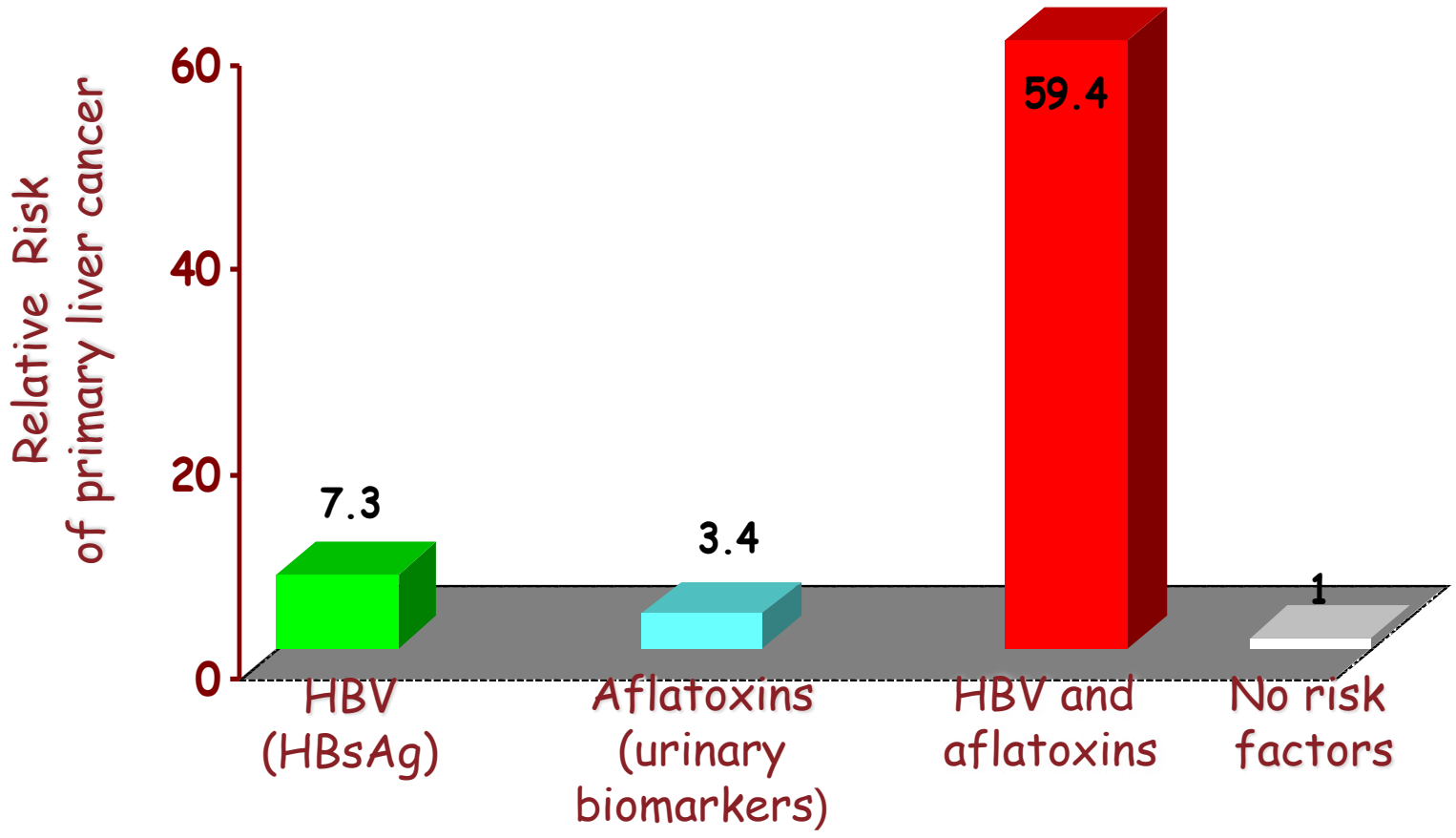
Flour	Cocoa
Corn meal	Cheese
Peanut	Sausage
Meat pies	Bread
Milk	Macaroni
Cottonseed	Copra
Cassava	Cooked meat
Brazil nuts	Pistachio nuts
Oilseeds	Rice
Pumpkin seeds	Soy

Prevalence of 249 ^{Ser} p53 mutation – Aflatoxin Exposure Incidence of Hepatocellular Carcinoma

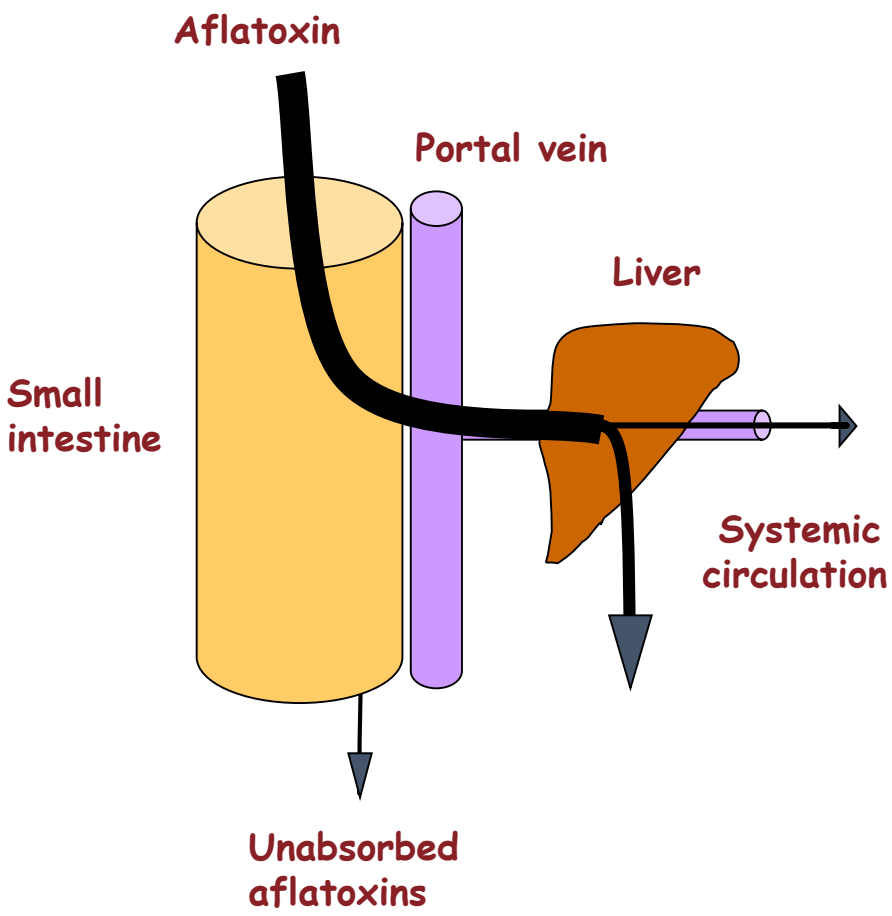
(Total number of cases: ~ 1000)

