UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NEPTUNE GENERICS, LLC, APOTEX INC., APOTEX CORP., TEVA PHARMACEUTICALS USA, INC., and FRESNIUS KABI, USA, LLC, Petitioners, -v- ELI LILLY & COMPANY, Patent Owner	Case IPR2016-00237 Case IPR2016-00240 Patent 7,772,209

VIDEOTAPED DEPOSITION OF

STEVEN H. ZEISEL, M.D., Ph.D.

Washington, D.C.

Tuesday, November 22, 2016

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Gail L. Inghram Verbano,

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November 22, 2016

8:13 a.m.

Videotaped deposition of STEVEN H. ZEISEL, M.D., PH.D., held at the offices of WILLIAMS & CONNOLLY, LLP, 725 Twelfth Street, N.W., Washington, D.C. 20005-5901, before GAIL INGHRAM VERBANO, Registered Diplomate Reporter, Certified Realtime Reporter, Certified Shorthand Reporter-CA (No. 8635) and Notary Public in and for the District of Columbia.

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STEVEN H. ZEISEL, M.D., Ph.D.

THE VIDEOGRAPHER: This is the start of the video deposition of Dr. Steven H. Zeisel in the matter Neptune Generics, LLC, et al. versus Eli Lilly & Company.

This deposition is taking place at 725 12th Street Northwest, Washington, D.C. on November 22nd, 2016, at approximately 8:13 a.m.

My name is Jordan Mummert from TSG Reporting, Inc. I am the legal video specialist. The court reporter is Gail Verbano in association with TSG Reporting.

Will the counsel please introduce yourselves.

MR. KRINSKY: David Krinsky from Williams & Connolly, LLP, on behalf of the patent owner, Eli Lilly & Company. With me are Dov Grossman and Adam Perlman, also of Williams & Connolly; and James Leeds of Eli Lilly & Co.

MS. SPIRES: Sarah Spires of Skiermont Derby, LLP, representing Neptune Generics.

MR. PARKER: Thomas Parker,

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P-A-R-K-E-R, Alston & Bird, for Mylan.

MS. LYDIGSEN: Laura Lydigsen of Brinks Gilson & Lione for Sandoz, Inc.

THE VIDEOGRAPHER: And on the phone?

MS. SPIRES: I believe we'll have some people on the phone at some point but we haven't been able to dial in yet.

THE VIDEOGRAPHER: The court reporter may swear in the witness.

STEVEN H. ZEISEL, M.D., PH.D.

called as a witness, having been duly sworn by a Notary Public, was examined and testified as follows:

EXAMINATION

BY MS. SPIRES:

Q Good morning, Dr. Zeisel.

A Morning.

Q Could you start by describing your experience working with cancer drugs up to 1999.

A Sure. I am a professor at the University of North Carolina, Chapel Hill. Prior to that I was trained in medicine, and I have a Ph.D. in neurochemistry and nutrition.

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And during my work as a professor, I've conducted research in nutrition and in nutrition as it relates to cancer; and have worked with studies on -- funded by the National Cancer Institute; and agents like genistein, a Phase 1 study, would be an example.

Q Prior to this case, have you ever done in any work for Eli Lilly?

A I was an expert witness in a case approximately two years ago in Indiana District Court.

Q In your work as a nutritionist, do you ever work with oncologists?

A I do. I've collaborated on a number of research studies, and I'm a member of the Lineberger Cancer Research Institute, an NIH-funded center focusing on cancer, the treatment and causes of cancer and its prevention.

Q Do you ever work with oncologists in conjunction with patient care?

A Again, I worked with oncologists in conjunction with patient care as part of

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research studies.

Q Have you ever worked with oncologists in conjunction with patient care outside of the context of research studies?

A Once I finished my residency in pediatrics, I've solely seen patients as part of research thereafter.

Let me correct that. I was a Fellow at Children's Hospital in Boston for a couple of years where I saw patients as well. But after that period of time, since 1982 or so, I've mainly done -- only done research and seen patients in the context of them being subjects in a clinical trial.

