The VDR and Metastasizing Cancers

PRESENTED BY PROF TREVOR MARSHALL
Director, Autoimmunity Research Foundation

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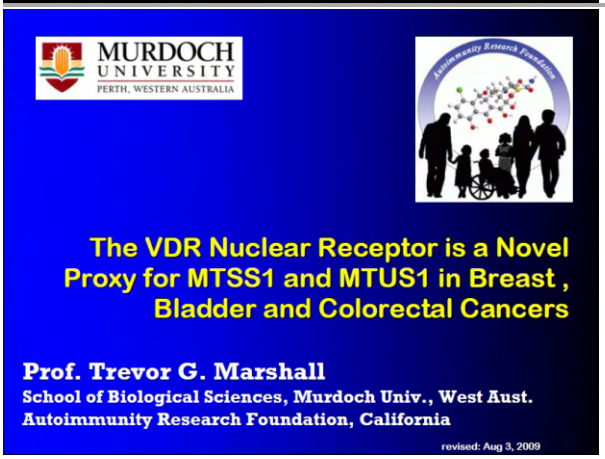
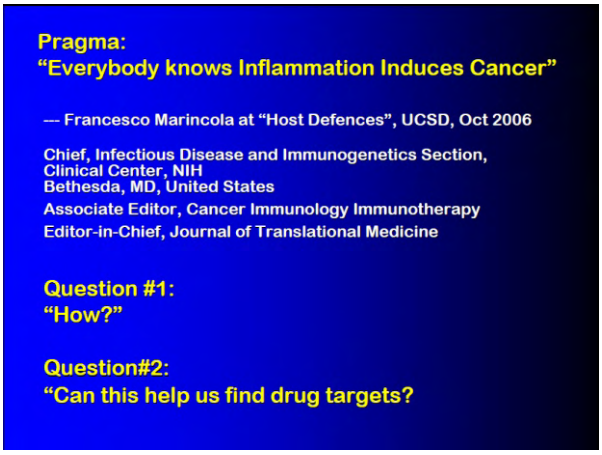
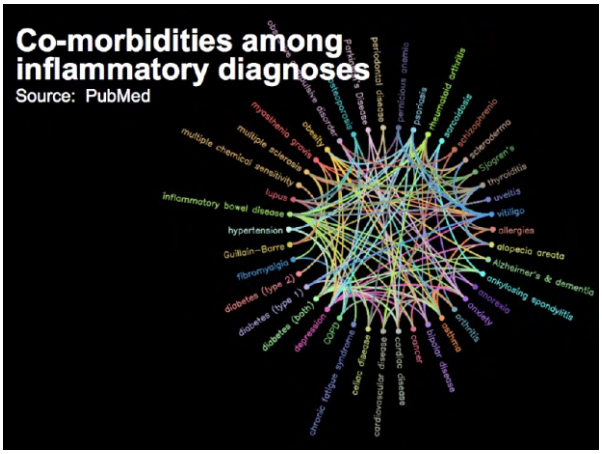






Thank you Mr. Chairman, and thank you all for coming here today.

00:00:30
The VDR Nuclear Receptor is a Novel Proxy for MTSS1 and MTUS1 in Breast, Bladder and Colorectal Cancers

I'm going to talk about Translational medicine, actual clinical data, and how that clinical data sits in the molecular regions.

What I am going to talk about is the VDR nuclear receptor and why it should be pointing us towards MTSS1, particularly, in some of the more common cancers.

00:00:43
Pragma: "Everybody knows Inflammation Induces Cancer"

So I'm going to start off with a Pragma:

"Everybody knows inflammation induces cancer." This was the way that Francesco Marincola of NIH started off his presentation about four years ago at UCSD. It took me totally by surprise and, I think, the rest of the audience. It is a very good way to start.

Everybody knows inflammation induces cancer.

Now what I am going to go on and talk about, though, is question number one: "How?" and question number two: "Can this knowledge, or can this pragma, help us find drug targets?"

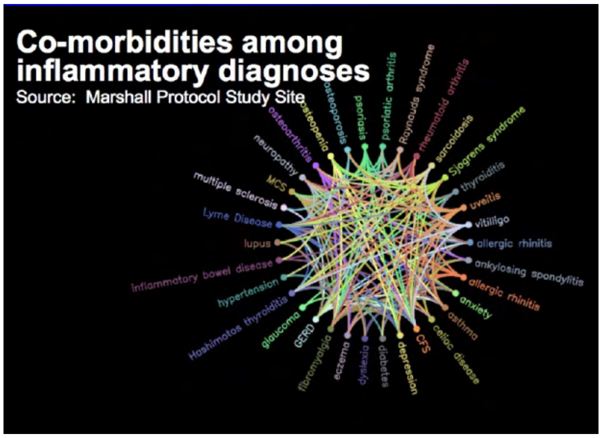
00:01:23
Co-morbidities among inflammatory diagnoses

Well, what is inflammation? There are a lot of inflammatory diseases.