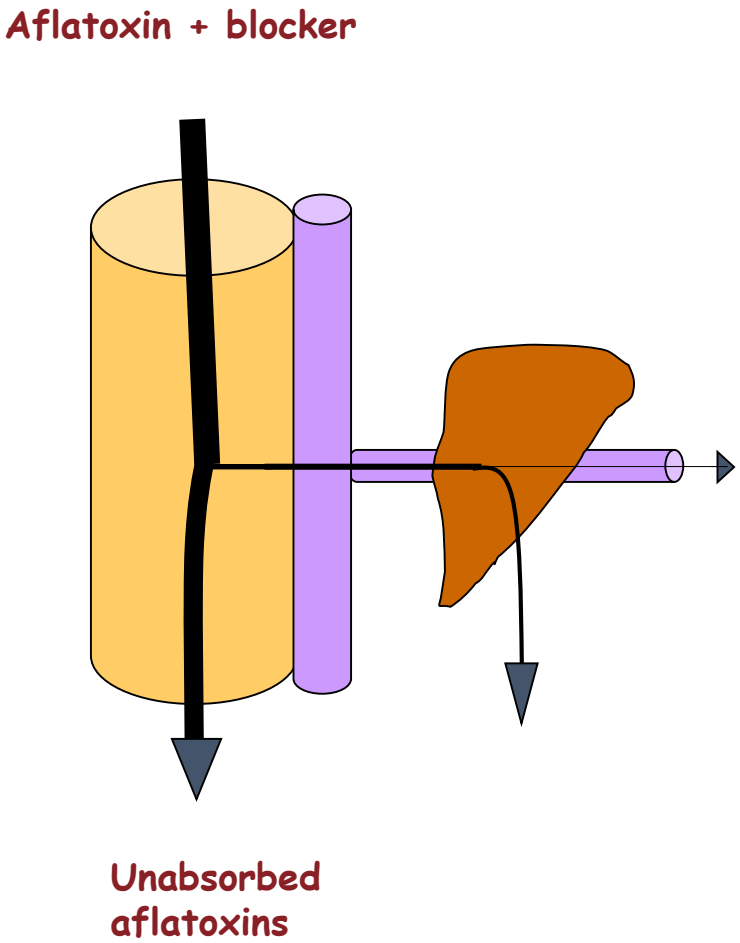


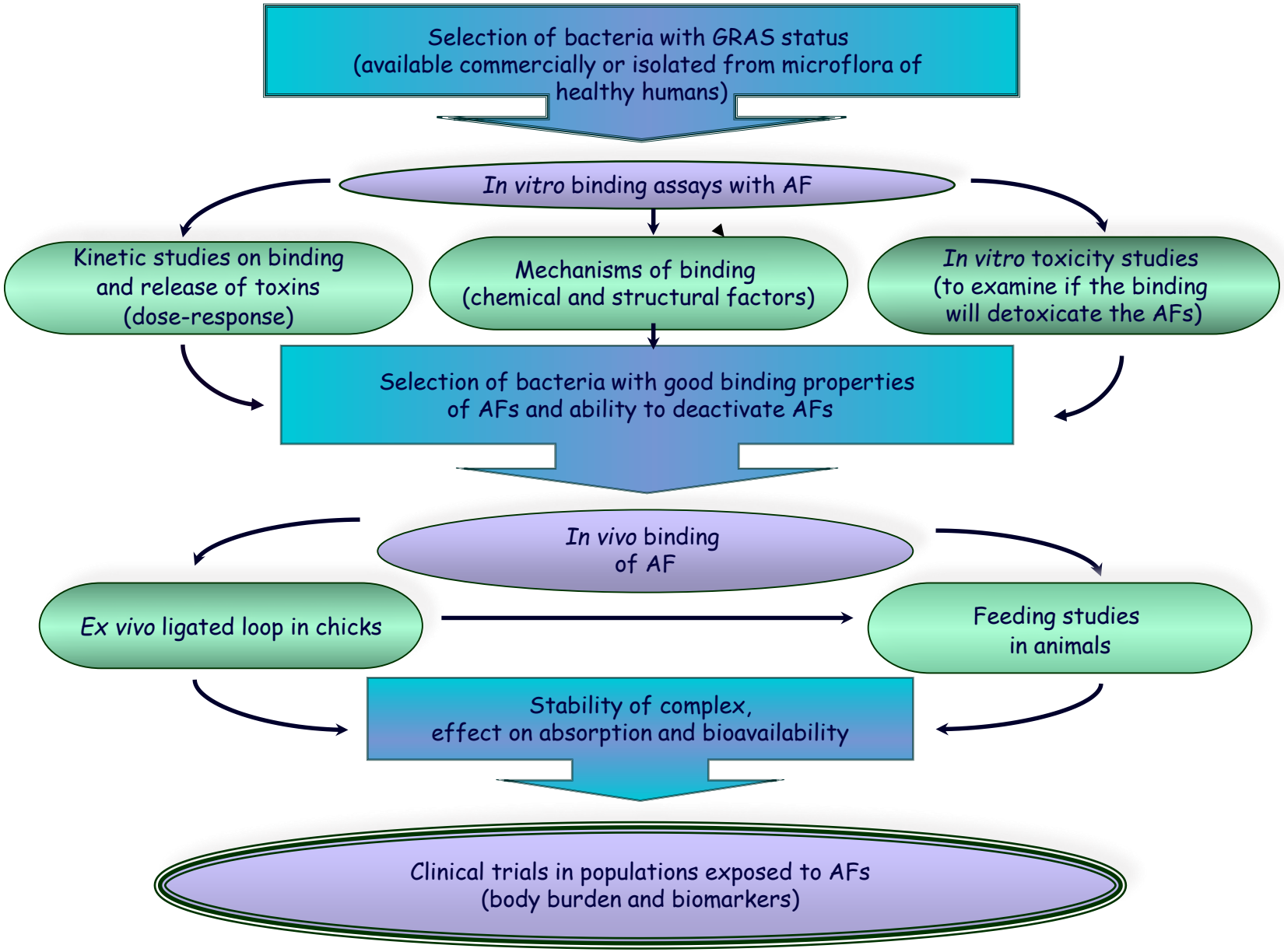
Chronic hepatitis B together with exposure to dietary aflatoxins increases the risk of liver cancer



Blocking/reducing absorption of AFB_1 from the small intestine

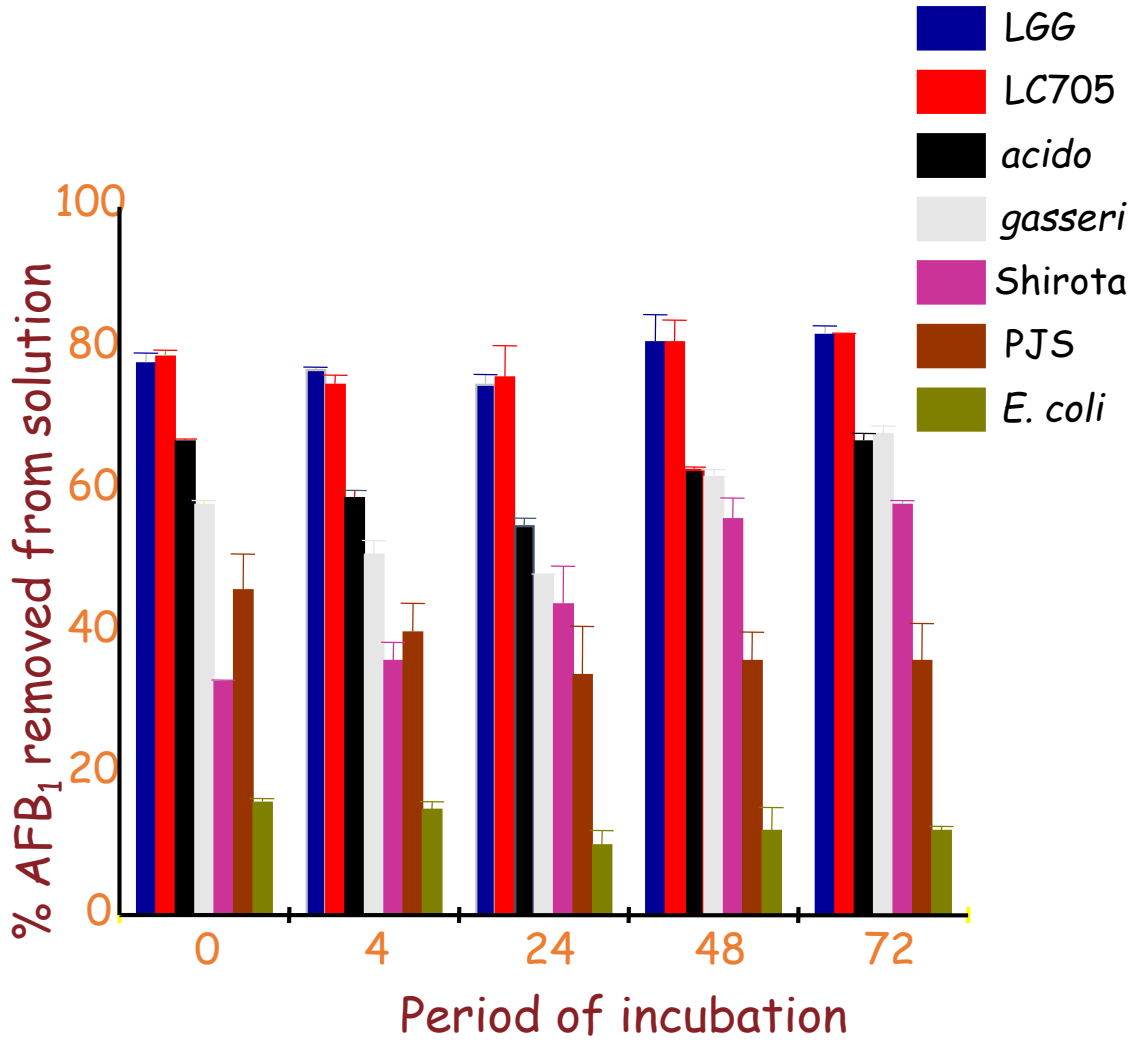





Selection of bacteria with GRAS status
(available commercially or isolated from microflora of healthy humans)
In vitro binding assays with AF
Kinetic studies on binding and release of toxins (dose-response)
Mechanisms of binding (chemical and structural factors)
In vitro toxicity studies (to examine if the binding will detoxicate the AFs)
Selection of bacteria with good binding properties of AFs and ability to deactivate AFs
In vivo binding of AF
Ex vivo ligated loop in chicks
Feeding studies in animals
Stability of complex, effect on absorption and bioavailability
Clinical trials in populations exposed to AFs
(body burden and biomarkers)

Aflatoxin is bound by probiotic bacteria – *in vitro* evidence

- Certain strains of lactobacilli are capable of binding up to 80% of AFB_1 *in vitro* (El-Nezami *et al*, 1996, 1998a,b,c), Fusarium toxins (El-Nezami *et al*, 2002a,b, 2004), PhIP and Trp-P-1 (Haskard *et al*, 2001)
- AFB_1 is predominantly bound to a carbohydrate moiety on the surface of the bacteria (Haskard *et al*, 2002)
- The complex formed between the bacteria and AFB_1 is stable under different conditions (Haskard *et al*, 2002, Lee *et al*, 2003)



The American Journal of Clinical Nutrition

Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from Southern China[1–3]

Hani S El-Nezami, Nektaria N Polychronaki, Jing Ma, Huilian Zhu, Wenhua Ling, Eeva K Salminen, Risto O Juvonen, Seppo J Salminen, Tuija Poussa, and Hannu M Mykkänen

ABSTRACT

Background: In vitro and in vivo studies suggest that selected strains of probiotic bacteria can form tight complexes with aflatoxin B_1 and other carcinogens.