Q So you don't have experience working with oncologists in conjunction with routine patient care?

A So again, patients in a clinical trial get routine patient care; but we are collecting information and data to develop new knowledge during that.

So it's a research study, and I don't get paid. I don't charge patients for the care. They're covered because they're part

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of the research program.

So they get routine care, but they're a part of a research study. I do not see patients where I'm seeing them, billing them for my services.

Q As part of your work in the research studies, do you work with oncologists to develop a protocol for patient cancer treatment?

MR. KRINSKY: Objection to the form of the question.

THE WITNESS: Could you repeat that again.

BY MS. SPIRES:

Q I'll ask a better question.

Do you work -- do you ever work with oncologists in developing a protocol for patient care for cancer treatment?

A So as part of these research studies, we have to put together a protocol that we will follow with each of the patients being studied.

So, for instance, let's take the genistein study. We would have -- I would have

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sat down with oncologists, and we would have discussed the dosing, the timing, the measurements we would make, and we would submit a protocol to a board called the Institutional Review Board together. And that would have been approved, and that's what we would have proceeded in the study.

So, yes, I would have worked with them for the research study clinical care design and the exact protocol that would be followed with every subject in the study.

MR. KRINSKY: Could we go off the record for a moment?

MS. SPIRES: Sure.

THE VIDEOGRAPHER: Time is 8:19. We're off the record.

(Discussion off the record.)

THE VIDEOGRAPHER: Time is 8:24. We're on the record.

BY MS. SPIRES:

Q So I understand you have experience developing protocols for research studies.

A I do.

Q Do you have experience developing a

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treatment protocol for a patient outside of a research study? A cancer patient?

A Not since I finished my residency.

Q You said that was in around 1992?

A I finished my residency in '77.

Q In 1999, it was not uncommon for an oncologist to consult with a nutritionist regarding the nutrition of a cancer patient; correct?

A No. When a nutritional issue came up with a cancer patient, an oncologist would consult; and normally that consultation was about the patient was losing weight for a treatment, et cetera.

MR. PERLMAN: Could we just go off the record for a second?

THE WITNESS: Time is 8:25. Off the record.

(Discussion off the record.)

THE VIDEOGRAPHER: Time is 8:27. We're on the record.

BY MS. SPIRES:

Q In 1999, a typical oncologist would have had limited knowledge in the field of

nutrition; correct?

A I think it depends on the training of the oncologist. In certain areas, the oncologist would have had a deeper understanding because the interventions they're performing involved some aspect of metabolism or nutrition. In other areas, they might have not been as well up-to-date.

But I think it's very hard to say what any individual oncologist's nutrition background might have been outside of the areas that directly impact on their oncology services.

Q When is the last time you treated patients with vitamin deficiencies?

A Again, as part of research studies, probably a year or two ago, maybe this year. Depends on the research protocol.

Q As part of these research studies, would you know from the outset that a patient had vitamin deficiencies?

A I'm sorry. Could you repeat that.

Q Would you know from the outset of the research protocols that the patients had

vitamin deficiencies?

A You would have had a set of laboratory studies that you would have interpreted, along with physical exam studies, that you would have drawn the conclusion that they have a health problem or an organ dysfunction problem relating to having too little of a nutrient; and you then would have treated that for some of the studies.

We were specifically studying people who had a problem and asking whether giving the vitamin or nutrient back made that problem go away.

Q Was it part of the research protocols to do this testing, this lab work that you mentioned prior to patients entering the clinical trials?

A So that -- you're asking me was it part of the protocol to do all of the tests?

It was a part of the protocol to do a set of tests to describe the patient and determine whether they were, quote, normal or not. And then you would do additional tests as indicated by the results of the first test that

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were part of the protocol.

All tests are part of the protocol, but there might have been some extra ones that had to follow-up tests after you got the first results back.

