Journal of Nephrology & Therapeutics

Dose Escalation, Safety and Impact of a Strain-Specific Probiotic (Renadyl™) on Stages III and IV Chronic Kidney Disease Patients

Natarajan Ranganathan[1]*, Bohdan Pechenyak[1], Usha Vyas[1], Pari Ranganathan[1], Stephanie DeLoach[2], Bonita Falkner[2], Alan Weinberg[3], Subodh J Saggi[4] and Eli A Friedman[4]

[1]Kibow Biotech Inc., Newtown Square, PA, USA
[2]Thomas Jefferson Univ., Philadelphia, PA, USA
[3]Mount Sinai School of Medicine, New York, NY, USA
[4]Downstate Medical Center, State University of NY, New York, NY, USA

Abstract

The primary goal of the open label study of Renadyl™ in stage 3 and 4 chronic kidney disease patients was to confirm the safety and tolerability of several doses of Renadyl™ (90, 180, 270 billion colony forming units). Secondary goals were to quantify quality of life improvement, to confirm efficacy in reducing commonly known uremic toxins, and to investigate the effects on several biomarkers of inflammation and oxidative stress. Participants underwent physical examinations and venous blood testing, and completed quality of life questionnaires. Data were analyzed with SAS V9.2. Of 31 subjects, 28 (90%) completed the study (2 lost to follow-up). The primary goal was met, as no significant adverse events were noted during the dose escalation phase. All patients tolerated the maximum dose (note: 1 subject reported nausea upon initial use). The escalation efficacy was shown in statistically significant changes of serum creatinine (months 2 to 6: -0.23 mg/dL, p<0.05), C-reactive protein (months 2 to 6: -0.28 mg/L, p<0.05), and hemoglobin (base to month 6: 0.35 mg/dL, p<0.01, months 1 to 6: 0.46 mg/dL, p<0.001, months 2 to 6: 0.58 mg/dL, p<0.0001). Trends, but not statistical significance, were noted in blood urea nitrogen (base to month 4: -3.56 mg/dL, p<0.09; months 1 to 4: -3.81 mg/dL, p<0.07). The secondary goal was also met, as QOL measure of physical functioning improved (base to month 6, p<0.05) and a strong trend in reduction of pain was observed (base to month 6, p<0.08).

Keywords: Probiotics; Renadyl™; Dose escalation; Chronic kidney disease; Dysbiosis

Abbreviations: CKD: Chronic Kidney Disease; ESRD: End-Stage Renal Disease; QOL: Quality Of Life; CRP: C-Reactive Protein; BUN: Blood Urea Nitrogen; CFU: Colony Forming Unit; SHIME: Simulated Human Intestinal Microbial Ecosystem

Introduction

Probiotics are defined by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (2002). Prebiotics, in turn, are defined as "non-digestible, but fermentable, foods/ingredients that allow specific changes, both in the composition and/or activity, in the gastrointestinal microflora that confers benefits upon host well-being and health [1]". They now appear with increasing frequency in various foods, beverages, supplements, and are readily utilized in alternative and complementary medical practice strategies. Due to direct consumer advertising, the average population has been convinced of the positive role that probiotics play in health and disease. This has led to marketing and consumption of a number of probiotic supplements available for purchase online or in pharmacies without their benefit being scientifically proven in a rigorous clinical trial. The role of digestive [2] and immune [3] systems, as well as inflammatory [4] and oxidative stress [5,6] functions in the progression of kidney disease has been emphasized by researchers in the past decade. Current data have highlighted an integrated and perhaps a causal relationship between the observed clinical outcomes and the role of an activated immune system in uremia. Most recently, the June 2013 issue of this very publication included a review of the role of microbial imbalance (dysbiosis) in Chronic Kidney Disease (CKD), which discussed the extent to which the gut and its microbial population might play a permissive role in the generation or assist in the degradation (perhaps even both) of many of the uremic toxins [2]. The June issue's cover was adorned by a hypothetical conceptualization of the relationship between a failing kidney and intestinal microbiota: the metabolic changes associated with the progression of CKD to End-Stage Renal Disease (ESRD) alter the balance of symbionts and pathobionts in a way that favors pathobiont overgrowth, i.e., dysbiosis. (Please see Figure 1 for further elucidation of dysbiosis).

Probiotic microbes are predominantly found in fermented dairy foods such as yogurt, kefir, cheese and other fermented foods. The expansion of our awareness and use of probiotics, however, has raced ahead of the scientific basis for the mechanisms by which they impact health. Probiotics are increasingly utilized in clinical settings. A simple search of the NIH clinicaltrials.gov registry for "probiotics" brought up 548 clinical studies [7]. As their safety and health benefits are established, it is reasonable to anticipate that probiotic bacteria will be incorporated into a growing number of clinical regimens, either on their own or as an adjunct/part of a combined treatment.
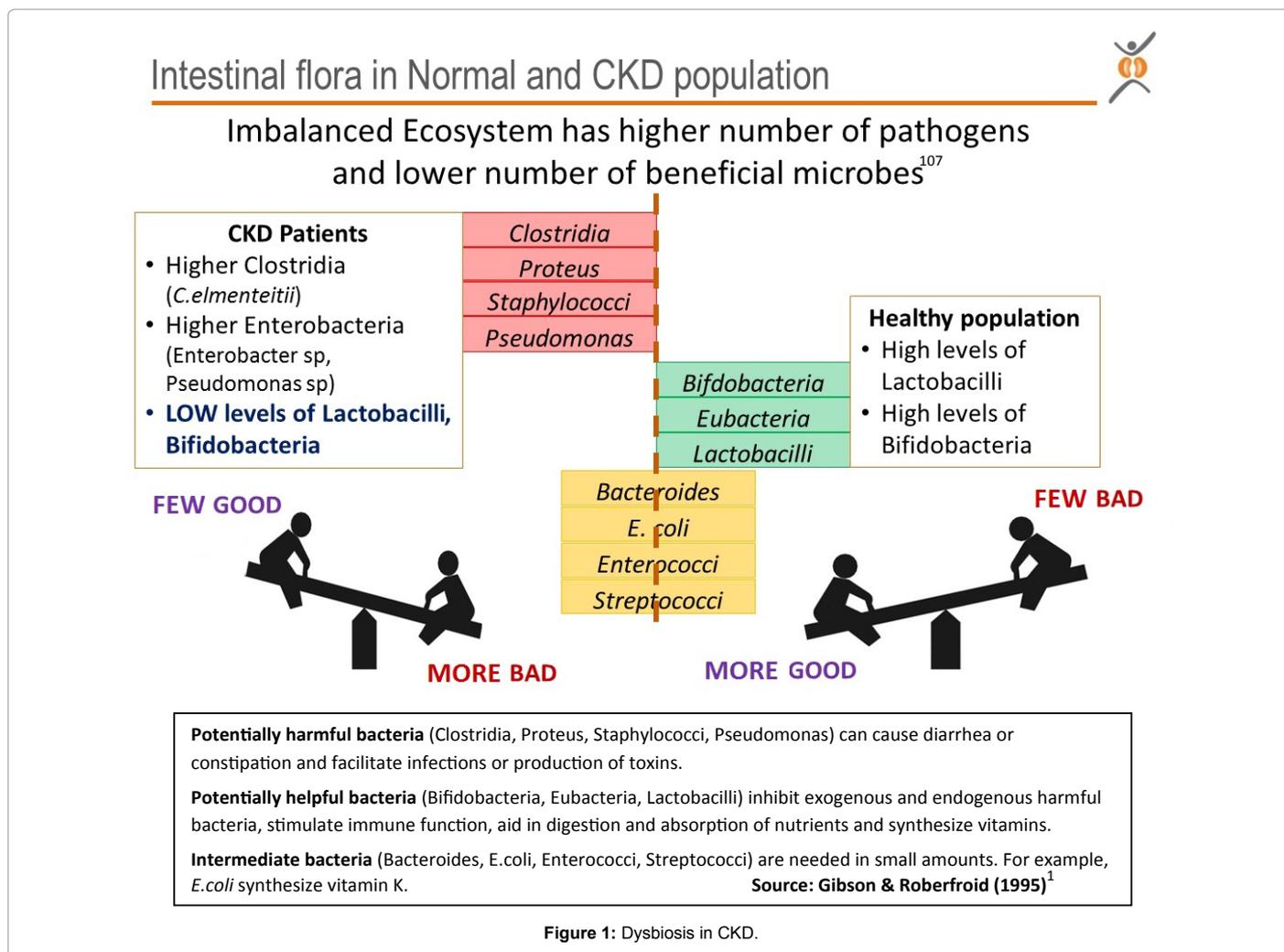
The National Institutes' of Health (NIH) Human Microbiome Project (HMP) targeted a goal of sequencing the genome of the microbial ecosystem found in a healthy human body and, if possible, of defining a core microbiome [8-11]. This collaborative project provided an excellent opportunity to examine the microbial diversity within and across body habitats and individuals [12], and with regard to geography and age [13]. This was the first, extremely important step in a new research direction, which has the potential of revolutionizing the practice of medicine [14] and public health [15], as well as the very