Objective: The aim of the present study was to determine whether administration of probiotic bacteria could block the intestinal absorption of aflatoxin B_1 and thereby lead to reduced urinary excretion of aflatoxin B_1-N^7-guanine (AFB-N^7-guanine), a marker for a biologically effective dose of aflatoxin exposure. Elevated urinary excretion of this aflatoxin-DNA adduct is associated with an increased risk of liver cancer.

Design: Ninety healthy young men from Guangzhou, China, were randomly assigned to 2 groups; one group received a mixture of *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp. *shermanii* strains 2 times/d for 5 wk, and the other group received a placebo preparation. The subjects provided 4 urine samples: at baseline, at 3 and 5 wk after starting the supplementation, and at the end of the 5-wk postintervention period.

Results: The percentage of samples with negative AFB-N^7-guanine values tended to be higher in the probiotic group than in the placebo group during the 5-wk intervention period (odds ratio: 2.63, $P = 0.052$), and a statistically significant decrease in the concentration of urinary AFB-N^7-guanine was observed in the probiotic group. The reduction was 36% at week 3 and 55% at week 5. The geometric means for the probiotic and placebo groups were 0.24 and 0.49 ng AFB-N^7-guanine/mL, respectively, during the intervention period ($P = 0.005$).

Conclusion: A probiotic supplement reduces the biologically effective dose of aflatoxin exposure and may thereby offer an effective dietary approach to decrease the risk of liver cancer. *Am J Clin Nutr* 2006;83:1199–203.

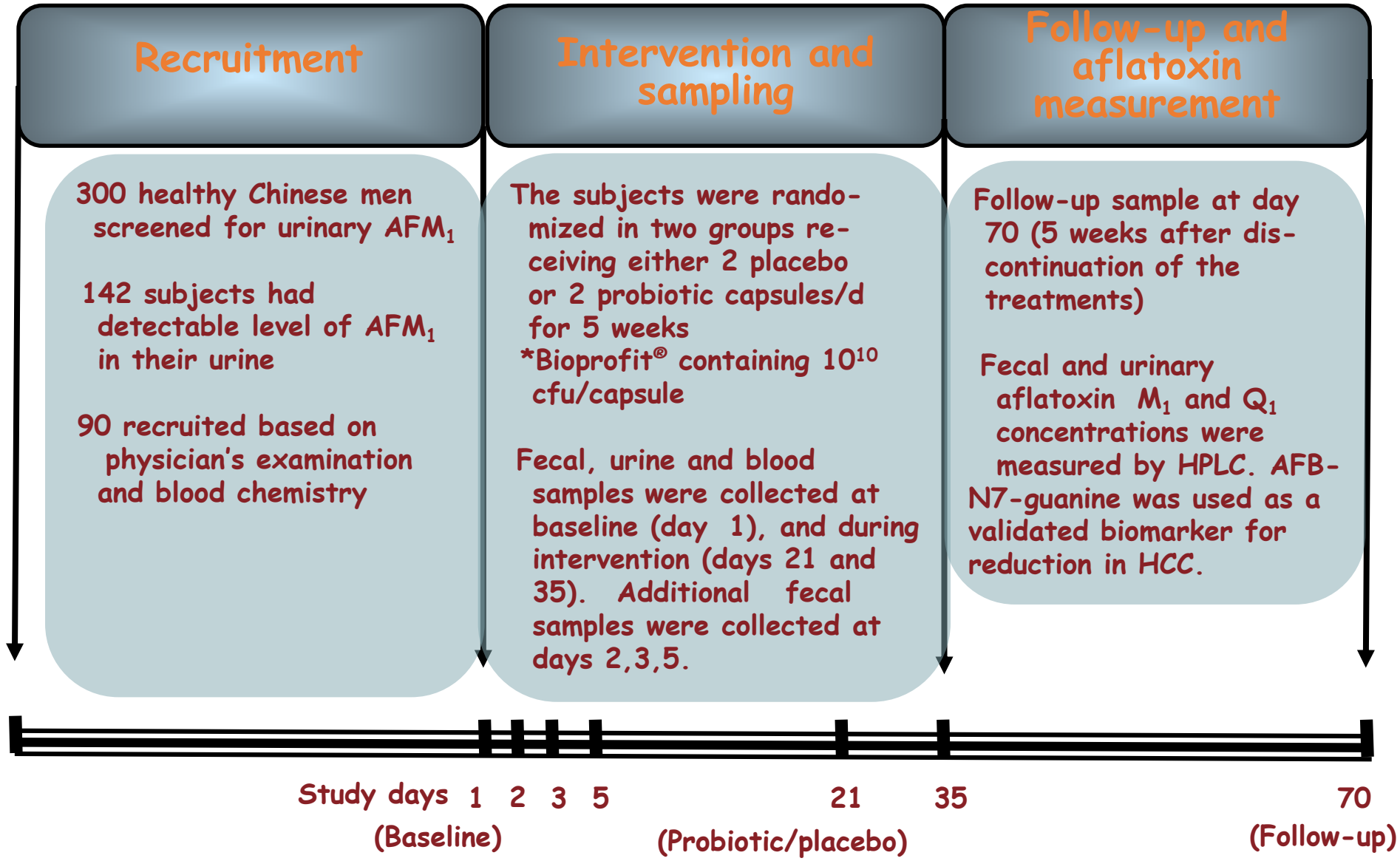
KEY WORDS Probiotic bacteria, aflatoxins, aflatoxin B_1-N^7-

Aflatoxins, a group of mycotoxins produced by the common fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are established human hepatocarcinogens (4–6) and are well-known HCC risk factors when present in foodstuffs (2, 7). They play an important role in modifying the risk of liver cancer associated with HBV. After being metabolized in the liver, the toxin can bind to guanine in DNA, resulting in mutations at codon 249 of the *TP53* tumor suppressor gene (8). In addition to being potent carcinogens, aflatoxins are cytotoxic, and associations between childhood aflatoxin exposure and growth faltering (9–12) and reduced concentrations of salivary IgA (12) have been reported. This highlights the need to reduce or eliminate exposure to aflatoxin (13). The approach to prevent exposure to aflatoxins has been to ensure that foods consumed have the lowest possible aflatoxin concentrations. Although this is achieved in developed countries via strict food regulations, it has clearly failed as a control measure in developing countries where the problem is more evident. Additional prevention strategies, such as chemo- or dietary prevention, need to be considered in the high-risk regions of developing countries. These strategies need to be completely safe, inexpensive, and mechanistically simple. Chemopreventive agents (oltipraz and chlorophyllin), which reduce the burden of harmful aflatoxin metabolites in the body, have been studied and shown to be potentially beneficial in targeted groups (14, 15).

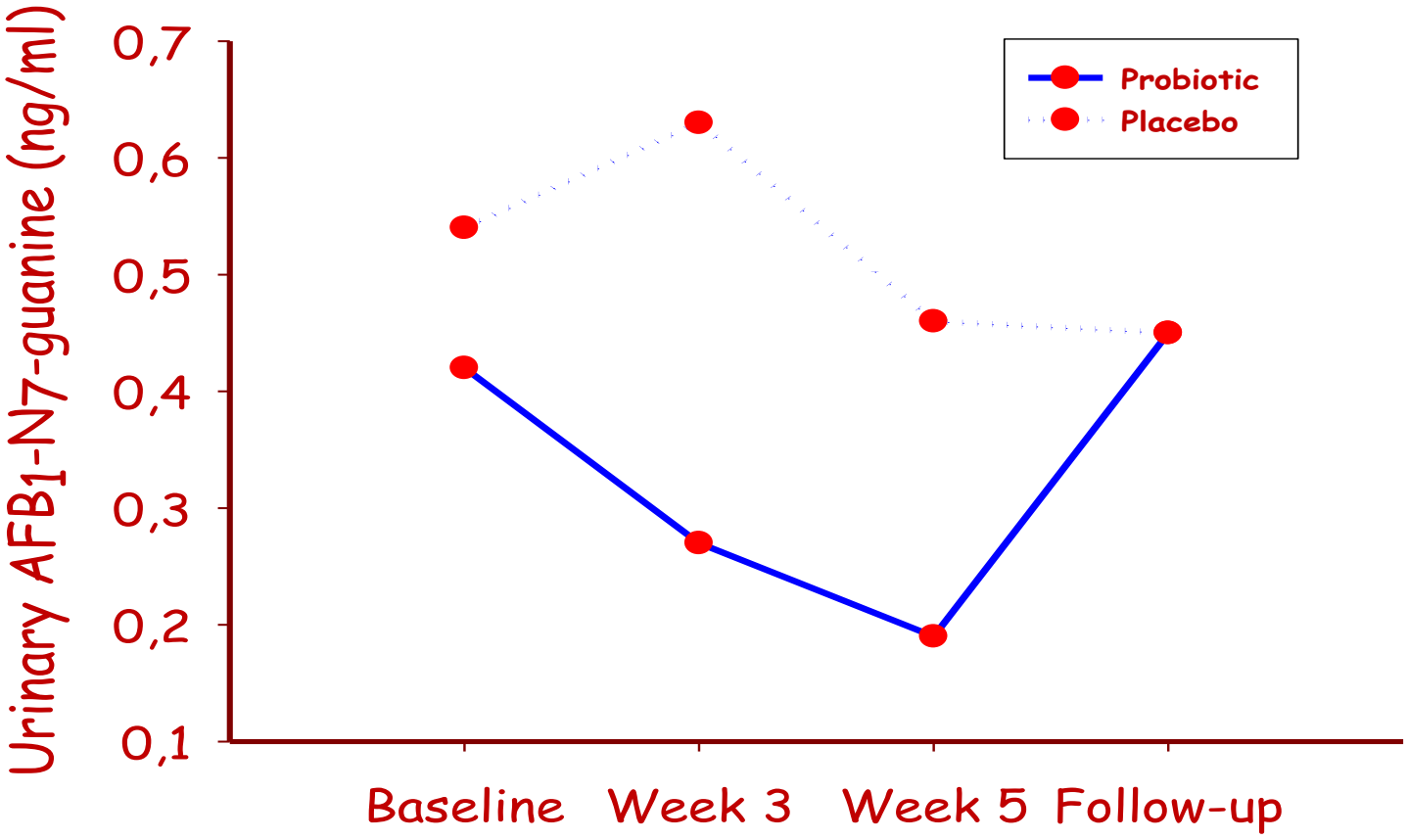
Our previous work with >250 strains of lactic acid bacteria isolated from either dairy products or healthy human microbiota showed that 2 *Lactobacillus rhamnosus* strains, LGG and LC705 (both possess probiotic properties), were the most efficient strains in binding a range of mycotoxins, including aflatoxins