Q Are you aware of whether similar testing is done outside of the research context?

MR. KRINSKY: Objection; scope.

THE WITNESS: So your question is, is -- would a physician be doing tests like this when they're seeing their patients?

And some of the tests are fairly standard tests that you would do on a patient at their yearly physical, and other tests you would do because you had some specific symptom or sign in the patient that told you you should follow up with more testing to figure out what is the cause or what's going on in the patient.

BY MS. SPIRES:

Q Is testing for vitamin deficiencies

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a standard part of these tests?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: So, again, would a physician test for vitamin deficiencies in every patient? No. But some of the tests that they would normally do on a yearly physical might be the first clue that there was a vitamin deficiency, and then they would follow up to look at that.

BY MS. SPIRES:

Q And would a physician, as of 1999, be conducting these tests?

A Yes, a physician in 1999 would be doing laboratory tests on patients and following up with more tests if the first test gave them indications to do so.

Q You state in your declaration that antifolates interfere with the natural action of folates; correct?

A Yes.

Q How do antifolates interfere with the natural action of folates?

A So there is a variety of drugs

called antifolates. Most of them act by competing with folate, reduced folate, for folates in general, for binding to some enzyme -- an enzyme being the protein that helps folate progress through the steps in metabolism that have to occur to make DNA or to be used in other ways.

And the different antifolates bind differently to different enzymes depending on their structure and properties. But in general, they are called an antifolate because they compete with folate, at least one of these important enzymes that normally folate would be published through metabolism by.

Q I think you mentioned that antifolates compete with reduced folate for binding to enzymes; is that correct?

A I'm sorry.

Q I think you mentioned -- trying to go without realtime here -- that -- I think you mentioned that antifolates compete with reduced folate for binding to enzymes; is that correct?

A I should correct that. They compete with folate because -- dihydrofolate reductase,

they would be competing with a nonreduced folate, a folic acid for binding to the dihydrofolate reductase.

So be more accurate, they compete with folate at some step for binding to an enzyme, whether it's reduced or not reduced.

Q So if the antifolate is competing with the folate for DHFR, then the antifolate is competing with nonreduced folate; correct?

A I'm sorry. With --

Q With nonreduced folate.

A So when folic acid comes into it, it's not in a form that could be used in folate metabolism and has to be converted. And so at that point, a drug like methotrexate is binding and preventing the folic acid completely or partially from being converted to the form, reduced form that it can be used in.

Q How does methotrexate prevent the folate from binding -- strike that.

How does methotrexate prevent the folate completely or partially from being converted to the reduced form that it can be used it?

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A It inhibits the activity of the enzyme that is converting folic acid to tetrahydrofolate.

Q And that enzyme is DHFR?

A Yeah.

Q Are there any other enzymes that DHFR competes with folates for binding?

MR. KRINSKY: Objection to the form of the question; scope.

THE WITNESS: Your question didn't make sense to me. That -- are there any other enzymes that dihydrofolate binds to?

BY MS. SPIRES:

Q Strike that.

Are there any other enzymes that methotrexate competes with folates for binding to?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: I am not sure whether methotrexate binds to other folate enzymes as well as it binds to dihydrofolate reductase. So I just don't know that answer.

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BY MS. SPIRES:

Q When antifolates are competing with folate for binding to an enzyme, do the antifolates bind more strongly to the enzyme than does folic acid?

A I'm trying to think if there are any exceptions. But usually there is a binding constant for folate and the antifolate. And the antifolate either has to be able to knock folate off, and so that it -- often they have a better binding affinity. But if you had enough of an antifolate, someone with a lower binding affinity still would be an effective competitor to some extent.

So that it depends on the ability of the structure, the antifolate, to bind to the binding site compared to the -- the endogenous folic acid or folate, reduced folate that's binding to it.

Q Do you happen to know the binding -- the binding constant for folic acid to DHFR?

A I don't.

Q Do you happen to know the binding constant for methotrexate to DHFR?

