So as an example of the important of nutrition in disease I’d like first of all to show the example of the power of epidemiology in colon cancer. And we’ve known for a number of years that colon cancer risk varies in credibly throughout the different parts of the world, and so does diet. And if you look at the epidemiology on this picture here, I don’t know if it goes through – is there an indicator that you can use? This thing. Okay, don’t worry I’ll just talk to it. But basically if you look through the picture of the world there you can see that less developed countries have a low incidence of colon cancer whereas the developed world has a high. And if you look at differences in dietary intake between those populations increased risk has been associated with a high red meat intake, high animal fat intake and high processed meats in particular; whereas there are other things such as fiber, vegetables and calcium which are associated with a low risk. But I will argue that it’s not the diet it’s the diet residue in the microbiota, in other words the milieu interior, whatever is in the colon that actually determines colon cancer risk.

So this brings us to the question of the microbiome. Up until recently we thought that the colon, the microbiome was simply something to allow us to survive in the desert, so we sort of reabsorb fluid, but in fact it’s extremely complex. And basically it consists of a lot of bacteria, but they are all anaerobic and they were difficult to culture and so we didn’t identify them. But with the onset of the genomic era there were major breakthroughs with culture independent techniques which actually look at conserved regions of ribosomal RNA in the 16S region, and basically they are different for different species. So if there is a sequence similarity of over 97% then that’s considered a species, if it’s greater than 95% then it’s a strain.
And this has allowed us to actually count the number of different strains and types of bacteria, and we now know that the numbers of microbes in our gut actually outnumber our total human cell number by 10 to 1, and more so that the amount of DNA that they contain exceed the amount of the DNA that we contain in our own bodies by 100 to 1. There are 800 species, the number is increasing with new indicators, 7000 strains and number 100 trillion with a weight of 1.2 Kg. Basically it has a metabolic mass which is equivalent to the liver. So you can see that living within us is a vibrant mass of DNA which is highly likely to influence our health.

As I’ve said this is the enormous number of bacteria and we know from situations where you, you raise mice for instance in microbiotic conditions where they don’t have any bacteria at all that they basically die very soon of an acute colitis. So it’s absolutely essential for stabilizing the mucosa. We also know that the GI or gut associated lymphoid tissue is trained by the microbiota and so therefore there are a whole host of disease that are immune to mediators which is actually dependent on a healthy microbiota. It produces between 70 and 85% of all the immune cells of the body, it’s the intestinal system is able to protect us from pathogenic microbes, so the community is highly organized and basically polices itself so that pathogens do not have a niche to fill.

All of these compounds present locally are in relationship to the whole organism, so we need to look at ourselves as a complex organism and Lederberg has emphasized the importance of having a broad ecological view of our relationships with our microbes. In his view we are seen as super organisms
composed of an amalgam of both microbial and homo sapiens cells where the survival of the microbe and the human are interdependent.

So when we look at the diversity by those 16S techniques that I mentioned before we see that there is an enormous diversity of the human microbiome, but within that diversity there are two major cleavages. And the one is characterized by firmicutes, I’m going to go back, and the other one by bacteroides. But what’s very important to note is that most of the OTUs, in other words the species, are specific to a single individual, and only 2% were shared in half of the individuals studied. And remarkably no single strain was shared by all individuals. So it’s truly remarkable, and we don’t really understand the meaning of this and why it should be so.

And if you look at a schematic sort of figure of what I think is going on is that we’ve got the microbiome within the lumen there and with all its enormous diversity. Within the microbiome is the metagenome, so all of the microbes have specific genes that have metabolic functions. And what’s remarkable is that despite all this diversity most of the bacteria have core metabolic genes and they are particularly related with fermentation, which makes sense because the way they survive is to eat what we don’t digest, in other words fiber that gets into the colon. So there is more of a core metagenome within our body than microbiome. And basically the metagenome interacts with digestive residues, so things that aren’t digested in the small intestine. And they produce metabolic products which then go into the lumen and that’s called the metabolome. And the metabolome is extremely important because then it allows the gut to interact with the rest of the body because it not
only has effects on the surface of the lumen but they are also absorbed and distributed around the body, so it can have effects in distal parts and therefore effect disease processes in different parts of the body.