Figure 1: Dysbiosis in CKD.

concept of who we are as human beings [16,17]. Unlike the human genome, the microbiome can be modulated and engineered, according to the therapeutic needs and diseased conditions [18,19]. The efficacy of microbiota-targeted therapies will need to be assessed using new diagnostic tools that measure community function rather than strictly microbial composition, including the temporal response of a microbial community to the introduction of external agents such as antibiotics or probiotics [20].

The human intestinal tract has been colonized by thousands of species of bacteria during the coevolution of man and microbes [21,22]. Gut-borne microbes outnumber the total number of cells in the human body by a factor of ten [23]. Recent metagenomic analysis of the human gut microbiota has revealed the presence of some 3.3 million genes, as compared to the mere 23 thousand known genes of the human body [24-26]. Microbial communities carry out the majority of the biochemical activity on the planet, and they play integral roles in metabolism and immune homeostasis in human physiology and function [27]. Evidence for various beneficial roles of the intestinal microbiota and, concomitantly, probiotic microbes in human health and disease has been expanding rapidly in recent years [28-31]. Beneficial impacts have been noted for gastrointestinal disorders [32-36] (incl. functional bowel disorders [37], inflammatory bowel disease and ulcerative colitis [38-41], diarrhea [42], and others),

cardiovascular disorders [43-46], cancer [47-49], hepatic function [50], metabolic conditions (incl. diabetes [51-53], obesity [54-60], lipid metabolism [61,62]), neuropsychiatric disorders (depression and anxiety [63,64], chronic fatigue syndrome [65,66], autism [67-69], psychoneuroimmunity [70-72], and neurodermatology [73]), immune function [74-77] (incl. immunomodulation [78-81], inflammation [82,83], allergies [84], autoimmune disorders [85,86]).

In recent years, the general awareness of the rising global prevalence of kidney disease has been steadily growing among the medical and public health professionals [87-89]. Kidney disease is the eighth leading cause of death in the U.S. [90], with close to 600 000 ESRD patients (most of them on dialysis) and more than 20 million in earlier stages of CKD [91]. As the population continues to age and as the epidemiological shift from acute infectious to chronic metabolic diseases progresses, contributing factors to kidney disease such as obesity, diabetes and high blood pressure have spiraled out of control. People in the U.S. and globally are likely to have a major health crisis in kidney disease.

Over the past 15 years, Kibow Biotech has continuously explored the potential utilization of oral sorbents and probiotics as complementary strategy for CKD. The first patented and proprietary probiotic product formulation to maintain kidney health was developed-Kibow Biotics® (now Renadyl™, containing *S. thermophilus* KB 19, *L. acidophilus* KB

27 and *B. longum* KB 31 strains, a total of 30 billion Colony Forming Units (CFU) per capsule). It uses "enteric toxin removal technology" to specifically target and reduce several uremic toxins that diffuse from circulating blood across the bowel and contribute to CKD. Initial *in vitro* R&D lab studies were performed including the use of a Simulated Human Intestinal Microbial Ecosystem (SHIME), a five-step biochemical reactor to mimic stomach, small intestine, ascending, transverse and descending colonic environments [92]. Further exploratory studies of orally administered probiotic bacteria were performed in 5/6th nephrectomized rats and minipigs [93,94], live cats [95] and dogs with diagnosed kidney failure, and in humans [96,97] with CKD and ESRD [98]. Human clinical trials included several pilot-scale studies to determine whether daily treatment with probiotic bacteria would improve or delay the onset of established signs and symptoms of CKD.

Through all of the studies listed above, the Renadyl™ probiotic formulation has shown the ability to utilize various nitrogenous uremic toxins as nutrients for growth of the beneficial gut microbial population. In this manner, the specifically formulated probiotic microbial strains keep the uremic toxins from accumulating to highly toxic levels in patients with CKD. Unlike many untested probiotic supplements available on the market, Renadyl™ has the advantage of having proven scientific validity. In accordance with its mission to market only the scientifically proven products, Kibow Biotech opted to invest its resources into performing a rigorous clinical trial for specific clinical indication use in CKD, before initiating direct consumer marketing. In December of 2012, the most recent 6-month dose-escalation study in human subjects was completed (Figure 2). The primary goal was to confirm safety and tolerability of several doses of Renadyl™ (from 90 to 180 to 270 billion CFU). Secondary goals were to quantify Quality Of Life (QOL) improvement [99-107], to confirm the product's ability to reduce the levels of commonly known uremic toxins, and to investigate the product's effects on some inflammation and oxidative stress biomarkers.

Methods

Study design

An open label, dose escalation observational study of an orally administered, strain-specific probiotic formulation (Renadyl™) in CKD Stage 3 and 4 patients with compromised renal function was initiated at the Thomas Jefferson University (TJU) Nephrology Division in Philadelphia, PA, with the enrollment of the first patient in August of 2011. The study was approved by the Institutional Review Board of TJU and written informed consent was obtained from each participant at enrollment. The participants in the study enrolled voluntarily and were prequalified and selected based on prior medical history and the inclusion/exclusion criteria. TJU Institutional Review Board approved this clinical study protocol and a summary is recorded in the NIH registry www.clinicaltrials.gov (Trial #NCT01450657). Primary endpoints were defined as measurable improvement in the levels of biochemical markers, such as urea and creatinine, in hematological values (CBC) and in hepatological function. Secondary endpoints

included the measurements of Quality Of Life (QOL) parameters in accordance with modified SF36 questionnaire. Finally, tertiary endpoints were measurements of the exploratory biomarkers of inflammation and oxidative stress (indoxyl sulfate, p-cresyl sulfate, serum pentosidine, β-2 microglobulin).