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A I don't.

Q Do you happen to know the binding content for pemetrexed to DHFR?

A I don't recall the numbers.

Q Those are the type of things that you could look up somewhere?

A Yes. I mean, they're available. Again, I would defer to an oncologist who is working about which antifolate to use to inhibit which set of enzymes; and I just don't memorize those constants.

Q So you're not an expert in which antifolates inhibit which enzymes?

MR. KRINSKY: Objection to the form; misstates.

THE WITNESS: I would not consider myself an expert. I'm aware of which enzymes pemetrexed inhibits; and methotrexate is a very common one, and I know what it inhibits.

BY MS. SPIRES:

Q And how are you aware of which enzymes pemetrexed and methotrexate inhibit?

A In articles in the public literature

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in 1999, they reported what enzymes pemetrexed had efficacy for.

Q And which enzymes does pemetrexed have efficacy for?

A Well, it's primarily a TS, thymidylate synthase, inhibitor. It also has some activity against DHFR. It has some activity against GARFT and AICARFT.

Q Why do you call pemetrexed primarily a TS inhibitor?

A That's what the literature said, that its primary activity was inhibiting TS.

Q Do you have a view as to what the literature meant when it said that?

A My -- my view would that it -- TS was most effectively inhibited by pemetrexed.

Q If I told you that the binding constant for DHFR by pemetrexed pentaglutamate is 7.2 nanomolar, does that sound right?

MR. KRINSKY: Object to the form; foundation, asked and answered.

THE WITNESS: As I said, I don't recall the binding affinity.

BY MS. SPIRES:

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Q Let's assume that the binding constant of DHFR by pemetrexed pentaglutamate is 7.2 nanomolar versus 1.3 nanomolar for TS.

What is the difference in inhibition level for these two enzymes?

MR. KRINSKY: Object to the form; foundation, scope.

THE WITNESS: The two numbers again were 7.2 and 1 point something?

BY MS. SPIRES:

Q 1.3.

A So that means that 1.3 micromolar, half of the activity was inhibited by one, and it took 7.2 micromolar to get to the same efficacy -- to get to the same inhibition.

Q In terms of enzyme kinetics, 7.2 and 1.3 are fairly equivalent; correct?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: It all depends on what the concentrations are in the actual situation. To get 7 times more concentration could be a huge difference.

BY MS. SPIRES:

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Q And to get 7 times more concentration could also be very little difference; correct?

A I have no way to assess that. I mean, seven times more concentration is a big change in concentration, normally, in a cell. So I just -- without details of what you're -- how you would conduct the experiment or whatever -- you treat the patient, it's hard to understand. But 7 times is a big difference.

Q What concentration -- strike that.

Are you aware of the strength of pemetrexed's inhibition of DHFR versus the strength of methotrexate's inhibition of DHFR?

A Again, I would defer to an oncologist around use of the drug. But the literature that I've read about the drug says that it is less effective than methotrexate at inhibiting DHFR.

I don't have -- haven't looked more deeply into that literature because it wasn't part of what I was asked to consider.

Q You agree that by June of 1999, a POSA would have known that elevated

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pretreatment plasma homocysteine levels were a predictor for pemetrexed toxicities; correct?

A Could I see my declaration just so I can be precise of what I said in the declaration?

Q Sure.

I'll hand you what's been previously marked as Exhibit 2118. And I'm handing you a declaration from the 237 proceeding, but I understand that you submitted the same declaration in both proceedings. Is that correct?

A I believe so.

And could you repeat the question again.

Q Sure. You would agree that by June of 1999, a POSA would have known that elevated pretreatment plasma homocysteine levels were a predictor for pemetrexed toxicities; correct?

A A POSA would have known that there were abstracts that had been published that reported that homocysteine above the level of 10 micromolar was predictive of toxicity when pemetrexed was administered.

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Q And you specify abstracts. Do you view abstracts differently than other publications?