Here is a list of most of the autoimmune inflammatory diseases. There are some other diseases, for example, depression here, which was mentioned in some of the keynotes this morning as being a key problem now in China. And ... and all the standard allergies, Sjogren's, thyroiditis, periodontal disease, inflammatory bowel disease; all of the autoimmune diseases.

Now what is interesting is this chart took a number of studies out of PubMed and it showed the co-morbitidies within the cohorts of people that had, for example, allergies, also typically had thyroiditis; typically had asthma. It shows the co-morbidities.

In other words, inflammation is not unique. It is so easy for us to think that because somebody has got a diagnosis of Rheumatoid Arthritis, that that is the only inflammatory disease they are suffering from. It is very unusual that it is a unique inflammatory disease.

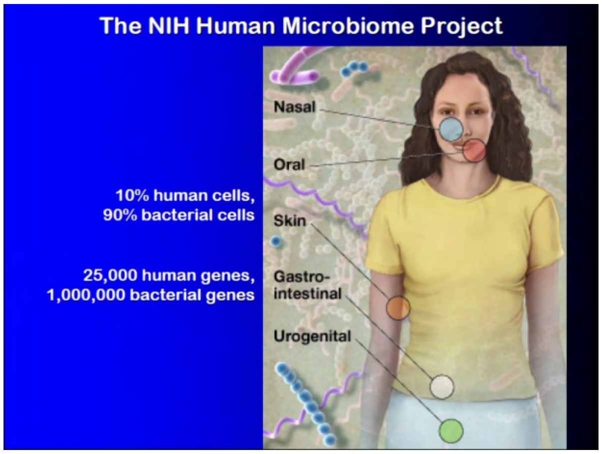


00:02:34
Co-morbidities among inflammatory diagnoses

In fact, in our own study, we did a lot more co-morbidity studies. You can see that the co-morbidity trajectories are even wider than they were in the PubMed search.

Why?

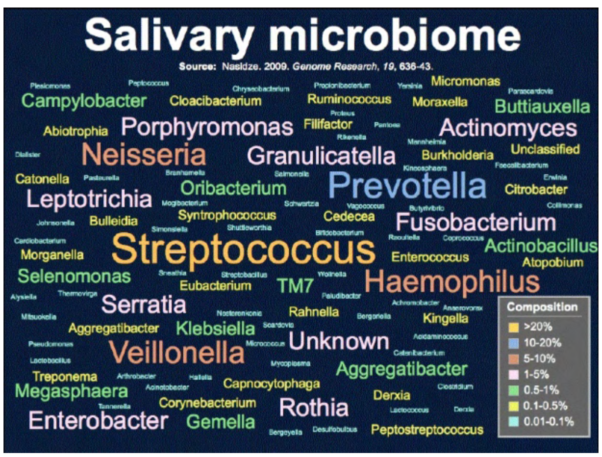
Why could this be?



00:02:49
The NIH Human Microbiome Project

Well, the NIH started up, a year or two ago, the Human Microbiome Project to try and identify whether the human body really is a sterile compartment as clinical medicine has assumed for so many years.

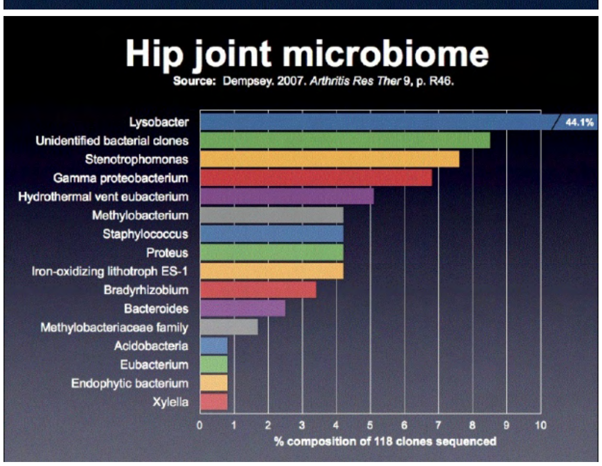
Initially, what is studied is the external cavities, obviously nasal — all the external cavities. Ultimately, the entire body will be studied and the expectation is that there will be about a million bacterial genes found active compared with about 25,000 human genes.



00:03:24
Salivary Microbiome

Some of the microbe biomes are starting to come out now. This is some salivary microbiome, and you can see the normal bacteria you had expected to find in saliva in the mouth: *Streptococcus*, perhaps *Haemophilus*, *Porphyromonas*, of course.

But look, *Neisseria* is there in large concentration, and in smaller concentrations, you have got *Yersinia* and some of the other (*Klebsiella*) really nasty pathogens. Over a hundred species can be isolated using DNA techniques from the saliva of healthy human subjects.