During the screening (T0), each patient was examined and the baseline values were obtained, after which the patient was initiated on the dose of 1 capsule containing 30 billion CFU thrice daily with meals (90 billion CFU/day). (Please refer to Figure 2 for the timeline of the study.) At the end of month 1 (T1), the dose was increased to 2 capsules (180 billion CFUs/day), and at month 2 (T2) – to the maximum of 3 capsules (270 billion CFUs/day) thrice daily with meals. At each visit, participants underwent routine physical examinations and blood draws, as well as were given modified SF-36 QOL questionnaires to complete and were monitored for compliance with the study protocol. The only exception was visit 3 (T3), where no physical examinations or blood draws were performed – only a QOL questionnaire and compliance monitoring were administered. After two months on the maximum dose (T3 and T4), the treatment was discontinued (T4) and the washout period began. Two months later, each patient came for the follow-up visit (T5) and completed the study.

Inclusion and exclusion criteria

The inclusion criteria defined the potential participant population as those between 18 and 75 years of age, diagnosed with CKD stages III or IV (pre-ESRD, stable for at least a year) and with serum creatinine levels greater than 2.5 mg/dL at the time of screening.

The exclusion criteria limited the study population by excluding (1) pregnant or nursing women, (2) those with HIV/AIDs or liver disease diagnoses, (3) those with active dependency on controlled substances and alcohol, (4) those on anticoagulant therapy regimen, (5) those refusing to sign the informed consent form, and (6) those with social conditions or medical debilitating disease/disorder, which, in the judgment of the investigator, would interfere with or serve as a contraindication to adherence to the study protocol or ability to give informed consent or affect overall prognosis of the patient.

Laboratory methods: biochemistry and hematology

Complete blood counts and serum biochemical testing were performed at the Nephrology Division of Thomas Jefferson University. The conversion of serum creatinine level to eGFR data was not considered important, since this study was a short-term 6-month study-a period that is too short for interpreting the eGFR data. Glucose was closely monitored, if the patient was diabetic.

While most blood tests were performed at the hospital, blood serum samples were also obtained and analyzed by Kibow's laboratory for biochemical markers of inflammation and oxidative stress. Serum pentosidine and β-2 microglobulin were analyzed using ELISA kits obtained, respectively, from Novateinbio (catalog number NB-E10646) and R&D systems (Catalog number DBM200). Indoxyl sulphate and p-cresyl sulphate were quantified by HPLC on a Waters Alliance 2695 using published method of Niwa [108] and Meyer [109]. In addition,
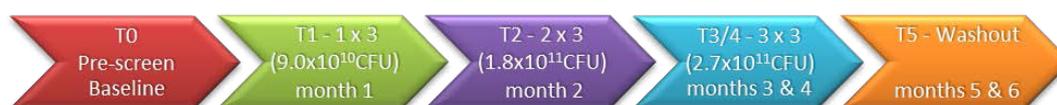

Figure 2: Dose escalation study design.

fecal samples were also obtained and analyzed at Kibow's lab for the presence of the three strains comprising the formulation in Renadyl™ using microbiological methods of plating, enumeration and counting the colonies on appropriate and specific growth media on agar plates.

Statistical methods

All data were analyzed by Dr. Alan Weinberg, an Associate Professor of Biostatistics at the Mount Sinai School of Medicine, using SAS system software V 9.2 (SAS Institute Inc., Cary, NC). All variables – phenol, p-cresol, indoxyl sulfate, BUN and creatinine – were measured over five time points. These repeated measures were each modeled via the PROC MIXED procedure in SAS, similar to an analysis of variance for repeated measures.

Due to the fact that repeated measurements within each patient may be correlated, the Mixed Model procedure allows one to model this "correlation structure", commonly referred to as a covariance pattern. This accurate estimate will allow for improved estimates of the standard errors of measurement, and therefore more powerful tests.

There are a number of various covariance structures to choose from. Three of the more common covariance structures include "Compound Symmetry" (CS), for correlations that are constant for any two points in time, "Auto-Regressive Order One" (AR1), for correlations that are smaller for time points further apart, and "Unstructured" (UN), which has no mathematical pattern within the covariance matrix. Other covariance structures that are usually tested include the Toplitz (TOEP) and the Heterogeneous Compound Symmetry Structure (CSH).

A likelihood ratio test or a procedure known as Akaike's information criterion (AIC) [110] is used to discern which covariance pattern allows for the best fit. We therefore chose the "Compound Symmetry" (CS) structure. Adjusted means at each time point were then generated with adjusted standard errors. P values were not adjusted for multiple comparisons and the inflation of the Type I error.

Results

Out of 31 participants, 28 (90%) completed the study, with additional 2 participants lost to the follow-up. No significant adverse events were noted with dose escalation. All patients tolerated the maximum dosage, with the exception of patient CK020, who reported nausea upon initial use (see Adverse Events section). All study results are presented in Tables 1-4 and Figures 3 through 8. Statistically significant changes were observed in creatinine (Figure 4) (months 2 to 6: -0.23 ± 0.09 mg/dL, p<0.05), C-Reactive Protein (CRP) (mos. 2 to 6: -0.28 ± 0.14 mg/L, p<0.05), hemoglobin (baseline to month 6: 0.35 ± 0.13 mg/dL, p<0.01, months 1 to 6: 0.46 ± 0.13 mg/dL, p<0.001, months 2 to 4: 0.35 ± 0.13 mg/dL, p<0.01, months 2 to 6: 0.58 ± 0.13 mg/dL, p<0.0001) and hematocrit (baseline to month 6: 1.17%, p<0.05, months 1 to 4: 1.00%, p<0.05, months 1 to 5: 1.69%, p<0.001, months 2 to 5: 1.36%, p<0.005). In addition, trends not reaching statistical significance were observed in BUN (baseline to month 4: -3.56 ± 2.07 mg/dL, p<0.09; months 1 to 4: -3.81 ± 2.07 mg/dL, p<0.07), potassium (months 1 to 6: 0.21 ± 0.11 mmol/L, p<0.06, months 2 to 6: 0.19 ± 0.11 mmol/L, p<0.09), hemoglobin (baseline to month 2: 0.23 ± 0.13 mg/dL, p<0.08, months 1 to 4: 0.23 ± 0.13 mg/dL, p<0.09, months 4 to 6: 0.23 ± 0.13 mg/dL, p<0.09) and CRP (baseline to month 2: 0.23 ± 0.14 mg/L, p<0.095). QOL results (Tables 3 and 4, Figures 6 and 8) indicated improvement in physical functioning (baseline to month 6, p<0.05), a trend toward reduction of pain (baseline to month 6, p<0.08), with no significant change in mental, emotional and social well-being.