A I think abstracts indicate a suggestion of the direction of a scientific result and that they're usually followed up by more detailed reports of the data. And so they're useful to a POSA for understanding the possible direction of an effect.

Q And you specified abstracts, I think you said, that reported homocysteine above the level of 10 micromolar was predictive of toxicity when pemetrexed is administered; is that correct?

A Let me just find what I said in my thing, but I believe that's correct. Let me just look a minute.

Yes. In the Niyakiza abstract, it was at or about 10 micromolar.

Q And I believe you've testified that a POSA would not consider 10 micromolar homocysteine levels to be elevated; is that correct?

A Yes. In 1999, the normal range

reported by laboratories would have been up to 13 to 15 to 16 micromolar as the upper end of the normal distribution of homocysteine. And many research studies would have also reported cutoff levels of somewhere that high before they would have considered that they were dealing with somebody with abnormally high homocysteine.

Q And you said that 13 to 16 micromolar were the upper end of normal; correct?

A In my declaration, I say 13 to 15, but EP-005 defines a cutoff level of 16.3. And in 1999 a clinical laboratory where a physician would have sent a homocysteine off to to be run routinely would have reported upper end of normal somewhere in the 13 to 15. But that -- and it varied by laboratory and the method used.

But those were still within normal range up to those levels. So 10 definitely was well within normal range for homocysteine.

Q So is it your view that, because a homocysteine level of, say, 10 or 11 micromolar

would not be viewed as an abnormal homocysteine level, that a POSA would ignore the correlation with pemetrexed toxicity at that level?

A So you're asking whether a POSA, because the normal range extended up to 15, would have said that a finding that patients with more than 10 -- or the range more than 10 would have had some increased risk of pemetrexed.

And the answer is that a POSA would have regarded that association as suggesting that there was an effect; and that the way these types of studies are done is you segment your population into pieces, quartiles and quintiles, and then you say, At what cut can I show that the group seems different statistically from the lower group?

And a POSA would have regarded that as indicative that there might be something there, certainly, worth consideration.

Q You said that the numbers at 10 were suggesting that there was an effect. What did you mean by that?

A So in the Niyakiza abstracts, they

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found statistically that patients who grouped in the group that had homocysteine concentrations greater than a cutoff level of 10 micromolar had an association with an increased toxicity from pemetrexed.

So that suggests that there might be something about having a higher level than 10 that was related to why people were getting toxicity from pemetrexed, and that's what a POSA would have taken out of that set of studies.

Q So a POSA would not have simply ignored a homocysteine level from 10 to, say, 13, so within the normal range?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: So, again, in the realm of thinking about hypotheses for how you might predict who might have toxicity from pemetrexed, this is useful.

In the realm of a physician in their clinical office seeing a patient, getting a laboratory value of 10 back, they would not have been compelled to do something

for that patient because they would have regarded that as a normal level.

So a physician thinking about how to structure pemetrexed therapy, and whatever, it would have been suggestive of something, but it would not have compelled treatment for lowering homocysteine. It just suggests there's some relationship.

BY MS. SPIRES:

Q Would a physician have taken any action based on receiving a lab result of a homocysteine level of 10 in a patient about to receive pemetrexed?

A In 1999, I don't think -- I don't think they would have.

But again, I would have to defer to an oncologist, because I'm dealing with nutrition. And if you're talking about what an oncologist would do, I'd defer to Dr. Chabner as oncologist for what they we would do.

As a nutrition person, I would have said this is not a level of homocysteine that I feel I'm compelled to treat.

Q As a physician, would you feel

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compelled to address the -- strike that.

Do you agree that based on the Niyakiza reference, homocysteine is a biomarker for increased pemetrexed toxicity?

A The Niyakiza abstract suggests that homocysteine could be associated -- homocysteines above 10 could be associated with greater risk for pemetrexed toxicity.

Q So is that a yes, you agree that Niyakiza would view increased homocysteine as a biomarker for increased pemetrexed toxicity?