There is a recent review article from the Human Microbiome Group in Nature and basically they conclude that taken together with microbial variation observed above throughout the human microbiome, functional variation among individuals might indicate pathways of particular importance in maintaining community structure in the face of personalized immune, environment or dietary exposures among the subjects. Determining the functions of uncharacterized core and variable protein families will be especially essential in understanding the role of the microbiota in health and disease. So you can see that there is a massive unexplored territory there. Why is there this diversity when the genes do the same sort of thing? So there are probably surface features which relate to interconnections between the microbiota and the pilae that allow the bacteria to interact with the mucosa and the surface proteins and so on that actually probably have an important effect on health and disease risk.

This shows the importance of the microbiome or metabolome, and this is some of Jeremy Nicholson’s work from England where they looked at germ free rats and then colonized them with normal flora. And they basically looked at hepatic metabolism and were able to show that there were major changes after colonization which triggered increases in hepatic triglyceride synthesis, the synthesis of bile acids and the absorption of fat. So this is one way of looking at the way that
bacteria can produce a substance that can then go be exported to the body and have profound effects.

We are currently doing studies looking at high risk and low risk communities of colon cancer around the world and looking at the microbiome to see in fact if there is specific biomarkers that yet are undetected that we can actually use clinically to detect or to decide whether somebody is at high or low risk of getting colon cancer.

We recently have written a review with Dr. Vipperla who is one of our hospitalists of the diseases associated with the microbiome. And because of this process of the interaction between the microbiome and the whole, and the host in general there are a whole variety of diseases which now have been linked with the microbiome. Everybody knows about obesity, it’s the biggest problem in America at the moment and it’s been shown that the microbiota is very different and it has a great propensity to be able to salvage undigested nutrients and so therefore become more efficient. The metabolic syndrome has also been associated with high rates of mucosal permeability, again associated with microbiota.

Colon cancer I will talk about in a minute. There is stronger and stronger evidence coming out that disturbances in the microbiota have a role in the development of irritable bowel syndrome, the commonest GI disease in the USA. And then allergic and autoimmune diseases are also – have also been associated with disturbances in the microbiota.
The problem is not just related to colon cancer, it’s related to the way that we practice in hospital, and I’m – my main hospital role is to look after nutrition of the patients and I’ve always been concerned that most of the elemental formula that we use do not contain fiber. And so we looked at the effect this might have on the microbiota. And this was a series of patients, as you can see there are many patients with severe acute pancreatitis which we are probably world leaders in knowing how to feed these patients and they are being fed for considerable lengths of time and they nearly always get into problems with severe diarrhea. And it’s really not surprising because if you look at the medications that they have on the right there, nearly everyone was on an acid suppressant which disturbs the microbiota and also broad spectrum antibiotics. So we are creating havoc with the microbiota. Secondly the elemental diets don’t contain residue and so therefore they are also starved. So not surprisingly when we looked at bacterial numbers in the colon in black in healthy controls and in white are the patients that I’ve just shown. You know they are basically decimated. So it’s really not surprising that patients develop severe diarrhea which in fact increases the risk of developing C- difficile colitis.

And when we looked at short chain fatty acids they basically were grossly depleted. And what was important as we then started supplementing to see if we could induce some sort of change. And we did, we increased short chain fatty acid production which is associated with mucosal health, but most patients stayed on their antibacterials and so therefore the problem remains. It is an unexplored area but it’s one more area that we need to improve the quality of our care.
So going back to the neoplastic situation, how can bacteria induce or suppress neoplastic change? And basically there are toxins that they can produce. In fermentation you produce hydrogen, and hydrogen in high quantities actually disturbs cellular metabolism. Hydrogen sulfide is another terminal product which in experimental situations has been shown to be genotoxic. There are bacterial enzymes that are produced which are inflammatory and there are metabolites such as secondary bile acids which are again carcinogenic.