[1] From the Department of Clinical Nutrition and Food and Health Re-

Probiotic intervention in China



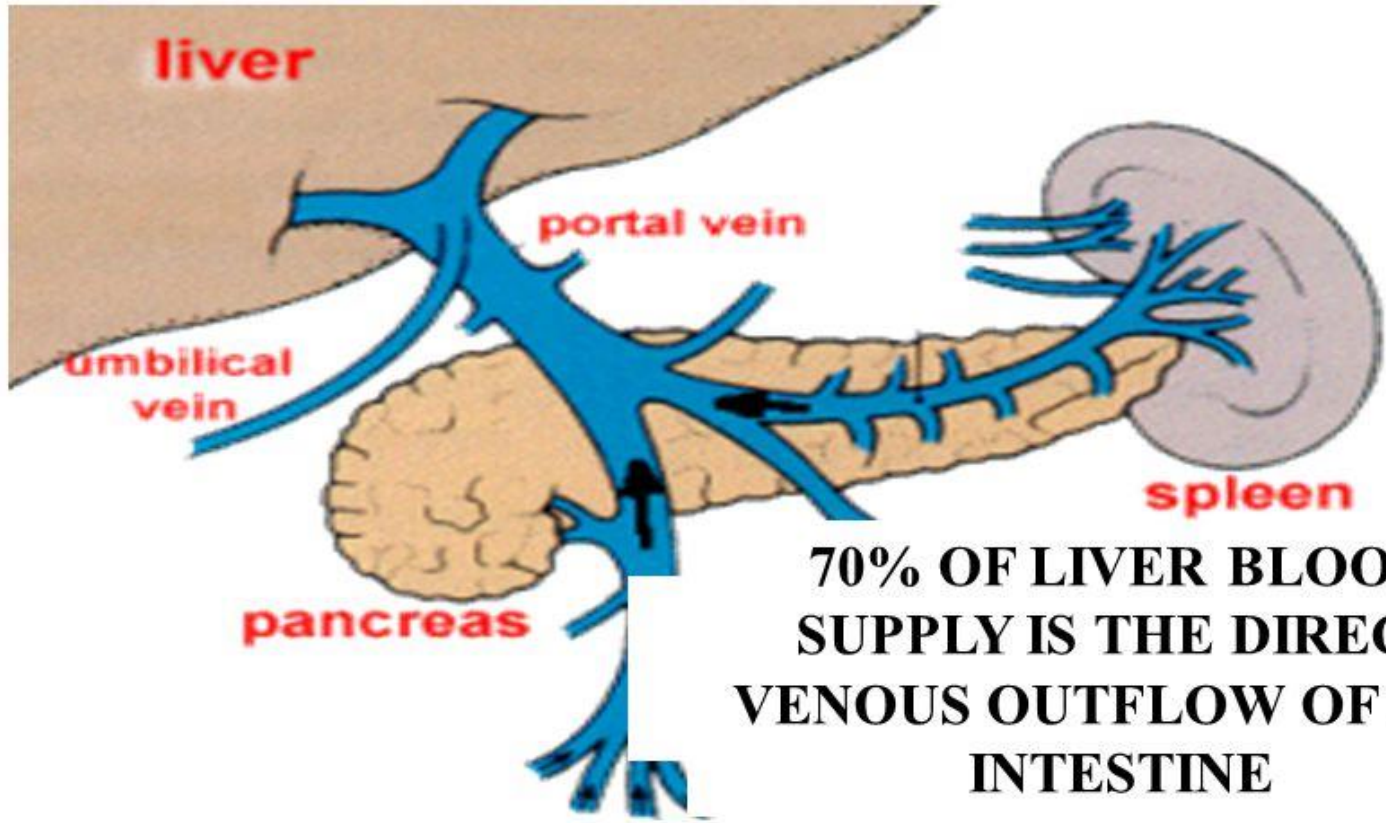
Probiotic supplementation reduces the urinary excretion of AFB_1-N^7-guanine, a biomarker of biologically effective dose of exposure to AFB_1



El-Nezami et al, Am J Clin Nutr, 2006

Probiotics and modulations of liver cancer development and progression

Gut-liver axis



70% OF LIVER BLOOD SUPPLY IS THE DIRECT VENOUS OUTFLOW OF THE INTESTINE

…LIVER IS CONTINUALLY EXPOSED TO GUT-DERIVED FACTORS INCLUDING BACTERIA AND BACTERIAL COMPONENTS

Microbiota affect the liver and act as a cofactor in aetiology of chronic liver damage.

Role of gut microbiota & metabolites in HCC development

- Patients with liver cirrhosis and HCC have significant increase in serum endotoxin levels
- Presence of *Helicobacter spp.* in the liver of HCC patients, but absent from that of control
- A study by Yoshimoto et al. 2013 Nature, dietary or genetic obesity alters gut microbiota and thus rises the deoxycholic acid (DCA) levels, a gut bacterial metabolite that cause DNA damage
    - DCA provokes the production of various inflammatory and tumour-promoting factors in the liver, thereby promoting HCC development in mice after exposure to chemical carcinogen
    - Blocking DCA production efficiently prevents HCC development in obese mice

Pilot study: Three commercial probiotics

Escherichia coli
Nissle 1917 (EcN)

Lactobacillus rhamnosus GG (LGG)