MR. KRINSKY: Object to form; asked and answered.

THE WITNESS: Again, I believe I just gave the answer, which was that what Niyakiza suggests is that there might be an association with homocysteines above 10 micromolar and increased risk for toxicity from pemetrexed. And a biomarker is something you can measure that might give you an idea as to whether this patient is at more or less risk of toxicity from pemetrexed.

BY MS. SPIRES:

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Q You agree that homocysteine levels can correlate with low levels of folate; correct? Strike that.

You agree that increased homocysteine level can correlate with low levels of folate; correct?

A I agree that there are many causes of increased homocysteine levels, one of which, among a number of them, is having inadequate folate, methylfolate, to methylate homocysteine and convert it to methionine. But there are many other reasons for it.

Q What are the other reasons, causes -- what are the other causes of increased homocysteine levels?

A I present that in my declaration, so let me just review it so I can present it in a similar way.

(Witness reviews document.)

Okay. So let's start -- you could be producing too much homocysteine. So if an individual took a high methionine load in our diet, they would produce a lot of homocysteine. That would cause higher homocysteines.

Balancing it would be the ability to remove homocysteine.

So homocysteine can be removed by methylating it. That requires folate, methylfolate and B12 to occur.

It can also be methylated from a parallel pathway called BHMT; and that pathway uses betaine, B-E-T-A-I-N-E, as the methyl donor to convert homocysteine to methionine.

In addition, homocysteine can be converted in a pathway to cystathionine, and taurine and cysteine down that pathway; and that pathway requires an enzyme, CBS, and a vitamin, vitamin B6.

So on balance, whether you have high homocysteine is determined by do you form it too much; do you remove it too slowly; and which of the removal pathways aren't working that cause you to have a high homocysteine.

So once you have that balance, you can then figure out why somebody has high homocysteine.

Q And in 1999 a POSA would have known all of these ways in which homocysteine can be

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elevated?

A Yes, this was common knowledge, basic biochemistry.

Q Would a POSA have known how to treat each of these methods to lower homocysteine?

MR. KRINSKY: Object to the form.

THE WITNESS: You're asking me would a POSA have understood what vitamins and nutrients were required for each of the steps? Yes. And could -- would -- they were -- people with very high homocysteines, people were treating them with various concentration of nutrients.

BY MS. SPIRES:

Q What were the combination of nutrients that people with high homocysteine were treated with?

A Folate would have been a treatment that they used. Betaine would have been a treatment that they might have tried. B6, B12.

Q Anything else?

A Not that I recall.

Q Would a POSA, in 1999 have selected only one of folate, betaine, B6 or B12 to treat

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a patient with high homocysteine?

MR. KRINSKY: Object to the form; incomplete hypothetical.

THE WITNESS: So I'm not certain what- an optimal combination was. They would likely have done some laboratory tests to try to determine which of the possible causes of high homocysteine were responsible and then focus their treatment on treating the cause of the high homocysteine.

They might have -- and, again, they might have found any of those causes contributing or combinations of those causes contributing.

BY MS. SPIRES:

Q For instance, it was common in 1999 to treat high homocysteine with a combination of folate, B6 and B12; correct?

A Very high folate, yes. You know, 30s and 15s would have been treatable at that point.

Q 30s and 15?

A I mean 30 micromolar folate -- I

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mean homocysteine might have prompted a physician to undergo a treatment.

Q Are you aware of what doses of folate were used to treat patients with high homocysteine around 1999?

MR. KRINSKY: Objection; scope.

THE WITNESS: I think doses of -- I don't know the exact doses of folate that people would have been treating with. There were a number of suggestions, from -- you know, at various levels of folate to use for that.

BY MS. SPIRES:

Q Do you recall any of those levels of folate that were suggested?

A Let me see if I talked about it in my declaration. I don't remember discussing that.

I don't recall the exact levels that were recommended, and I don't think I discussed it in my declaration.