	Month	N	Mean	Std Dev	Min	Max
BUN	0	28	48.82	17.91	16.00	88.00
	1	28	49.07	20.02	13.00	100.00
	2	28	45.96	19.95	15.00	107.00
	4	27	44.85	14.72	14.00	72.00
	6	26	46.50	17.63	15.00	80.00
Creat.	0	28	2.38	0.86	1.20	4.60
	1	28	2.38	0.82	1.10	4.20
	2	28	2.31	0.91	0.90	4.00
	4	27	2.29	0.89	1.00	4.10
	6	26	2.51	1.13	1.00	6.20
CRP	0	27	0.44	0.48	0.10	2.30
	1	2	0.20	0.28	0.00	0.40
	2	2	0.15	0.21	0.00	0.30
	4	25	0.40	0.33	0.10	1.20
	6	25	0.50	0.48	0.10	1.90
HGB	0	28	10.79	1.42	8.40	14.60
	1	28	10.68	1.53	8.30	14.70
	2	28	10.55	1.39	8.20	13.70
	4	27	10.93	1.50	8.10	14.10
	6	26	11.06	1.40	9.10	14.50
K	0	27	4.52	0.82	2.60	5.90
	1	28	4.56	0.75	2.70	5.90
	2	28	4.54	0.72	3.10	6.00
	4	27	4.44	0.74	2.60	6.50
	6	26	4.39	0.45	3.50	5.20

Table 1: Clinical Lab Data - The MEANS Procedure.

CRP was not measured in months 1 and 2 (T1 and T2, please refer to Figure 2), with the exception of the first two patients. These two patients' data from T1 and T2 were included in our statistical analysis. As the graph of CRP means in Figure 5 shows, this inclusion suggests that between months 2 and 4, when the patients were on the highest dose of Renadyl™, the levels of CRP actually increased. However, it would be more accurate to compare baseline and month 4 values, as they represent the data from all patients. If such comparison is made, it indicates that the mean value of CRP decreased slightly between baseline and month 4, and then started to rise during the washout period, when Renadyl™ supplementation was withdrawn.

Patient baseline demographics and epidemiology

Among the 28 participants that completed the study, the average age was 58 (range 22-77), the predominant sex was female (n=18, 64.3%). Systolic blood pressure (BP) averaged at 133 mmHg (range 110-190 mmHg), diastolic BP – 71 mmHg (50-100 mmHg), and pulse – 72 min (50-92 min). Most patients' medical history included at least one of the following conditions: hypertension (n=23, 82.1%), diabetes (n=12, 42.9%, one of them borderline), dyslipidemia (n=18, 64.3%, includes hypercholesterolemia – 11, hypertriglyceridemia – 1, and hyperlipidemia – 6), and anemia (n=11, 39.3%). In addition, a sizeable proportion of the population exhibited such conditions as gastric esophageal reflux disorder (GERD, n=8, 28.6%), gout (n=5, 17.9%) and (auto) immune disorders (n=9, 32.1%, including allergic rhinitis – 3, seasonal allergies – 3, lupus – 2, asthma – 3, sarcoidosis – 1). A minority of participants also exhibited potassium imbalance (hyperkalemia – n=3, 10.7%; hypokalemia – n=1, 3.6%) Figure 7, endocrine dysregulation (hyperpara-/hypothyroidism – n=3 each, 10.7%), polycystic kidney disease (PKD, n=3, 10.7%), or glomerulonephritis (n=3, 10.7%). All medications, prescribed and administered to each patient prior to the initiation of the study and the Renadyl™ regimen, were either continued without change or reassessed and substituted by

	Months	Estimate	Std. Error	DF	t value	Pr > |t|		Months	Estimate	Std. Error	DF	t value	Pr > |t|
BUN	0 to 1	-0.2500	2.0477	105	-0.12	0.9031	**Creat.**	0 to 1	5.55x10^{-17}	0.08810	105	0.00	1.0000
	0 to 2	2.8571	2.0477	105	1.40	0.1659		0 to 2	0.06071	0.08810	105	0.69	0.4923
	0 to 4	3.5557	2.0710	105	1.72	**0.0889**		0 to 4	0.05658	0.08911	105	0.63	0.5268
	0 to 6	1.9326	2.0958	105	0.92	0.3586		0 to 6	-0.1682	0.09018	105	-1.87	0.0649
	1 to 2	3.1071	2.0477	105	1.52	0.1322		1 to 2	0.06071	0.08810	105	0.69	0.4923
	1 to 4	3.8057	2.0710	105	1.84	**0.0689**		1 to 4	0.05658	0.08911	105	0.63	0.5268
	1 to 6	2.1826	2.0958	105	1.04	0.3001		1 to 6	-0.1682	0.09018	105	-1.87	0.0649
	2 to 4	0.6986	2.0710	105	0.34	0.7365		2 to 4	-0.00413	0.08911	105	-0.05	0.9631
	2 to 6	-0.9245	2.0958	105	-0.44	0.6600		**2 to 6**	-0.2290	0.09018	105	-2.54	**0.0126**
	4 to 6	-1.6231	2.1194	105	-0.77	0.4455		**4 to 6**	-0.2248	0.09120	105	-2.47	**0.0153**
CRP	0 to 1	0.1811	0.1356	49	1.33	0.1881	**HGB**	0 to 1	0.1071	0.1284	105	0.83	0.4057
	0 to 2	0.2311	0.1356	49	1.70	**0.0948**		0 to 2	0.2321	0.1284	105	1.81	0.0734
	0 to 4	0.02527	0.04630	49	0.55	0.5877		0 to 4	-0.1182	0.1298	105	-0.91	0.3647
	0 to 6	-0.04454	0.04630	49	-0.96	0.3408		**0 to 6**	-0.3527	0.1314	105	-2.68	**0.0084**
	1 to 2	0.05000	0.1638	49	0.31	0.7615		1 to 2	0.1250	0.1284	105	0.97	0.3323
	1 to 4	-0.1558	0.1358	49	-1.15	0.2569		1 to 4	-0.2253	0.1298	105	-1.74	0.0855
	1 to 6	-0.2256	0.1358	49	-1.66	0.1031		**1 to 6**	-0.4599	0.1314	105	-3.50	**0.0007**
	2 to 4	-0.2058	0.1358	49	-1.52	0.1361		**2 to 4**	-0.3503	0.1298	105	-2.70	**0.0081**
	2 to 6	-0.2756	0.1358	49	-2.03	**0.0479**		**2 to 6**	-0.5849	0.1314	105	-4.45	**<0.0001**
	4 to 6	-0.06981	0.04769	49	-1.46	0.1496		4 to 6	-0.2345	0.1329	105	-1.77	0.0805
K	0 to 1	-0.04319	0.1106	104	-0.39	0.6970							
	0 to 2	-0.02177	0.1106	104	-0.20	0.8444							
	0 to 4	0.09543	0.1119	104	0.85	0.3956							
	0 to 6	0.1699	0.1132	104	1.50	**0.1363**							
	1 to 2	0.02143	0.1094	104	0.20	0.8451							
	1 to 4	0.1386	0.1106	104	1.25	0.2130							
	1 to 6	0.2131	0.1119	104	1.90	**0.0597**							
	2 to 4	0.1172	0.1106	104	1.06	0.2919							
	2 to 6	0.1917	0.1119	104	1.71	**0.0898**							
	4 to 6	0.07449	0.1132	104	0.66	0.5119							

Table 2: Clinical Lab Data - Differences of Least Squares Means.

an alternative therapeutic modality, in accordance with the accepted standards of care.