VSL #3
8 strains of 3 genus:
Streptococcus ,
Bifidobacterium,
Lactobacillus

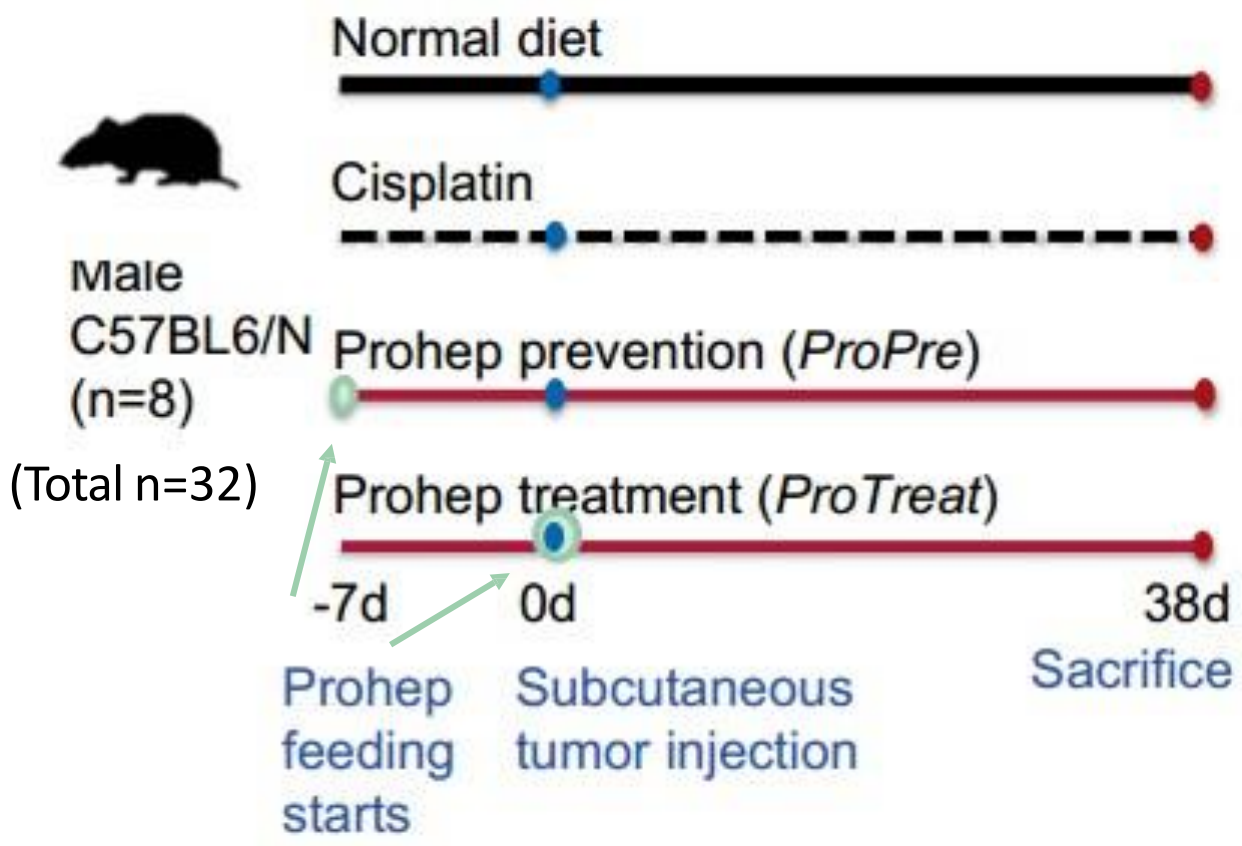
Probiotics and Cancer

	Lactobacillus *acidophilus* (Moro) Hansen and Mocqu	**Eschericha coli Nissle 1917 (EcN)**	**VSL#3**
Skewing of T cell response	•Maturation and activation of dendritic cell •Affect TH1/TH2 paradigm	•Maturation and activation of dendritic cell •Affect TH1/TH2 paradigm •Induction of Treg	•Induction of Treg
Anti-inflammation	•TGF-β, IL-10 • Induction of Treg •Ulcerative colitis Allergic airway inflammation Eczema Crohn's disease Arthritis	**IL-17 mediated diseases**	
Anti-tumor immunity	•IL-12, IFN-γ •Activation of cytotoxic T cell, natural killer cell •Bladder, colon (in vitro), stomach (in vitro)	Breast, B16 melanoma (in vivo)	Colitis-associated colorectal cancer (CRC)

Hypothesis

- Feeding selected probiotic bacteria orally to hepatocellular carcinoma-bearing mouse leads to changes in T cell response, and thereby remodeling the tumor microenvironment, resulting in slowing down of tumor progression

Effect of Prohep in mice study



PNAS

Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice

Jun Li[a,1], Cecilia Ying Ju Sung[b,1], Nikki Lee[c], Yueqiong Ni[a], Jussi Pihlajamäki[d,e], Gianni Panagiotou[a,2], and Hani El-Nezami[b,d,2]

[a]Systems Biology and Bioinformatics Group, School of Biological Sciences, Faculty of Sciences, The University of Hong Kong, Hong Kong S.A.R., China; [b]School of Biological Sciences, Faculty of Science, The University of Hong Kong, Hong Kong S.A.R., China; [c]Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong S.A.R., China; [d]Insititute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio 70211, Finland; and [e]Clinical Nutrition and Obesity Center, Kuopio University Hospital, Kuopio 70211, Finland

Prohep

USA, EU, China Patents

I hereby certify that this correspondence is being electronically transmitted via EFS to the United States Patent and Trademark Office on the date shown below:

21 MARCH 2018

Jeff Lloyd, Patent Attorney, Reg. No. 35,589

AMENDMENT AFTER ALLOWANCE
UNDER 37 C.F.R. §1.312
Examining Group 1651
Patent Application
Docket No. UHK.182
Serial No. 14/460,732

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kade Ariani
Art Unit	:	1651
Applicants	:	Hani El-Nezamy, Nikki Pui-Yue Lee, Cecilia Ying Ju Sung, Jiandong Huang
Serial No.	:	14/460,732
Filed	:	August 15, 2014
Confirm. No.	:	7911
For	:	Method and Compositions for Treating Cancer Using Probiotics

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

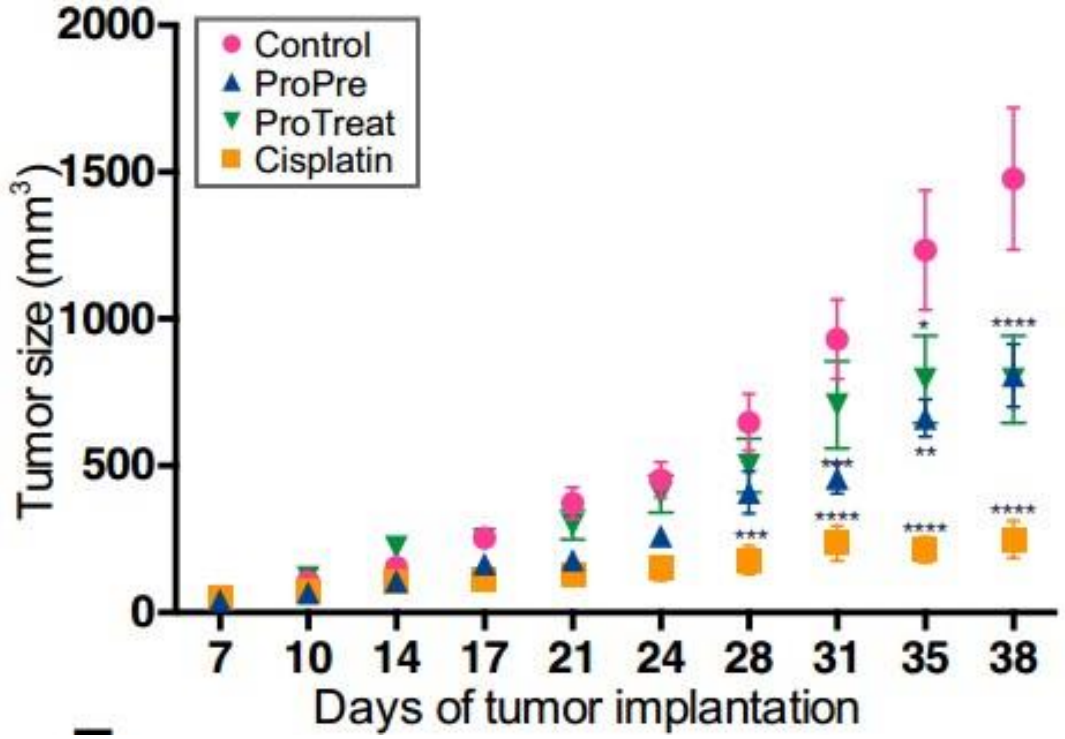
AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. §1.312

Sir:

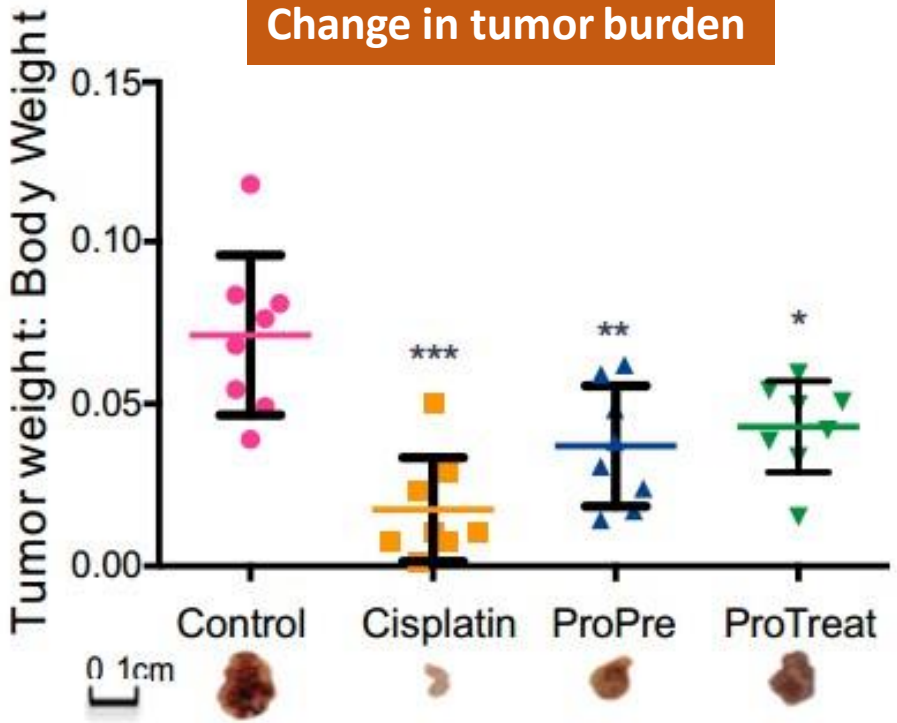
This Amendment is being submitted to the Patent Office in response to the Notice to File Corrected Application Papers dated March 19, 2018. Please amend the application identified above as follows:

Prohep suppress tumor development

Change in tumor size over time



Change in tumor burden



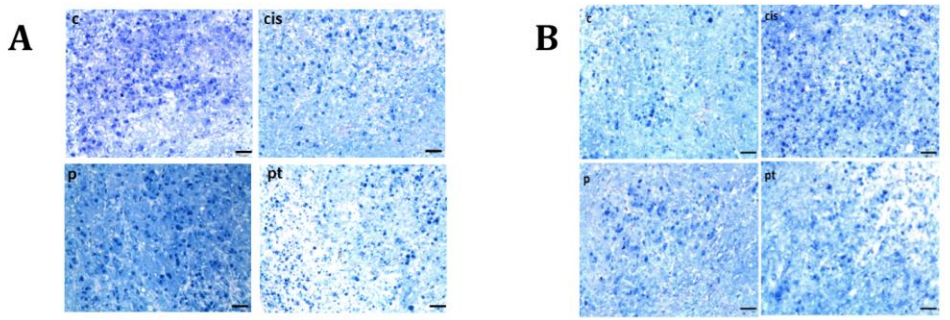
Compared with Control

*0.01 < P value < 0.05;