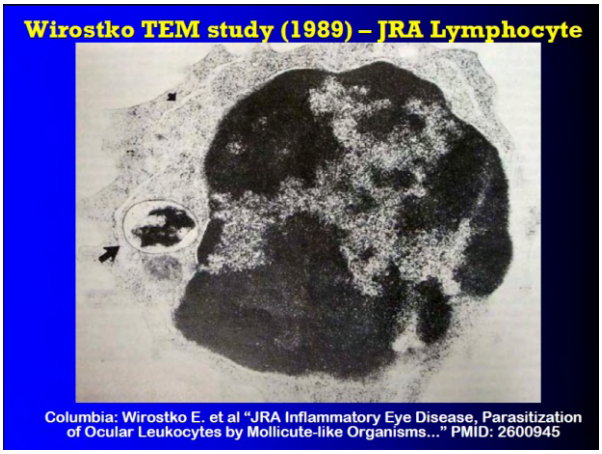


00:04:11
Hip joint microbiome

You go deeper inside the body and go into the hip joint and look at the composition of the hip joint, you find a different makeup; a different mix of the bacteria being involved. You have got *Lysobacter,* which is a gliding bacteria you would expect to find that in biofilms, and that is predominant.

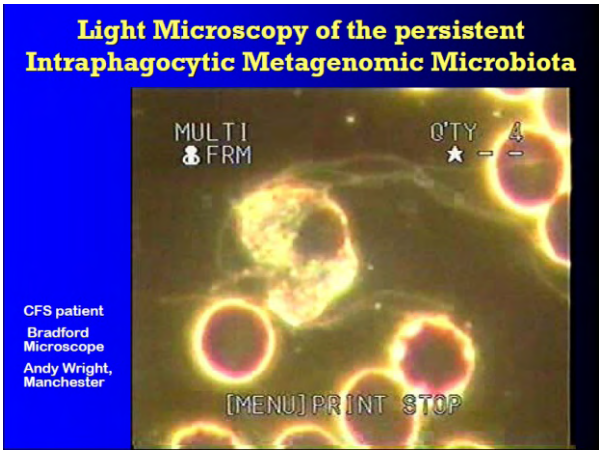
But look, down here you have got hydrothermal-vent eubacterium. That is a eubacterium that is previously identified in hydrothermal vents under the ocean, and here it is showing up in the joints during revision arthroplasty.

Many people say that it is contamination; all these pathogens that are found in the body by researchers are contamination. Well, I'm not quite sure where you will get hydrothermal vent eubacterium in a carefully controlled surgical environment.



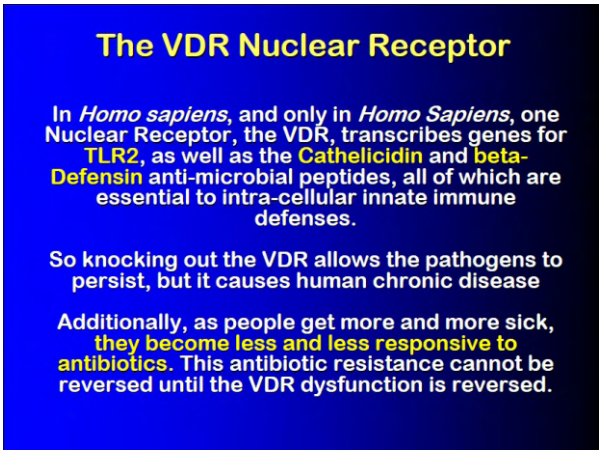
00:05:11
Wirostko TEM study (1989) — JRA Lymphocyte

But there are other microbiomes as well. Wirostko E. et al., at Columbia University back in the 1980's, isolated colonies of bacteria, or certainly, entities that stained as bacteria inside the cytoplasm of phagocytes. This is Juvenile Rheumatoid Arthritis, inflammatory eye disease, Ocular Leukocytes — what I think is a Leukocyte — yes. [There are] molicute-like organisms, biofilm-like organisms. There are some long ones and a round one.



00:05:48
Live action video

We have been able to image the cytoplasms of some of these infected cells exploding, in patients that are very seriously ill, when the blood is allowed to age a little bit — aged about 6 hours. You can see the whole cytoplasm of this monocyte has disintegrated, and throwing out long extruded polymers here, into the blood stream.

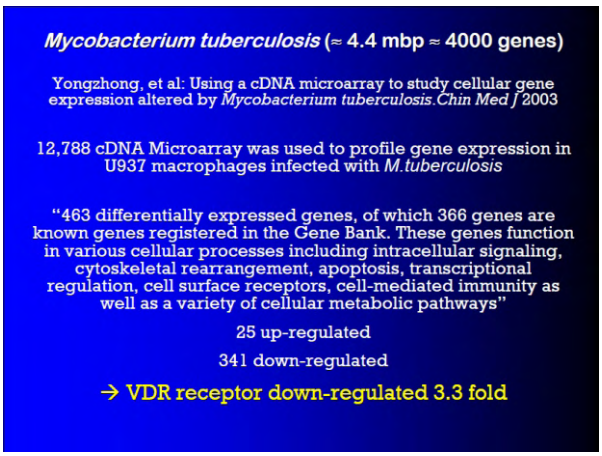


00:06:15
The VDR Nuclear Receptor

Well, what do we know about what is happening? What could possibly be allowing persistent pathogens to remain in what we thought was the sterile compartment in the body?