Adverse events

The study was monitored according to the best clinical practices as per the nephrology institutional clinical standards of Thomas Jefferson University, Philadelphia, PA. With one exception, most events were judged as non-serious, not related to the study, and non-severe. The one exception was a patient who experienced mild nausea after starting on the study formulation. In all cases, appropriate actions were taken to resolve the underlying condition, to the best of the clinical staff's abilities. One of the patients was taken off the study formulation for one week, when experiencing sore throat symptoms. One patient failed the inclusion criterion 3. Another patient was admitted to a hospital on June 8th, 2012, diagnosed with congestive heart failure, and subsequently discharged on June 15th. Following this episode, the patient decided to withdraw from the study due to the required multiple cardiologist visits, involving blood draws each time. This patient did not want to also have blood drawn at study visits, because the concept appeared overwhelming. Thus, a total of three patients out of thirty one who enrolled did not complete the study.

Discussion

Analysis of the study cohort indicated that administration of the probiotic mixture induced statistically significant improvements in serum creatinine, C-reactive protein, hemoglobin, hematocrit and physical functioning. Trends toward reduction were noted in the levels of BUN, potassium, hemoglobin, CRP and pain.

Toxicity due to the accumulation of various uremic toxins is a concern for CKD and ESRD patient populations. Concentrations of uremic solutes have been shown to increase as the disease progresses from CKD to ESRD [99]. The European Toxin workgroup (EUTOX) has classified many uremic toxins based on their molecular weights and their protein binding property [100]. Though urea is generally non-toxic, it can degrade to the highly toxic cyanate, which, in turn, binds to proteins by carbamylation, including serum albumin, and modifies them. Recent studies by Berg et al. [101]. Have shown that carbamylated serum albumin is a risk factor for mortality in patients with kidney failure. As early as 1998, it was shown that CKD patients are at a higher risk of cardiovascular problems. Death due to cardiovascular disease is higher by 10 to 20 times in these patients as compared with the general population [102]. Therefore, some believe it to be necessary to reduce the levels of urea in chronic renal failure patients by medication or other interventions and strategies, such as a probiotic therapy (some representatives of the lactic acid bacteria population have the capacity to metabolize urea).

Probiotics and prebiotics have been reported to enhance intestinal health for centuries [103]. Scientific proof has now been obtained that confirms their positive effects on human health in general [104]. Recently, the application of probiotics in various diseases has intensified, as understanding of how the gut microbiota shapes human health and how their composition changes in unhealthy populations is gained through extensive research efforts [105].

	Month	N	Mean	Std Dv	Min	Max
Physical Function	0	23	60.27	24.46	10.00	100.00
	4	23	69.01	25.49	20.00	100.00
	6	23	71.16	24.94	20.00	100.00
Pain	0	23	36.20	27.02	0	87.50
	4	23	33.91	29.91	0	100.00
	6	23	26.63	23.39	0	77.50

Table 3: Quality of life – The MEANS Procedure.

	Months	Estimate	Std. Error	DF	t value	Pr > |t|
Physical Function	0 to 4	-8.7379	4.9615	44	-1.76	0.0852
	0 to 6	-10.8877	4.9615	44	-2.19	**0.0335**
	4 to 6	-2.1498	4.9615	44	-0.43	0.6669
Pain	0 to 4	2.2826	5.1966	44	0.44	0.6626
	0 to 6	9.5652	5.1966	44	1.84	**0.0724**
	4 to 6	7.2826	5.1966	44	1.40	0.1681

Table 4: Quality of life – Differences of Least Squares Means.
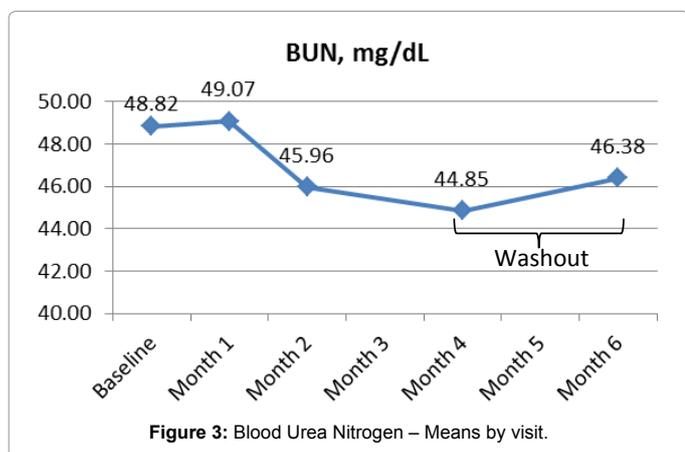

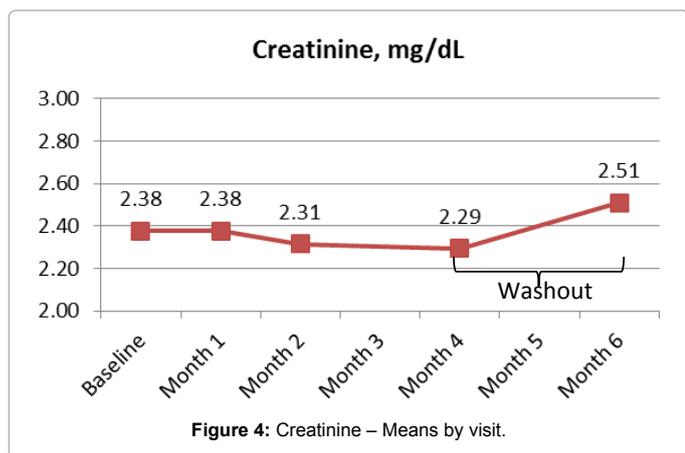**Figure 3:** Blood Urea Nitrogen – Means by visit.

Figure 4: Creatinine – Means by visit.

Our previous multicenter trials in patient cohorts with CKD stages 3 and 4 showed that the concentrations of uremic toxins – principally urea, uric acid and creatinine – were reduced, when the study subjects were treated with Kibow Biotics® (now Renadyl™) at a dose of 90 billion CFU per day [97]. The present study was conducted to assess the safety of Renadyl™ in a dose escalation regimen. As the results show (Figure 3), the levels of urea started decreasing as the patients began taking Renadyl™. These levels decreased until month 4,

indicating that the probiotic formulation could reduce the toxic levels of urea. Upon discontinuation, after a two-month washout period, the urea concentrations increased again, indicating that it was indeed the bacterial strains in Renadyl™ that were able to metabolize urea.