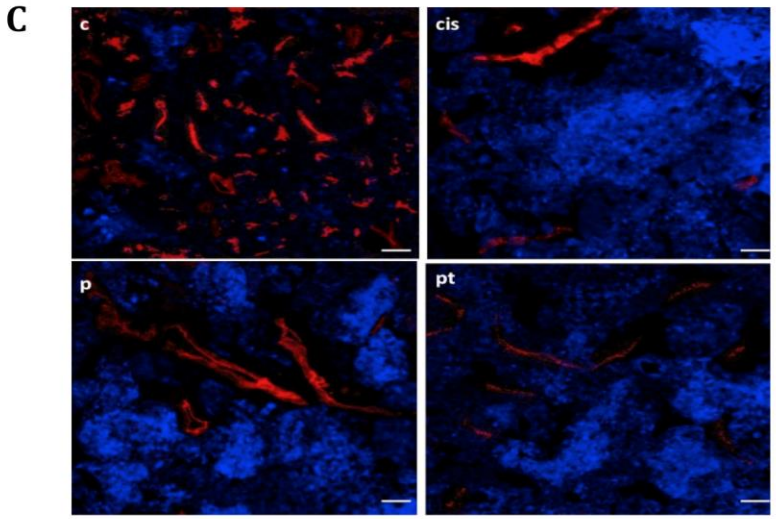
**0.001 < P value < 0.01;

***P value < 0.001

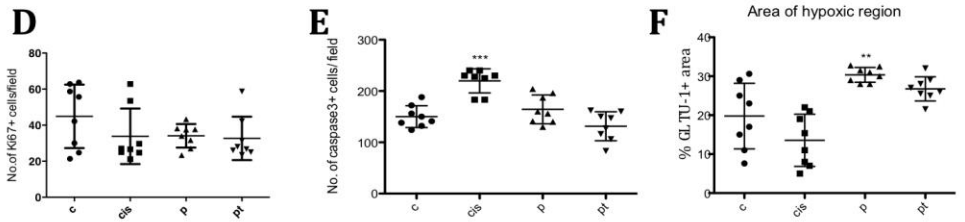
- Reduced tumor size is likely to be related to hypoxia-induced cell death



No significant difference in no. of apoptotic cells and proliferating cells



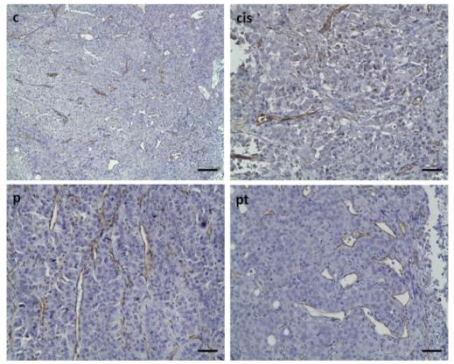
Significant increase in hypoxic area (blue) near hot spot (Red) in probiotic gps as compared to control

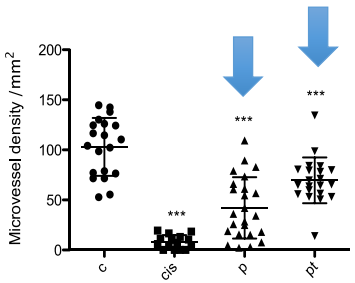


Li et al. 2016, PNAS

Prohep inhibit angiogenesis in subcutaneous HCC model

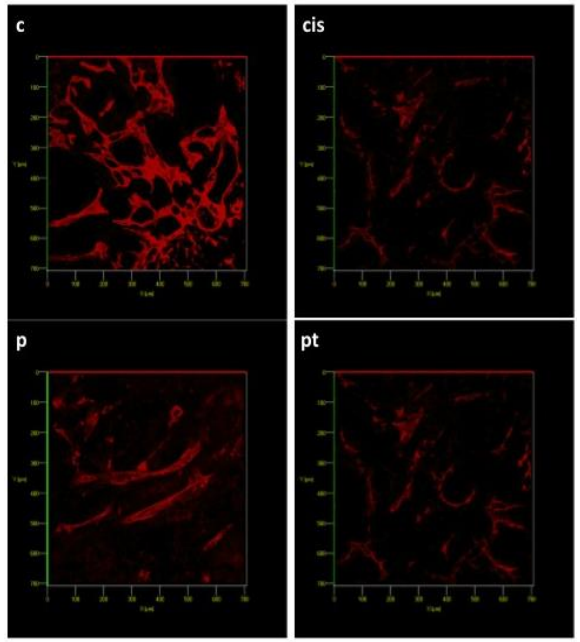
A

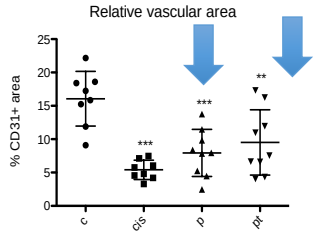




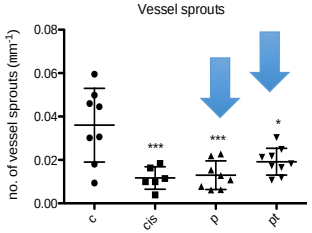
Significant reduction in microvessel density (MVD)

B





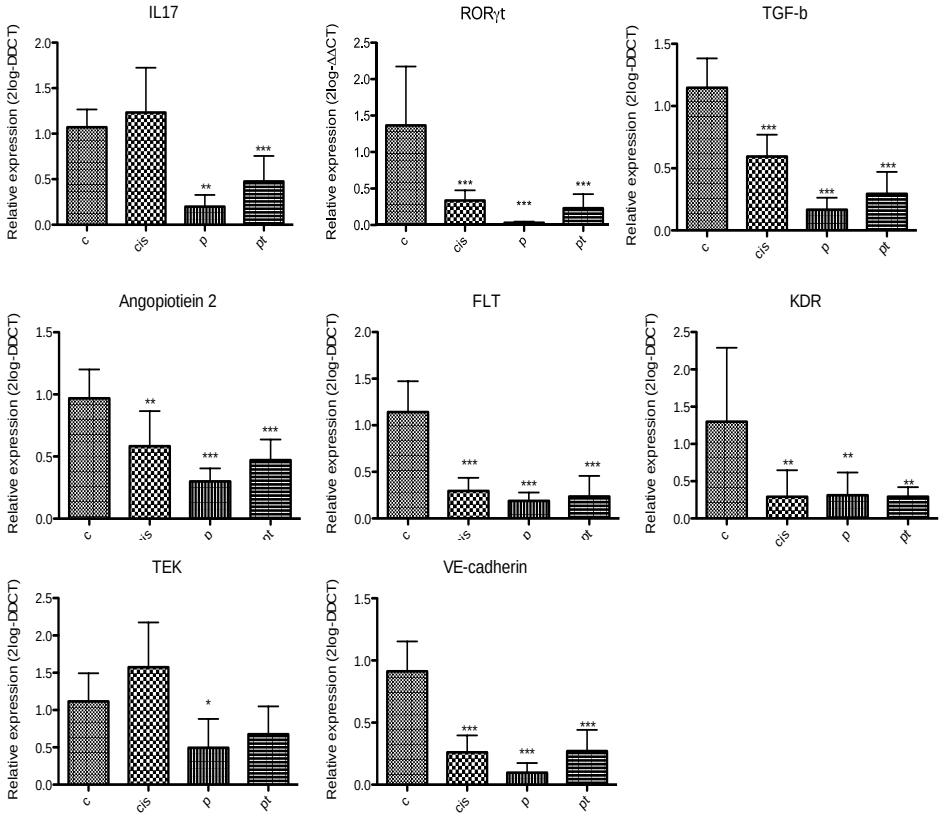
Significant reduction in relative vascular area