Q Do you recall the doses of vitamin B12 that were typically used to treat patients with high homocysteine?

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A Again, I don't recall the exact numbers that were being used at that time.

Q Do you know about what portion of the patients with high homocysteine levels have a folate deficiency?

MR. KRINSKY: Objection; scope, incomplete hypothetical.

THE WITNESS: So it's a complicated question and difficult to answer because in 1998, the US food supply was fortified with folate, and the number of people with higher homocysteines due to low folate decreased. And so by 1999, I am not sure what likely portion had low folate.

But some portion of the people in the United States did have higher homocysteines due to low folate.

BY MS. SPIRES:

Q It wasn't rare, in 1999, for high homocysteine to be caused by low folate?

A It was not rare.

Q Do you know what portion of high homocysteine levels were caused by deficiencies in B12 in 1999?

A I don't.

Q Do you know whether deficiencies -- it was rare for deficiencies in B12 to cause high homocysteine levels in 1999?

MR. KRINSKY: Objection; scope.

THE WITNESS: Again, I just don't know what portion of the population would have had high homocysteine due to inadequate levels of B12.

BY MS. SPIRES:

Q Are you aware of what portion of the population would have had high levels of homocysteine due to a betaine deficiency in 1999?

A No. Again, at that time, we didn't have good information on the betaine and choline content of foods, and we would not have been able to figure out that until we could calculate what dietary intake was and what pool sizes were. So we didn't know, and I don't think -- I don't know it for sure.

Q So in 1999, you're saying that it was not established yet what a baseline level of betaine was?

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A I don't understand the interpretation of "baseline." But if you meant what a -- how many -- what portion of the population had low dietary betaine and its precursor, intake, we didn't know that until more recently.

We know now that a large portion of the population has inadequate intake.

Q Would a POSA in 1999 have believed it was possible that deficiencies in a patient's pretreatment nutritional status could have been the cause of increased pemetrexed toxicity seen in the high homocysteine levels in Niyakiza?

MR. KRINSKY: Object to the form; asked and answered.

THE WITNESS: So based on the Niyakiza abstracts, people would have -- a POSA would have known that having a homocysteine greater than 10 suggested that the patient could be at greater risk of developing pemetrexed toxicity.

A POSA wouldn't have known whether the high homocysteine was due to

nutritional problems or due to other problems, such as lower activity of cystathionine betasynthase, CBS. So I don't know that they could have concluded that nutritional status was the sole reason for being more sensitive to pemetrexed toxicity.

BY MS. SPIRES:

Q But a POSA would have known that it was possible that nutritional status was the reason for being sensitive to pemetrexed toxicity; correct?

A A POSA would have known that the nutrients important for maintaining homocysteine concentrations at less than 10 micromolar would have been important in the nutrition of those people. I don't know about the other elements of nutritional status.

Q So when you say a POSA would have known that the nutrients important for maintaining homocysteine at less than 10 micromolar would have been important in the nutrition of those people, what do you mean by that?

A So I think it was "could have been," because we agreed that it wasn't the only reason and that it might be a contributor. And that it is -- a POSA would have known that it's possible that low folate status, low B12 status, low betaine/choline status, low B6 status could have been contributing to higher homocysteines and that these people had and could have been but might not have been the reason for the high homocysteines they had and their risk for pemetrexed toxicity.

Q In your declaration, you cited a reference called Vidal; correct?

A Yes, I did.

Q What is Vidal?

A Vidal is a French publication similar to the Physician's Desk Reference used in the United States. Includes the package labels and warnings associated with prescription medications.

Q And when you cited portions of Vidal, which you say is similar to the PDR, did you look at the PDR to see if it contained equivalent disclosures?

A I did.

Q And what did you find?

A The PDR didn't state that B12 was contraindicated because of its effect on rapidly dividing cells.

Q I'm sorry. I didn't catch the last part.

A What I said is that Vidal says that B12, vitamin B12 is contraindicated in patients because B12 can give rise to exacerbation of cancer progress, and the PDR did not include that line.