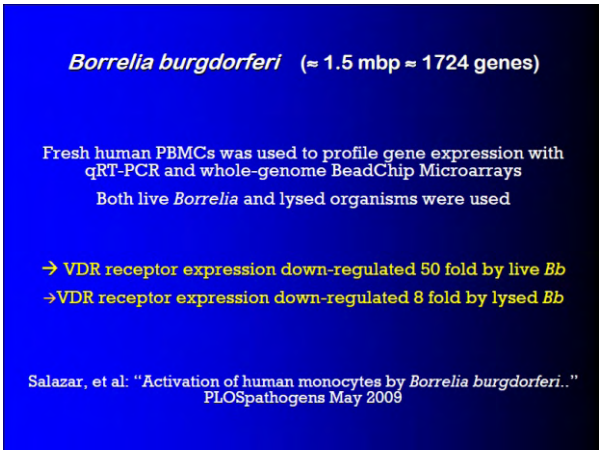
Well, in *Homo sapiens*, and only in *Homo sapiens*, not even in the higher primates, only in *Homo sapiens*, there is one Nuclear Receptor called the VDR Type 1 Nuclear Receptor, which transcribes genes for TLR2, as well as the Cathelicidin, antimicrobial peptides, which are the primary body's intraphagocytic protection mechanism, and also beta-Defensin anti-microbial peptides.

These are essential to the intra-cellular innate immune defenses, so if we knocked out the VDR, then pathogens would be more readily able to persist. However, when we knock out the VDR, we also cause human chronic disease.



00:07:16
Mycobacterium tuberculosis

If we look at some of the nasty pathogens that have been pretty well studied, *Mycobacterium tuberculosis*, a study here done, published in the Chinese medical journal in 2003, showed that the VDR receptor was down-regulated in monocytic cell line by an MTB infection by 3.3 times.



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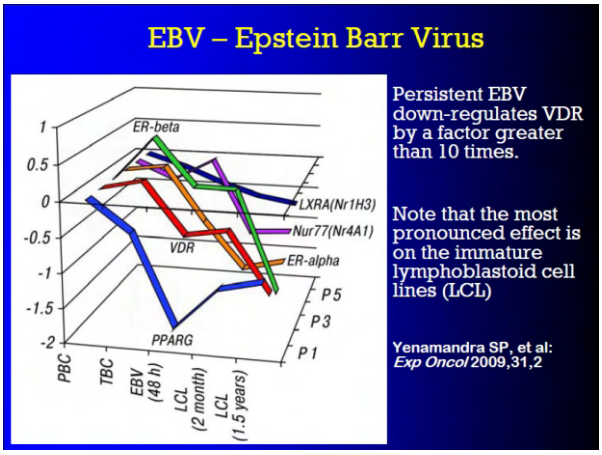
Borrelia burgdorferi

Borrelia burgdorferi, a pathogen that is getting increased attention these days, using a bead (BeadChip) assay which is more sensitive, found a 50 fold down-regulation of the VDR by live Bb, and 8 fold by lysed *Borrelia*.



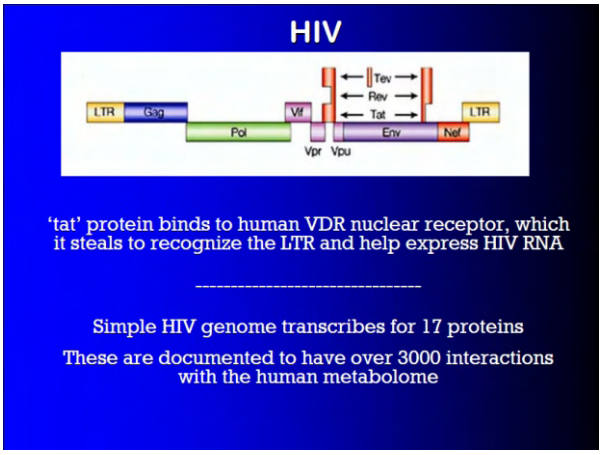
00:08:02
EBV -- Epstein Barr Virus

EBV, which is one of these persistent pathogens that you will find in all of the inflammatory diseases. If you think of an inflammatory disease, search for PubMed/the disease name/EBV, and there will be somebody saying that that disease is caused by EBV because it is an omni-present pathogen.

It [EBV] is a very persistent pathogen and when the innate immune system gets weak, it is hard for the innate immune system to clear it.

What is particularly interesting, is you can see VDR (you can see there are a number of Nuclear Receptors quoted here), but VDR is downregulated, particularly in the lympho-blastoid cell lines, the persistent, long-lasting, immature cell lines, after two months and after one and a half years the VDR is downregulated about 15 times. Very, very strong effect on VDR.