Recent studies accomplished at Stanford University have indicated that metabolites that are also uremic toxins, such as phenol and indoles, come from colonic fermentation [106]. In CKD, protein digestion is impaired. Undigested proteins enter the large intestine and are fermented by pathogenic bacteria, thus leading to the formation of indoles and phenols, which are sulphated and converted to indoxyl and
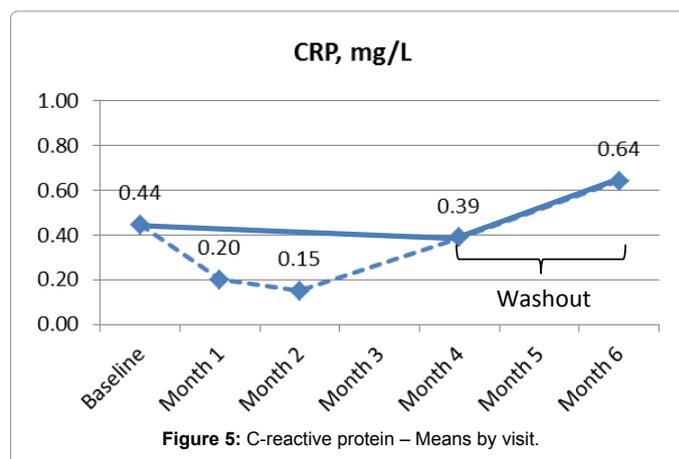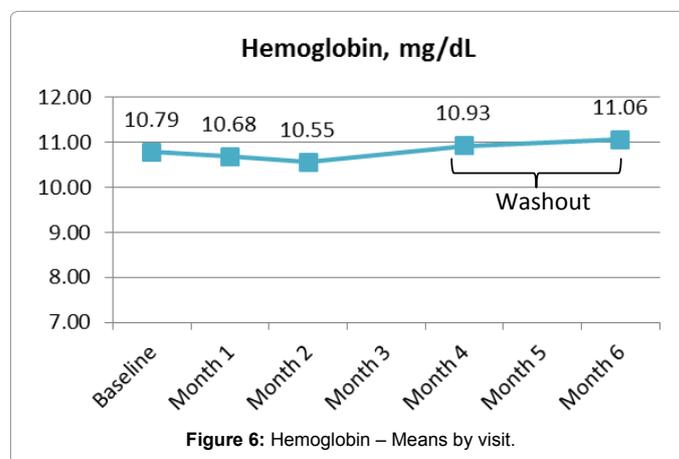

Figure 5: C-reactive protein – Means by visit.
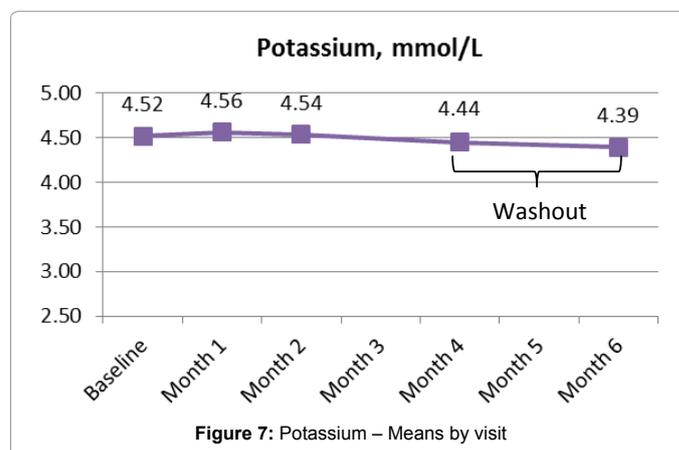

Figure 6: Hemoglobin – Means by visit.

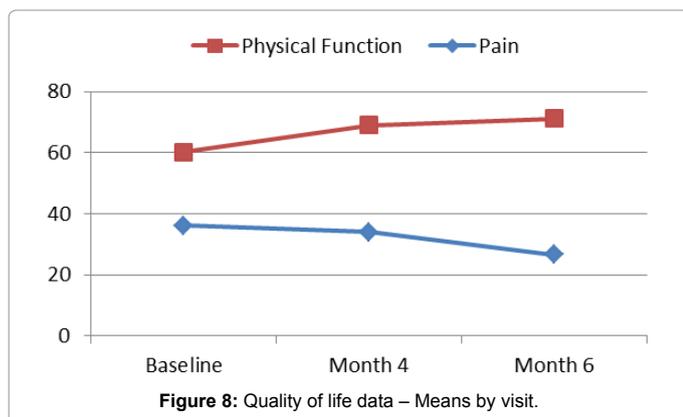**Figure 7:** Potassium – Means by visit

Figure 8: Quality of life data – Means by visit.

p-cresyl sulphates. Studies by Vaziri et al. have shown that renal failure patients have an imbalanced gut microflora [107].

This study investigated whether Renadyl™ supplementation could lower the concentrations of these putrefactants. Indoles, for example, are produced from amino acid tryptophan, and the administration of probiotics might be able to alter their generation rates. Although indole generation tended to decrease in this study, the results varied widely and did not reach statistical significance (data omitted). The reason for this variation could have been the suboptimal procedure, which necessitated obtaining the samples from the study site at Thomas Jefferson University and subsequently conducting assays in the Kibow's laboratory. Performing all laboratory analyses on the study site would have been optimal in a clinical study such as this, to avoid unnecessary time lapses and factors related to transportation.

Study limitations

The most significant limitation was sample size, affecting the statistical power of the study results. Due to the prohibitive costs associated with conducting clinical trials, a larger sample size was not feasible to attain under this particular study design. To confirm the results, especially those trends not reaching the level of statistical significance, a study with a larger sample size is warranted. Other limitations included the laboratory arrangements – clinical samples for inflammatory/oxidative stress marker analysis were delivered after long periods of storage, therefore not fresh. This may be one of the reasons for highly variable and inconsistent results that preclude any conclusions.

No major issues were encountered with regard to patient adherence with the treatment regimen. Average adherence amounted to 94%, with a standard deviation of 17%.

Conclusions

Administration of Renadyl™ for 4 months in CKD Stage 3 and 4 patients, reaching the highest dose of 270 CFUs per day, appears safe and well-tolerated. Statistically significant improvements were noted in creatinine, C-reactive protein, hemoglobin, and physical functioning. Trends toward reduction were noted in BUN and pain. Other markers of inflammation and oxidative stress exhibited a lot of variation. The study did not have sufficient statistical power to ascertain changes in other molecular toxins.

Disclosure

Kibow Biotech Inc., a privately owned biotechnology company focused on probiotics, financed this clinical investigation at the Thomas Jefferson University. Part of the data was also obtained in Kibow's own fully equipped research laboratories.

Prior Abstract Publication

A preliminary analysis of an incomplete data set from this trial (n=24) was presented in May 2013 as a poster at the World Congress of Nephrology in Hong Kong. A preliminary analysis of the completed data set (n=28) was presented in June 2013 at the conference "Probiotics, Prebiotics, and the Host Microbiome: The Science of Translation" at the New York Academy of Sciences.

Sources of Support

References

1. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125: 1401-1412.

2. Schepers E, Glorieux G, Vanholder R (2010) The gut: the forgotten organ in uremia? Blood Purif 29: 130-136.

3. Anders HJ, Andersen K, Stecher B (2013) The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. Kidney Int 83: 1010-1016.

4. Stenvinkel P (2006) Inflammation in end-stage renal disease: the hidden enemy. Nephrology (Carlton) 11: 36-41.

5. Shah SV, Baliga R, Rajapurkar M, Fonseca VA (2007) Oxidants in chronic kidney disease. J Am Soc Nephro l 18: 16-28.

6. Karamouzis I, Sarafidis PA, Karamouzis M, Iliadis S, Haidich AB, et al. (2008) Increase in oxidative stress but not in antioxidant capacity with advancing stages of chronic kidney disease. Am J Nephrol 28: 397-404.