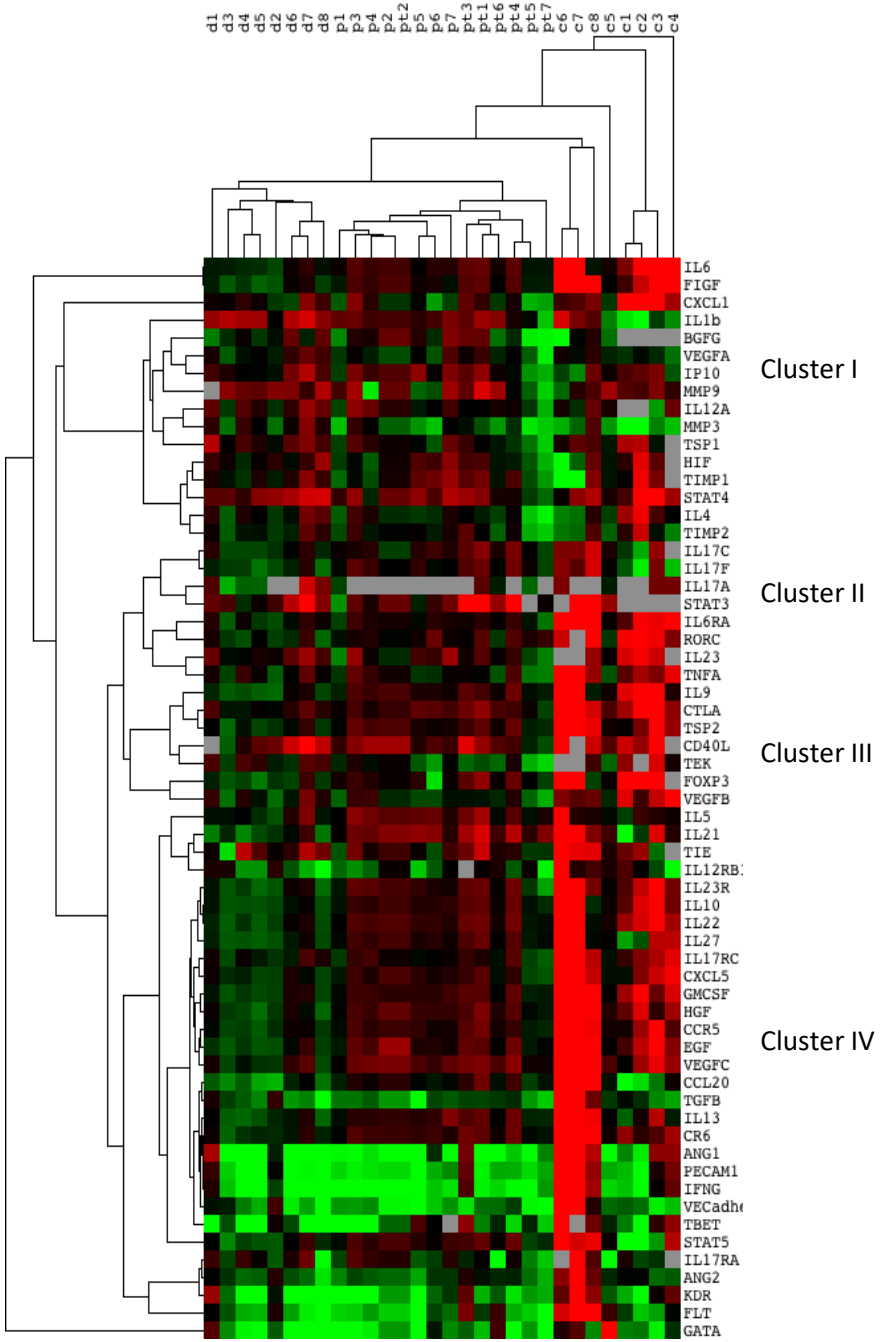


Significant reduction in no. of vessel sprouts

Li et al. 2016, PNAS

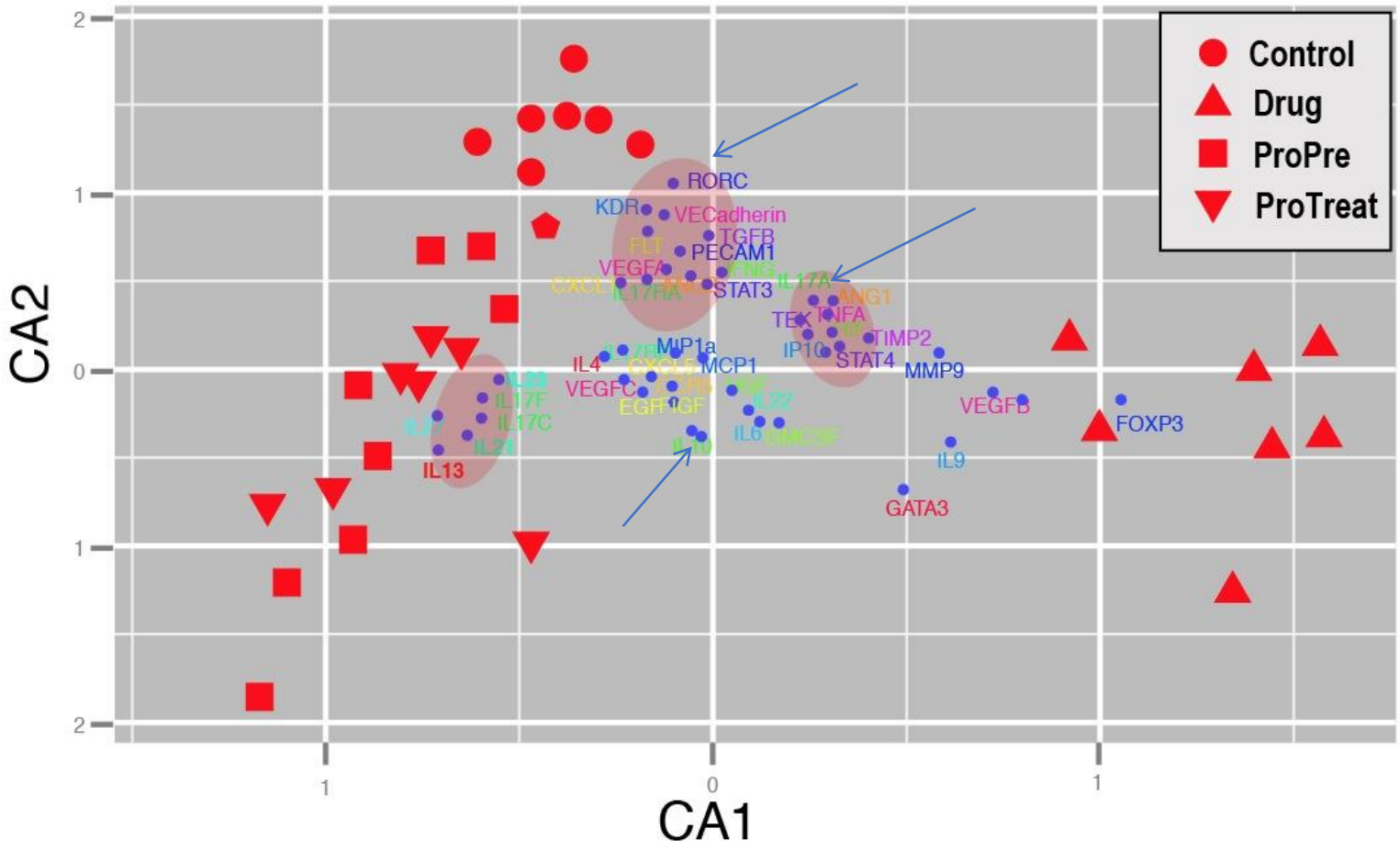
- **Prohep treatment downregulates the expression of proangiogenic genes in tumors**





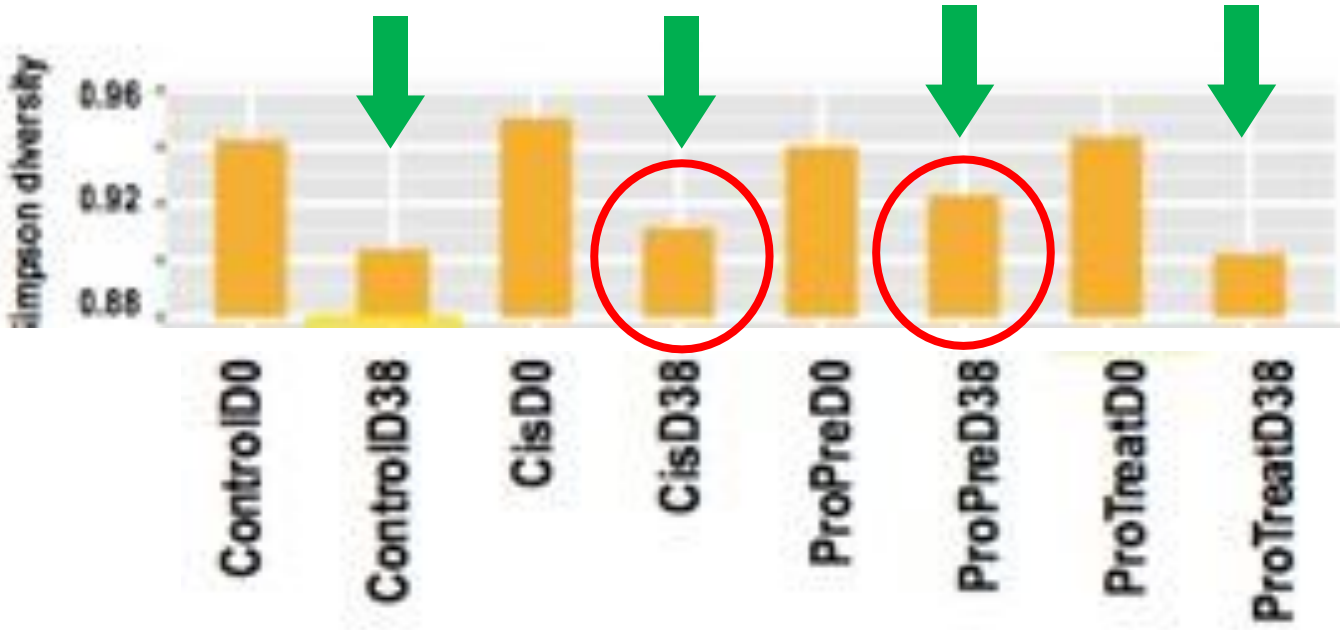
Li et al. 2016, PNAS

Corresponding analysis of qPCR revealed the angiogenesis related genes have similar expression profiles (decreasing)



Li et al. 2016, PNAS

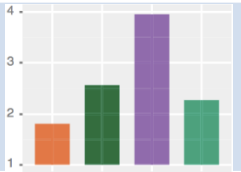
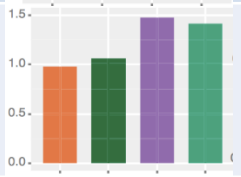
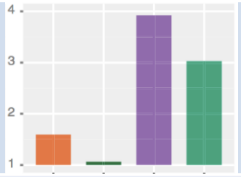
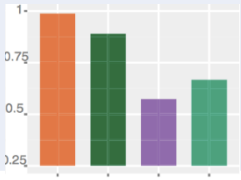
ProPre group rebalances gut microbiota