There is also a strong effect on Estrogen Receptor-beta, and that is not unreasonable because Estrogen Receptor-beta is believed to transcribe VDR — at this point in time — or express VDR.



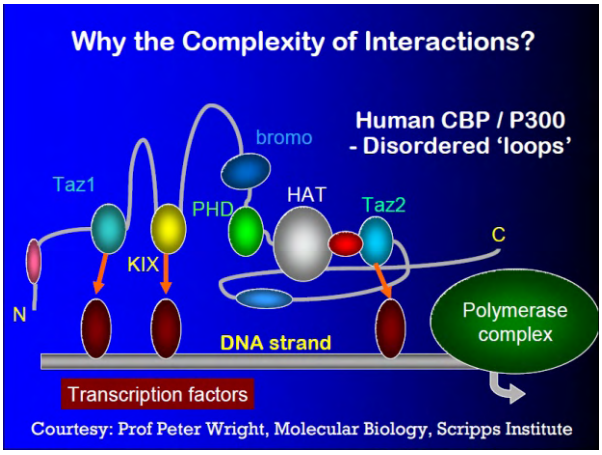
00:09:10
HIV

And then of course, the big daddy of them all, HIV.

HIV totally takes the VDR, it binds, the 'tat' protein binds, to human VDR and it then steals the VDR so that it can transcribe its own genome, so its long terminal repeats can be recognized and express the HIV RNA from the reverse DNA. So it is actually part of the survival mechanism of HIV to a much greater extent even than of the bacteria that we were talking about earlier.

Now what I want to talk about here is a simple HIV genome transcribes for about seventeen proteins. They are cleaved in to a number of other little proteins, but let us say that about seventeen proteins come from the HIV genome. But yet these seventeen proteins are documented to have over 3000 interactions with the human metabolome.

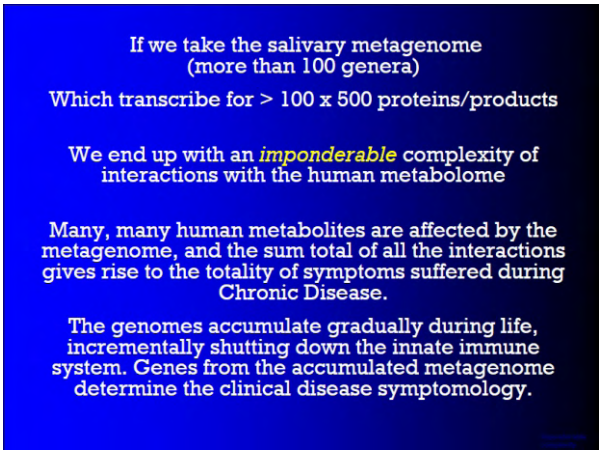
These seventeen proteins from HIV are documented to have over 3000 interactions within the human body.



00:10:25
Why the Complexity of Interactions?

Why does this occur [interactions within the human body]? Well, if we take a human CBP / P300, which has some disordered 'loops' in it, you can see you have got these colored balls [I am indebted to Prof Peter Wright of the Scripps Institute for this image], we have got the colored balls which are structured areas of CBP and then you have got the disordered loops. And depending on what those disordered loops bind to, that will determine where the transcription factors are actually working along the DNA strand.

So what happens in the viruses, particularly, is that most of their proteins are disordered. They can really go after many, and go into many, many different conformations [or conformal shapes], depending on what proteins they are binding to.

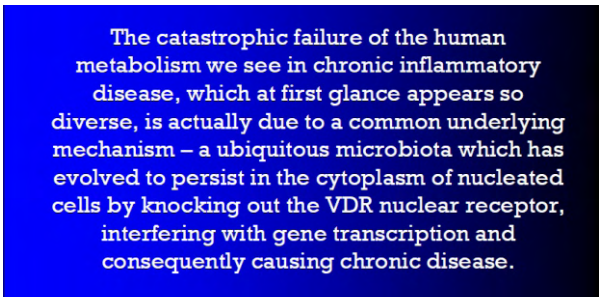


00:11:17
If we take the salivary... metagenome....

So if we just look back at that salivary genome of about 100 species, and let us say each specie has about 500 proteins in its genome, and you realize that we are starting off then with 50,000 proteins rather than the seventeen of HIV, you can see we end up with an imponderable complexity of potential interactions between the human metabolome and any pathogen which is capable of persisting inside the cytoplasm of nucleated cells, particularly, inside the cytoplasm of the phagocytes.