7. The clinical trials. gov website is a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world.

8. Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. Nature 486: 207-214.

9. Human Microbiome Project Consortium (2012) A framework for human microbiome research. Nature 486: 215-221.

10. Huse SM, Ye Y, Zhou Y, Fodor AA (2012) A core human microbiome as viewed through 16S rRNA sequence clusters. PLoS One 7: e34242.

11. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, et al. (2009) A core gut microbiome in obese and lean twins. Nature 457: 480-484.

12. Li K, Bihan M, Yooseph S, Methé BA (2012) Analyses of the microbial diversity across the human microbiome. PLoS One 7: e32118.

13. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, et al. (2012) Human gut microbiome viewed across age and geography. Nature 486: 222-227.

14. Blumberg R, Powrie F (2012) Microbiota, disease, and back to health: a metastable journey. Sci Transl Med 4: 137rv7.

15. Gordon JI, Dewey KG, Mills DA, Medzhitov RM (2012) The human gut microbiota and undernutrition. Sci Transl Med 4: 137ps12.

16. Gordon JI (2012) Honor thy gut symbionts redux. Science 336: 1251-1253.

17. Benezra A, DeStefano J, Gordon JI (2012) Anthropology of microbes. Proc Natl Acad Sci U S A 109: 6378-6381.

18. Holmes E, Kinross J, Gibson GR, Burcelin R, Jia W, et al. (2012) Therapeutic modulation of microbiota-host metabolic interactions. Sci Transl Med 4: 137rv6.

19. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK (2011) Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol 19: 349-359.

20. Lemon KP, Armitage GC, Relman DA, Fischbach MA (2012) Microbiota-targeted therapies: an ecological perspective. Sci Transl Med 4: 137rv5.

21. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, et al. (2012) Host-gut microbiota metabolic interactions. Science 336: 1262-1267.

22. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489: 220-230.

23. Kunz C, Kuntz S, Rudloff S (2009) Intestinal flora. Adv Exp Med Biol 639: 67-79.

24. Relman DA (2012) Microbiology: Learning about who we are. Nature 486: 194-195.

25. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, et al. (2006) Metagenomic analysis of the human distal gut microbiome. Science 312: 1355-1359.

26. Frank DN, Pace NR (2008) Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol 24: 4-10.

27. Abubucker S, Segata N, Goll J, Schubert AM, Izard J, et al. (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol 8: e1002358.

28. Parvez S, Malik KA, Ah Kang S, Kim HY (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100: 1171-1185.

29. Kinross JM, Darzi AW, Nicholson JK (2011) Gut microbiome-host interactions in health and disease. Genome Med 3: 14.

30. Murthy M, Venkitanarayan K, Rangavajhyala N, Shahani K (2000) Delineation of beneficial characteristics of effective probiotics. JAMA 3: 38-43.

31. de Roos NM, Katan MB (2000) Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 71: 405-411.

32. Girardin M, Seidman EG (2011) Indications for the use of probiotics in gastrointestinal diseases. Dig Dis 29: 574-587.

33. Zuccotti GV, Meneghin F, Raimondi C, Dilillo D, Agostoni C, et al. (2008) Probiotics in clinical practice: an overview. J Int Med Res 36 Suppl 1: 1A-53A.

34. Ghosh AR (2012) Appraisal of Probiotics and Prebiotics in Gastrointestinal Infections. WebMedCentral Gastroenterology 3: WMC003796.

35. DuPont AW, DuPont HL (2011) The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol 8: 523-531.

36. Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, et al. (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121: 580-591.

37. Ringel-Kulka T, Palsson OS, Maier D, Carroll I, Galanko JA, et al. (2011) Probiotic bacteria Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. J Clin Gastroenterol 45: 518-525.

38. Kim N, Kunisawa J, Kweon MN, Eog Ji G, Kiyono H (2007) Oral feeding of Bifidobacterium bifidum (BGN4) prevents CD4(+) CD45RB(high) T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clin Immunol 123: 30-39.

39. Marteau P (2006) Probiotics, prebiotics, synbiotics: ecological treatment for inflammatory bowel disease? Gut 55: 1692-1693.

40. Fedorak RN, Madsen KL (2004) Probiotics and the management of inflammatory bowel disease. Inflamm Bowel Dis 10: 286-299.

41. Hart AL, Stagg AJ, Kamm MA (2003) Use of probiotics in the treatment of inflammatory bowel disease. J Clin Gastroenterol 36: 111-119.

42. Delia P, Sansotta G, Donato V, Frosina P, Messina G, et al. (2007) Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 13: 912-915.

43. Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, et al. (2008) Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 453: 396-400.

44. Lam V, Su J, Koprowski S, Hsu A, Tweddell JS, et al. (2012) Intestinal microbiota determine severity of myocardial infarction in rats. FASEB J 26: 1727-1735.

45. Hazen SL, Smith JD (2012) An antiatherosclerotic signaling cascade involving intestinal microbiota, microRNA-10b, and ABCA1/ABCG1-mediated reverse cholesterol transport. Circ Res 111: 948-950.

46. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, et al. (2013) Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A 110: 4410-4415.

47. de Moreno de LeBlanc A, Matar C, Perdigón G (2007) The application of probiotics in cancer. Br J Nutr 98 Suppl 1: S105-110.

48. Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, et al. (2007) Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. Am J Clin Nutr 85: 488-496.

49. Osterlund P, Ruotsalainen T, Korpela R, Saxelin M, Ollus A, et al. (2007) Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. Br J Cancer 97: 1028-1034.

50. Stadlbauer V, Mookerjee RP, Hodges S, Wright GA, Davies NA, et al. (2008) Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. J Hepatol 48: 945-951.

51. Yadav H, Jain S, Sinha PR (2007) Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition 23: 62-68.

52. Asemi Z, Khorrami-Rad A, Alizadeh SA, Shakeri H, Esmaillzadeh A (2013) Effects of synbiotic food consumption on metabolic status of diabetic patients: A double-blind randomized cross-over controlled clinical trial. Clin Nutr.

53. Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, et al. (2003) Antidiabetic effect of Lactobacillus GG in streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem 67: 1421-1424.

54. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, et al. (2013) Richness of human gut microbiome correlates with metabolic markers. Nature 500: 541-546.

55. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, et al. (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 102: 11070-11075.

56. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 104: 979-984.

57. Mestdagh R, Dumas ME, Rezzi S, Kochhar S, Holmes E, et al. (2012) Gut microbiota modulate the metabolism of brown adipose tissue in mice. J Proteome Res 11: 620-630.

58. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334: 105-108.

59. Cani PD, Delzenne NM (2009) The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 15: 1546-1558.

60. Cani PD, Delzenne NM (2009) Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol 9: 737-743.

61. Schaafsma G, Meuling WJ, van Dokkum W, Bouley C (1998) Effects of a milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in male volunteers. Eur J Clin Nutr 52: 436-440.

62. Kiessling G, Schneider J, Jahreis G (2002) Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. Eur J Clin Nutr 56: 843-849.

63. Logan AC, Katzman M (2005) Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 64: 533-538.

64. Benton D, Williams C, Brown A (2007) Impact of consuming a milk drink containing a probiotic on mood and cognition. Eur J Clin Nutr 61: 355-361.