And then for suppression of cancer risk there is the metabolome itself, the healthy metabolome produces a lot of fermentation, short chain fatty acids, butyrate, lactic acid, methane and the antiinflammatory substances.

And how does this actually relate to cancer? And there is now you know considerable evidence that if you have a chronic inflammatory state it in fact predisposes you to developing neoplastic change. And if you look at the, the functional elements of what happens in an acute injury on the left hand side and on the right hand side a neoplastic process, all the cellular events are exactly the same, it’s just that one is organized and the other one is disorganized. So it’s easy to see that one might well lead onto the other.
And there have been a whole series of studies looking at basically mice that have an increased risk of developing neoplastic change and then giving them different types of bacteria and seeing that it accelerates the neoplastic process.

And then there are human studies with HP for instance is well associated with gastric cancer, IPSID, it’s an overgrowth of bacteria in the bowel is associated with an inflamer of the bowel, EB virus and papilloma virus, all things that produce a chronic inflammation which then can go out of, out of track and become neoplastic.

This is a case of a patient with IPSID that I saw in Africa. It happens in malnourished communities and it’s associated with severe bacterial overgrowth of the small intestine. And if you look in the intestine you can see it’s swollen and nodular. And on the left was a patient who came in with it, on the right is what happened to him 3 months later after simply giving him a course of broad spectrum antibiotics, namely Tetracycline. And it basically reversed the whole process. If left untreated that develops into full grown lymphoma, so again showing that link between bacteria and the neoplastic process.

And there are an increasing number of studies looking at the specific role of specific microbes in carcinogenesis. And this is one study where they looked at the microbes inherent to adenomas and to normal tissue, and basically you can see down on the left corner that the pattern of microbes on colonic polyps and cancer is completely different from normal healthy subjects. And the right
picture shows the close proximity of the microbes to the mucosa, again making it not difficult to understand the importance of bacteria in the etiology.

We have been looking at the products of colonic microbiota that might affect colon cancer risk in high risk and low risk populations, particularly with regard to butyrate, secondary bile acids, folate and biotin. And when we compared Africans, native Africans who rarely get cancer to African-Americans who have got the highest rate of colon cancer in America we found that in fact bacteria associated with butyrate production were much higher in Africans versus African-Americans. And when we looked at their products, their metabolomic products namely short chain fatty acids, again it was not surprising to see that acetate, propionate and butyrate were all significantly higher in Africans than non-Africans, again showing the importance of butyrate.

Butyrate is a remarkable substance, the bacteria – sorry, the colonocytes are not dependent on glucose as its main energy source, it’s actually butyrate. And butyrate is not something in the normal diet, so basically the undigested residues go through to the colon, particularly carbohydrate residues produce sarcolytic fermentation and they produce short chain fatty acids and butyrate. And it’s been shown experimentally that it’s the chief regulator of epithelial growth, it suppresses epithelial proliferation, enhances differentiation. It suppresses the inflammatory response through suppression of TNF alpha and nitric oxide release. It also has tumor suppression functions and increases apoptosis. Furthermore it works together and promotes good growth of other types of bacteria such
as probiotic species such as lactobacilli, which in fact reduce the inflammatory response as well. And then finally studies have shown that it suppresses the growth of C-dificile.

The other major factor is bile acids and if you eat a westernized diet you eat a lot of fat, it increases the synthesis of bile acids by the liver, they go through to the colon. A proportion are not absorbed and they metabolize by bacteria to secondary bile acids which are carcinogenic. And again looking at fecal concentrations they were all considerably higher in African-Americans and Caucasian Americans compared to Africans which are the black dots down below. So you’ve got an excess of bile acids in high risk communities and low amounts of butyrate, therefore increasing your risk.