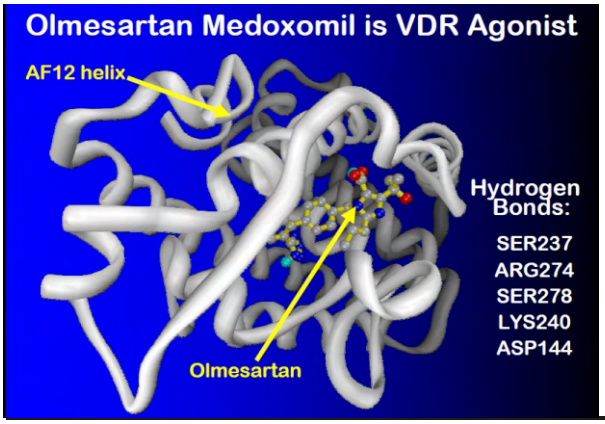
Many, many human metabolites are affected by the metagenome, and the sum total of all these interactions gives rise to the totality of symptoms which are suffered during Chronic Disease; and also, the commonality of many of the symptoms, as well.

The genomes accumulate gradually during life, incrementally shutting down the innate immune system. Genes from the accumulated metagenome determine the clinical disease symptomology.



00:12:24
The catastrophic failure...

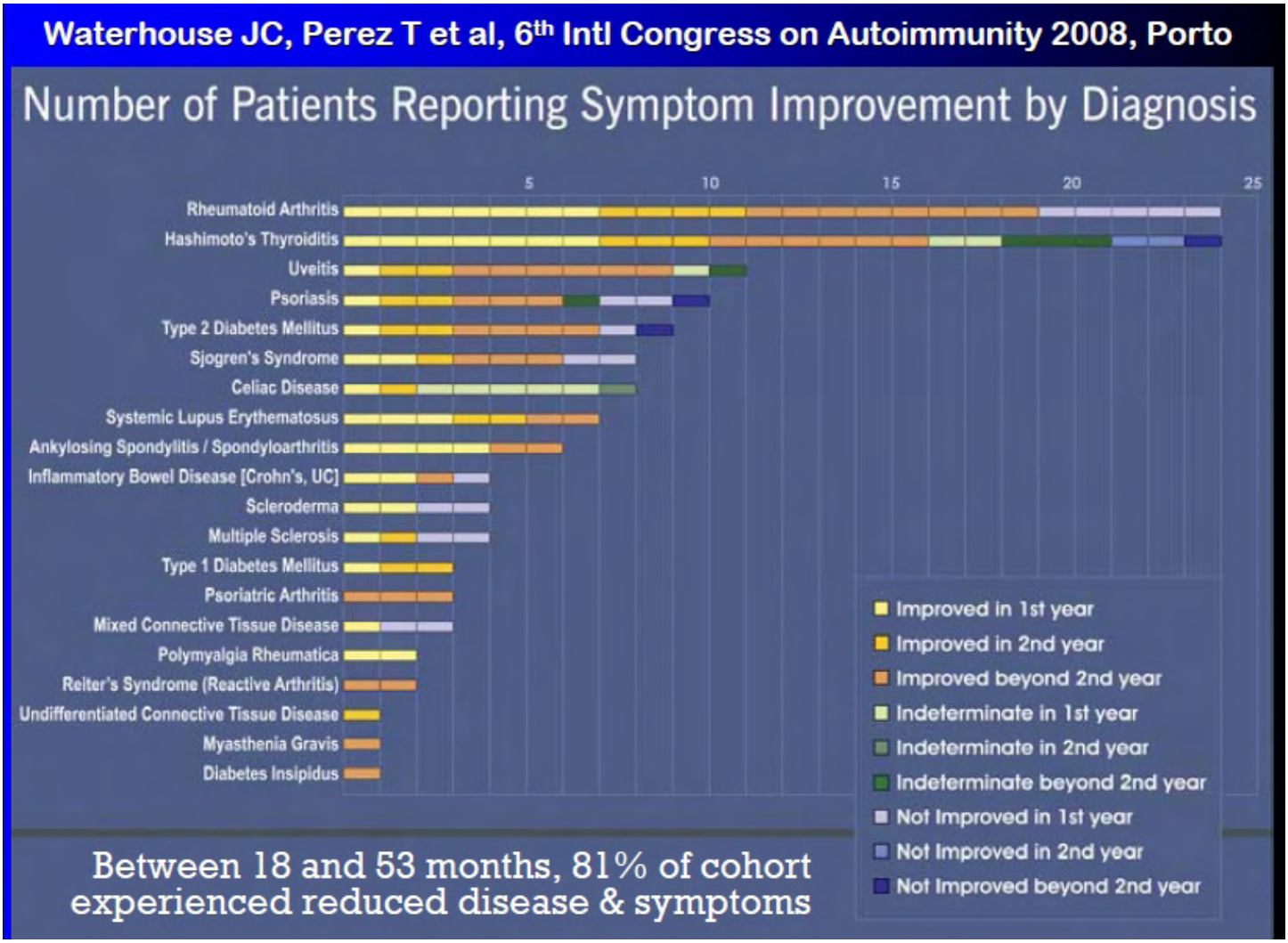
The catastrophic failure of the human metabolism, which we see in chronic inflammatory disease, which at first glance appears to be so diverse, is actually due to a common underlying mechanism — an apparent ubiquitous microbiota which has evolved to persist in the cytoplasm of nucleated cells by knocking out the VDR nuclear receptor.