65. Logan AC, Venket Rao A, Irani D (2003) Chronic fatigue syndrome: lactic acid bacteria may be of therapeutic value. Med Hypotheses 60: 915-923.

66. Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, et al. (2009) A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1: 6.

67. Mulle JG, Sharp WG, Cubells JF (2013) The gut microbiome: a new frontier in autism research. Curr Psychiatry Rep 15: 337.

68. Bolte ER (1998) Autism and Clostridium tetani. Med Hypotheses 51: 133-144.

69. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, et al. (2002)

Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35: S6-6S16.

70. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, et al. (2010) Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170: 1179-1188.

71. Gruenwald J, Graubaum HJ, Harde A (2002) Effect of a probiotic multivitamin compound on stress and exhaustion. Adv Ther 19: 141-150.

72. Lyte M (2011) Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. Bioessays 33: 574-581.

73. Bowe WP, Logan AC (2011) Acne vulgaris, probiotics and the gut-brain-skin axis - back to the future? Gut Pathog 3: 1.

74. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S (2001) Probiotics: effects on immunity. Am J Clin Nutr 73: 444S-450S.

75. Ménard S, Laharie D, Asensio C, Vidal-Martinez T, Candalh C, et al. (2005) Bifidobacterium breve and Streptococcus thermophilus secretion products enhance T helper 1 immune response and intestinal barrier in mice. Exp Biol Med (Maywood) 230: 749-756.

76. Benyacoub J, Czarnecki-Maulden GL, Cavadini C, Sauthier T, Anderson RE, et al. (2003) Supplementation of food with Enterococcus faecium (SF68) stimulates immune functions in young dogs. J Nutr 133: 1158-1162.

77. Chin J, Turner B, Barchia I, Mullbacher A (2000) Immune response to orally consumed antigens and probiotic bacteria. Immunol Cell Biol 78: 55-66.

78. Galdeano CM, de Moreno de LeBlanc A, Vinderola G, Bonet ME, Perdigón G (2007) Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clin Vaccine Immunol 14: 485-492.

79. Christensen HR, Larsen CN, Kaestel P, Rosholm LB, Sternberg C, et al. (2006) Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. FEMS Immunol Med Microbiol 47: 380-390.

80. Christensen HR, Frøkiaer H, Pestka JJ (2002) Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J Immunol 168: 171-178.

81. Matsuzaki T, Chin J (2000) Modulating immune responses with probiotic bacteria. Immunol Cell Biol 78: 67-73.

82. Riedel CU, Foata F, Philippe D, Adolfsson O, Eikmanns BJ, et al. (2006) Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation. World J Gastroenterol 12: 3729-3735.

83. Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, et al. (2004) Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut 53: 821-828.

84. Broekaert IJ, Walker WA (2006) Probiotics and chronic disease. J Clin Gastroenterol 40: 270-274.

85. Berer K, Krishnamoorthy G (2012) Commensal gut flora and brain autoimmunity: a love or hate affair? Acta Neuropathol 123: 639-651.

86. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, et al. (2011) Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 479: 538-541.

87. Grams ME, Chow EK, Segev DL, Coresh J (2013) Lifetime incidence of CKD stages 3-5 in the United States. Am J Kidney Dis 62: 245-252.

88. Perazella MA, Khan S (2006) Increased mortality in chronic kidney disease: a call to action. Am J Med Sci 331: 150-153.

89. Ayodele OE, Alebiosu CO (2010) Burden of chronic kidney disease: an international perspective. Adv Chronic Kidney Dis 17: 215-224.

90. CDC FastStats for 2010. Leading Causes of Death in the U.S.

91. USRDS Annual Data Report 2012, Volume 1: Atlas of CKD and Volume 2: Atlas of ESRD.

92. Ranganathan N, Patel BG, Ranganathan P, Marczely J, Dheer R, et al. (2006) In vitro and in vivo assessment of intraintestinal bacteriotherapy in chronic kidney disease. ASAIO J 52: 70-79.

93. Ranganathan N, Patel B, Ranganathan P, Marczely J, Dheer R, et al. (2005) Probiotic amelioration of azotemia in 5/6th nephrectomized Sprague-Dawley rats. ScientificWorldJournal 5: 652-660.

94. Ranganathan N, Patel B, Ranganathan P, Marczely J, Dheer R, et al. (2005) Probiotics reduces azotemia in Gottingen mini-pigs. Proceedings of the 3rd World Congress of Nephrology Poster Presentation, Singapore.

95. Palmquist R (2006) A Preliminary Clinical Evaluation of Kibow Biotics®, a Probiotic Agent, on Feline Azotemia. Journal of the American Holistic Medical Association 24(4): 23-7.

96. Ranganathan N, Friedman EA, Tam P, Rao V, Ranganathan P, et al. (2009) Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada. Curr Med Res Opin 25: 1919-1930.

97. Ranganathan N, Ranganathan P, Friedman EA, Joseph A, Delano B, et al. (2010) Pilot study of probiotic dietary supplementation for promoting healthy kidney function in patients with chronic kidney disease. Adv Ther 27: 634-647.

98. Vitetta L, Gobe G (2013) Uremia and chronic kidney disease: the role of the gut microflora and therapies with pro- and prebiotics. Mol Nutr Food Res 57: 824-832.

99. Wu IW, Hsu KH, Lee CC, Sun CY, Hsu HJ, et al. (2011) p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrol Dial Transplant 26: 938-947.

100. Vanholder R, Glorieux G, De Smet R, Lameire N; European Uremic Toxin Work Group (2003) New insights in uremic toxins. Kidney Int Suppl: S6-10.

101. Berg AH, Drechsler C, Wenger J, Buccafusca R, Hod T, et al. (2013) Carbamylation of serum albumin as a risk factor for mortality in patients with kidney failure. Sci Transl Med 5: 175ra29.

102. Foley RN, Parfrey PS, Sarnak MJ (1998) Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol 9: S16-23.

103. Quigley EM (2012) Prebiotics and probiotics: their role in the management of gastrointestinal disorders in adults. Nutr Clin Pract 27: 195-200.

104. Vyas U, Ranganathan N (2012) Probiotics, prebiotics, and synbiotics: gut and beyond. Gastroenterol Res Pract 2012: 872716.

105. Sekirov I, Russell SL, Antunes LC, Finlay BB (2010) Gut microbiota in health and disease. Physiol Rev 90: 859-904.

106. Aronov PA, Luo FJ, Plummer NS, Quan Z, Holmes S, et al. (2011) Colonic contribution to uremic solutes. J Am Soc Nephrol 22: 1769-1776.

107. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, et al. (2013) Chronic kidney disease alters intestinal microbial flora. Kidney Int 83: 308-315.

108. Taki K, Niwa T (2007) Indoxyl sulfate-lowering capacity of oral sorbents affects the prognosis of kidney function and oxidative stress in chronic kidney disease. J Ren Nutr 17: 48-52.

109. Martinez AW, Recht NS, Hostetter TH, Meyer TW (2005) Removal of P-cresol sulfate by hemodialysis. J Am Soc Nephrol 16: 3430-3436.

110. Akaike H (1974) A new look at the statistical model identification. IEEE Transaction on Automatic Control AC-19: 716-23.