α diversity (Simpson diversity)

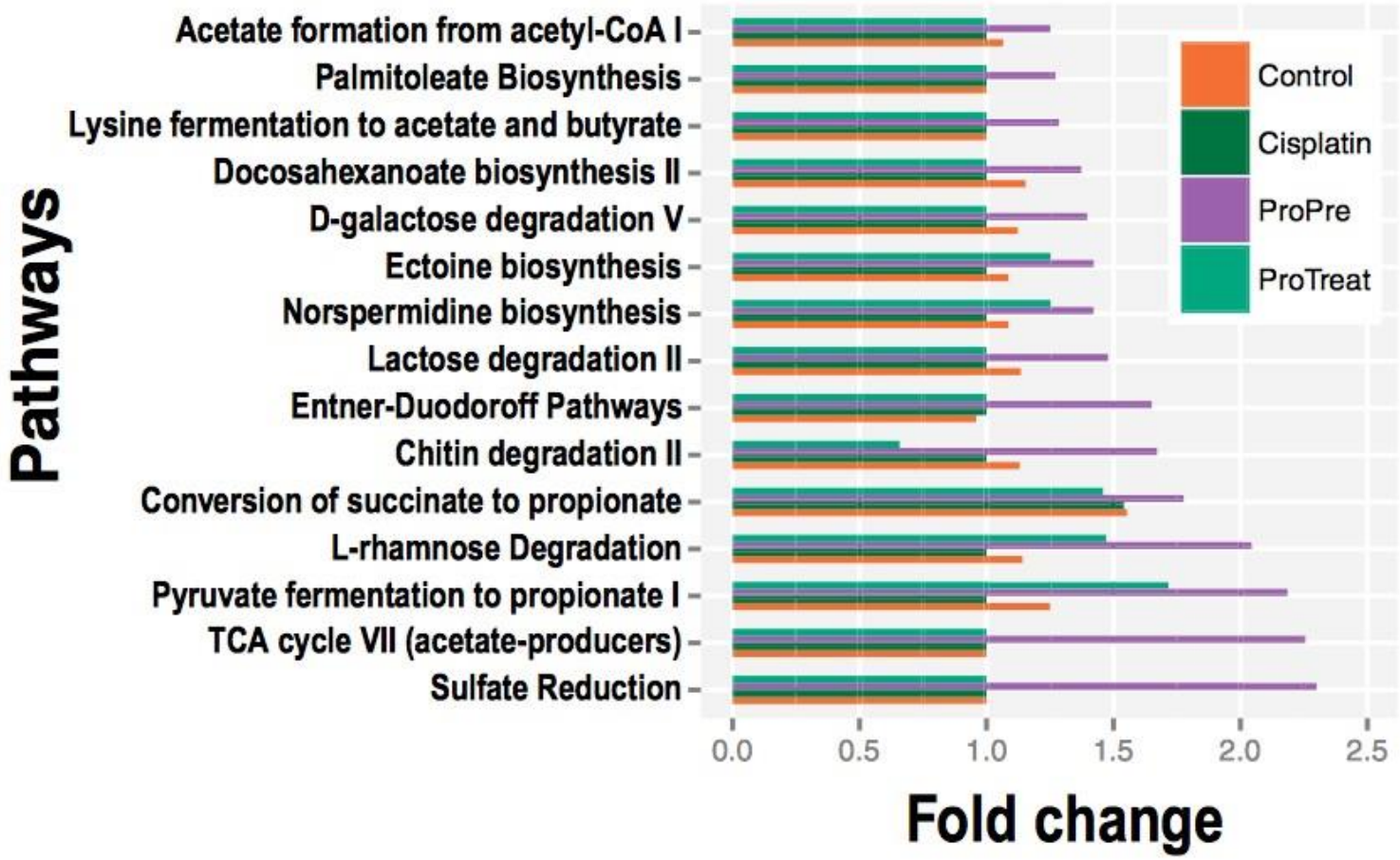
- ProPre and Cisplatin groups are significantly higher after 38 days
- Rebalancing Microbiota

Species level change: 4 significantly* enriched species in ProPre group

Species	Function	Change in Propre
Bacteroides fragilis	Gut immunoregulatory	Increase 
Alistipes shahii	Modulator in the suppression of tumor growth	Increase 
Parabacteroides distasonis	Antiinflammatory	Increase 
Segmented filamentous bacteria (SFB)	Th17-inducing	Decrease 

* Bonferroni adjusted P value <0.05 in Wilcoxon rank-sum test using 100 bootstraps for each sample

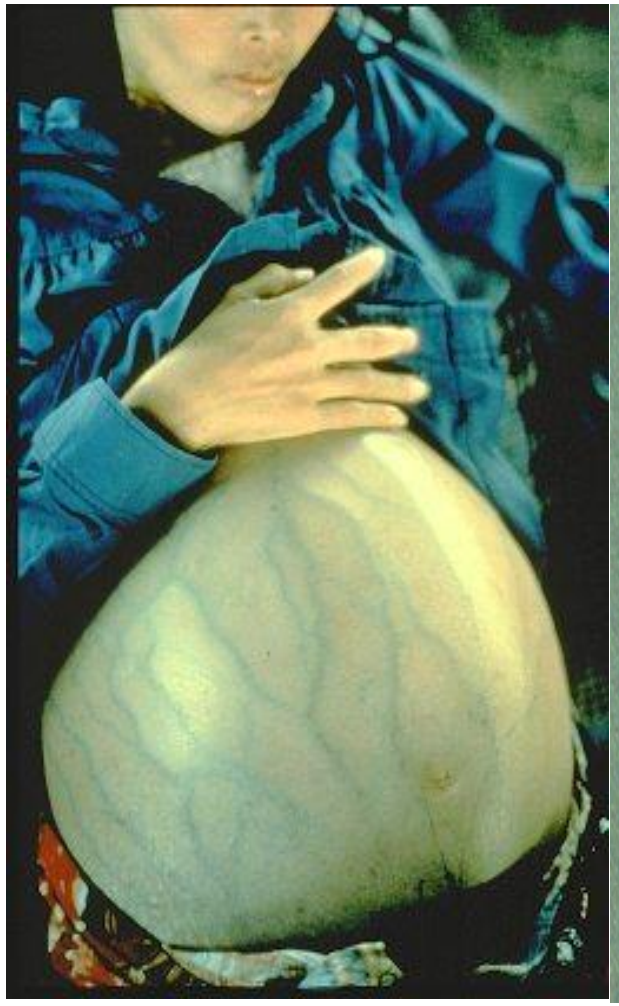
Metabolic Pathway: Top 15 enriched in *ProPre group*

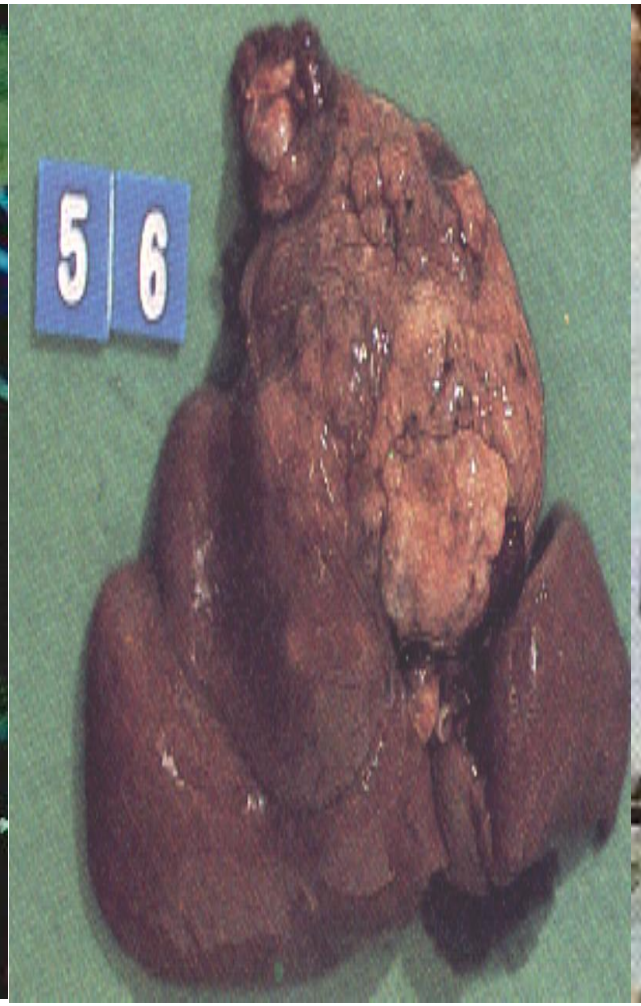


- 6 are related to SCFAs
- 2 are long-chain fatty acids: reduce the pro-inflammation cytokines in endothelial cells

Conclusion

- **Probiotic reduce the tumor growth and inhibit angiogenesis in mouse**
- **The anti-angioenenis in tumor is related to reduced Th17 and angiogenesis factors**
- **Th17 in tumor are mainly migrated and significantly reduced in intestine and peripheral blood**
- **Gut microbita were reshaped by probiotic intake**
- **The polarization of the gut microbial community in both taxonomy and functional aspects are towards SCFA producing and hense**
  - **Reduce Th17 differentiation**
  - **Enhance Treg/Tr1 production**





Thank you