00:12:47
Olmesartan Medoxomil is VDR Agonist

So what will you do? Well, of course you get a VDR agonist. Turn the VDR back on again.

It turns out that olmesartan medoxomil is quite an adequate VDR agonist. It forms hydrogen bonds with key amino acids, fits nicely there in the binding pocket, and reactivates the VDR.



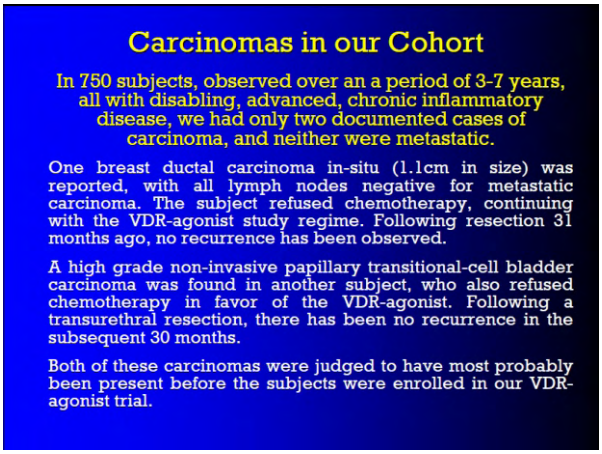
00:13:08
Number of Patients Reporting Symptom Improvement by Diagnosis

When you do that, you suddenly find that you can reverse autoimmune disease. Well, actually, you do not *suddenly* find you can reverse autoimmune disease, because the reversal recovery process is just about as chronic and as slow as the actual disease process, typically taking three to six years.

We have been running a study now for seven years, a Phase 2 study, in a variety of autoimmune diagnoses.

This [chart] was dated, we presented at the 6th International Congress on Autoimmunity last year [2008] in Porto, Portugal. And you can see a variety of diagnoses: rheumatoid arthritis, Hashimotos thyroiditis, uveitis, psoraisis, psoriatic arthritis, etc., down to diabetes insipidus. The different colors reflect patients who improved in first year, second year, improved beyond second year, etc. (I will give you a copy of these slides afterward if you want more details.)

But the main thing is that eighty-one percent of the cohort experience reduced disease and symptoms between eighteen and fifty-three months, and a significant portion had total disease reversal.



00:14:24
Carcinomas in our Cohort

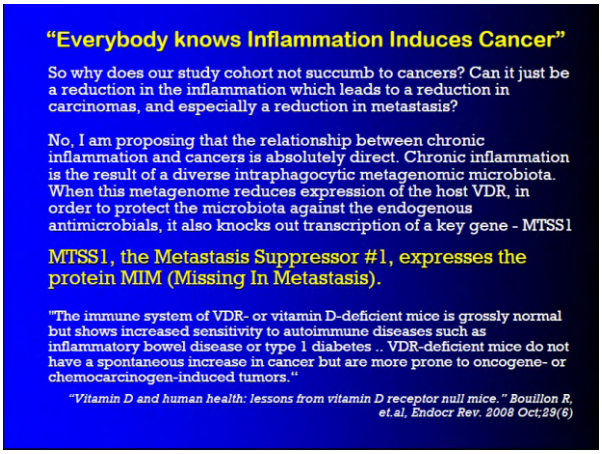
But the thing that I am here to talk about, is carcinomas. Because in 750 subjects, our current reporting cohort, observed over a period of three to seven years, all with disabling, advanced, chronic inflammatory disease, we only had only two documented cased of carcinoma, and neither were metastatic.

There was a breast ductal carcinoma in-situ. All lymph nodes negative for metastatic carcinoma. The subject refused chemo, continuing with the VDR-agonist study-regime. Following resection thirty-one months ago, no recurrence has been observed.

And in another subject, a high grade non-invasive papillary transitional-cell bladder carcinoma was found. That subject also refused chemo in favor of continuing the VDR-agonist therapy. Following a resection, there has been no recurrence in the subsequent thirty months.

Thirty months is just getting to the stage where we can start to say, "Hey, this looks interesting." That is why I am here.

Both of these carcinomas, incidentally, were judged to have most probably been present before the subjects were enrolled in our VDR-agonist trial.