And there is a most – a very recent publication in Nature again showing the importance of fats and bile acids. If you in fact produce a lot of bile acids a lot of them are conjugated taurine. Taurine goes through the colon, it stimulates the growth of specific types of bacteria which produce hydrogen sulfide and hydrogen sulfide itself is also a carcinogenic substance.

And then finally there is a critical role of the microbiota in production of vitamins, particularly folate, biotin, B12 and thiamin. And these are measurements in colonic evacuates that we’ve performed. And previously it was thought well this is no use because you can’t absorb them from your colon, but studies recently have shown that there are specific transporters for all of these vitamins in the colon, and so therefore it might be extremely important in patients – sorry in subjects from undeveloped countries who do not have a good balanced diet.
And then going on from our studies in Africans and African-Americans it was always thought that the reason why Africans don’t have colon cancer is because of the Burkett’s Hypothesis, they ate ground cereals that were rough, included a lot of fiber. In that bowl there you can see they are hammering beans and maze together to form a local cereal which they would consume. But nowadays in fact you know the population in Africa is massive and so therefore people don’t grow their own foods and they use commercial maze meal which is highly refined and white and low fiber so we all thought well maybe Burkett had the wrong hypothesis after all. But what happens is if you boil maze meal for a long period of time it polymerizes and it polymerizes into resistant starch which produce exactly the same effect. It goes through the colon, increases fermentation.

And if you look at the microbiota between Africans and African-Americans on the left you can see African-Americans on the right, they are basically all individual subjects, there are 12 African-Americans and then 12 native Africans on the right. And that shows the pattern of microbiota in each individual subject. You can see the pattern is completely different between the two different populations, again tying in the concept that bacteria are important in cancer risk.

Now I’ll leave you finally with the thought that you can change, if you change your diet you can change your risk of colon cancer. And these are studies we are currently doing now where we in fact give African-Americans an African diet for 2 weeks and then examine biomarkers of cancer risk. And basically so we are changing them from a high fat diet to a low fat, high fiber diet and we are
examining them – this is the type of food that they eat, lots of beans, so they are a little bit antisocial, but actually tolerate it remarkable well. And we did all the right things, so we measured colonic short chain fatty acids, they all go up, bile acids all go down, and there is an induction of hydrogen acceptors, changes in the microbiota pattern so that you can get rid of toxic hydrogen and toxic hydrogen sulfide. And most importantly when then you look at mucosal biopsies at the end of that intervention period you can measure proliferation rate, which is a biomarker of colon cancer risk, and there is a significant change. I don’t know if it shows well on your photo there but the black dots and the crips indicates stained cells that are rapidly turning over, and on the right is after intervention and you can see that there is a dramatic difference, a highly significant drop in proliferation and therefore cancer risk. And that’s only after 2 weeks of dietary change.

So basically I leave you with this concept that food goes into my ideally – idealized version of the gut, the top part is the small bowel, the bottom is the large bowel, you get digestion in the small bowel, absorption of nutrients which are then used for maintenance of body function. But you get a residue that goes through that is then utilized by the microbiota and depending upon the composition of the microbiota it can either produce good things such as short chain fatty acids and it can detoxify hydrogen and maintain mucosal health; or alternatively if you have a high meat diet, high fat diet it goes the other way, it produces toxic substances which produce chronic inflammation and therefore increase cancer risk.
So in conclusion, a balanced microbiota is absolutely essential for health whether you are talking about the gut, the heart, the brain or anything else, it’s absolutely essential. The effect of diet on cancer risk is indirect and is mediated through the microbiota, and the chief determinants of butyrate and bile acids but the situation is far more complex and it’s the net balance of the good versus the bad metabolites that predicts whether or not you are going to get colon cancer. In most cases - this is the most important take home message, that most cases of colon cancer can be prevented by dietary modification, over 95% of cases. And that means that we’ve got a lot of hope.

The next study is to look at Alaskan native people who have the highest risk of colon cancer in the world, probably because they eat too much meat and too much fat, but I’ll tell you about that next time. And I acknowledge my lab. Thank you very much.