00:15:44
"Everybody knows Inflammation Induces Cancer"

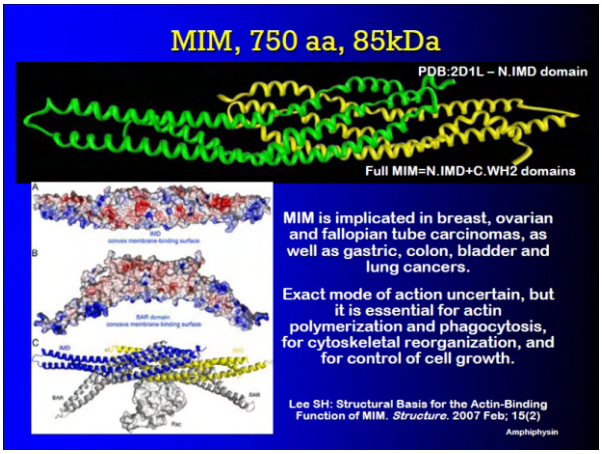
So, everybody knows inflammation induces cancer. So why does our study cohort not succumb to cancer? Can it just be a reduction in inflammation which leads to a reduction in carcinomas?

No. I am proposing a more direct relationship between chronic inflammation and cancers.

Chronic inflammation is a result of a diverse intraphagocytic metagenomic microbiota. And when this metagenome reduces expression of the host VDR, in order to protect the microbiota against the endogenous antimicrobials, it also knocks out transcription of a key gene — MTSS1.

If you do an expression map for the VDR, and it was done by Wang, et al., in about 2003. Of the 913 genes whose expression is affected when the VDR is liganded, of the 913 — confirmed with array — genes, the number one is CYP24 and the number two is MTSS1, in terms of the degree of expression changed when the receptor is liganded.

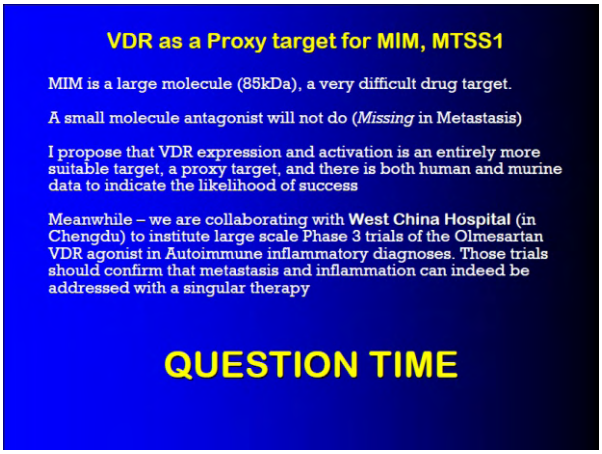
This is very important: MTSS1, the Metastasis Suppressor, has been identified as a drug target in carcinomas but it is devilishly hard to make a drug to deal with.



00:17:10
MIM, 750 aa, 85kDa

It is a large molecule, the exact mode of action is uncertain but it is something to do with actin polymerization and phagocytosis or maybe cytoskeletal reorganization.There is a paper which goes into things with a bit more detail.

But MIM is the protein which is transcribed from the Metastasis Suppressor Number 1 gene. MIM is "Missing in Metastasis," so obviously, when the VDR is knocked out, MIM is indeed missing. MIM is implicated in breast, ovarian and falopian tube carcinomas, as well as gastric, colon, bladder, and lung cancers, and implicated closely.



00:17:54
VDR as a Proxy target for MIM, MTSS1

So, what I want to propose here today, is that the VDR is a proxy target for MIM and MTSS1. It has not been reported up until now. Most people ignore the VDR as a Nuclear Receptor.
But MIM is a large molecule, it is a very difficult drug target on its own. A small molecule antagonist will not do and, obviously antibodies are very difficult to design.

I propose that VDR expression and activation is an entirely more suitable topic, a proxy target, and there is both human and murine data to indicate the liklihood of success (I had a murine citation on the previous slide).

Meanwhile, we are collaborating with West China Hospital in Chengdu to institute large-scale Phase 3 trials of the olmesartan VDR agaonist in Autoimmune inflammatory diagnoses.

These trials should also confirm that metastasis and inflammation can indeed be addressed with a singular therapy.

So West China Hospital is moving forward with us to do large Phase 3 trials in inflammatory diagnoses. The first inflammatory diagnoses we are collaborating on is Ankylosing Spondylitis. Over the next few years, it will be very interesting to see how things evolve.

Thank you very much.

Questions

Moderator: Professor Marshall — any questions?

Question: Is there some example of a functional foundation for MIM, for example if a cell is missing MIM does it make more metastatic enzymes? For example, some cells which have MIM, and in those cells that MIM is knocked-down with [antisense] RNA, to see how the metacells will be inhibited or downgraded, is there such an example?

Marshall: No, there has been no... well, actually, there has been work on MIM in those cancers, which I think include knocked-out mouse models. But I have not been paying a lot of attention to the mouse, the murine, work because as I said in my first slide, the VDR Nuclear Receptor is unique to *Homo Sapeans*.

In fact, in the murine model, neither Cathelicidin or TLR2 or beta-Defensins are transcribed by the VDR. The murine VDR does not express those. So I guess that is why it has been so hard to get decent animal models for many of the inflammatory diseases.

Moderator: Thank you for your presentation.