Q Did the PDR say anything about vitamin B12 being contraindicated in cancer patients?

A No.

Q Did you look at the PDR to see whether it said anything about folic acid being contraindicated in pemetrexed patients?

A I didn't look.

Q Do you consider the PDR to be an important reference?

A It's one of many medical references that a POSA would be aware of.

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Q Would a POSA typically rely on the PDR?

A A PDR or the Vidal are listing pharmaceutical company package inserts approved by the appropriate regulatory authority. So people would take it as one of the references that tells you about a medication, and it would have been considered in forming an opinion, as one of many sources.

Q If a POSA ran across, say, a recent peer-reviewed publication that contradicted the PDR, would the POSA still heed the PDR's warning?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: So your question is if there were other evidence from publications, would the POSA balance the two evidence and try to draw a conclusion based on their balancing the two? Yes. I mean, that's the job -- that's what a POSA does, is try to take all of the available information, weigh once against the other, and come to a conclusion.

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So it would depend what is in that published reference and, you know, its quality and how strong it was compared to the PDR.

MR. KRINSKY: Counsel, I think -- I know we had some fits and starts at the beginning. I think we've gone about an hour on the record. Is this a good time for a break?

MS. SPIRES: Sure.

THE VIDEOGRAPHER: The time is 9:18. We're off the record.

(Recess taken from 9:18 a.m. to 9:36 a.m.)

THE VIDEOGRAPHER: Time is 9:36. We're on the record.

BY MS. SPIRES:

Q Dr. Zeisel, going back to one thing you mentioned earlier, you said that the percentage of the population that is folate deficient has decreased since the government started supplementing the food supply with folic acid; is that correct?

A I believe I said the people with

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homocysteine that is higher would be responsive to adding folate to the diet has dropped because there's more folate being taken in in the diet right now. I'll stop there.

Q Do you know the portion of the population that would be responsive to adding folate prior to the addition of folic acid in the food supply in 1998?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: So the question was do I know how many people would have had higher homocysteine that would respond to getting more folic acid or folate in the diet.

I don't know the exact proportion, but it would have been a proportion of the population that was reasonable, significant. I don't know how big it would have been.

BY MS. SPIRES:

Q You say it would have been a significant portion of the population.

A It would have been a measurable

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portion, yes.

Q What do you consider to be a measurable portion of the population?

A I don't know the exact amount, but it might have been one of the more common causes of having an elevated homocysteine.

Q So in 1999, a POSA would have been aware of folate deficiency as a cause of high homocysteine; correct?

A Yes.

Q In 1999 would a POSA believe that nutritional deficiencies secondary to cancer would be common?

MR. KRINSKY: Object to the form of the question; scope.

THE WITNESS: So there are many kinds of cancers. Some of those cancers in which the treatment makes people unable to eat, a POSA would have realized that they developed nutritional deficiencies. And some of those cancers where eating properly was difficult, as part of the cancer itself, a POSA would have realized that there was potential for developing --

for malnutrition, yes.

BY MS. SPIRES:

Q And sometimes the cancer treatment would cause eating problems or nausea that would also cause nutritional deficiencies; correct?

A Yes.

Q And a POSA in 1999 would have been aware of this?

A Yes, a POSA would have been aware of that.

Q Cisplatin is one of those cancer treatments that causes nausea and sometimes nutritional deficiencies; correct?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: So I defer to an oncologist for the side effects of Cisplatin because it's not something that I was asked to think about.

BY MS. SPIRES:

Q Would a POSA in 1999 often direct cancer patients to take vitamin supplements to address the nutritional deficiencies we've just

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talked about?

MR. KRINSKY: Object to the form; scope, incomplete hypothetical.

THE WITNESS: Could you repeat the question, please. I lost it.

BY MS. SPIRES:

Q Would a POSA in 1999 direct cancer patients to take vitamin supplements to address the nutritional deficiencies secondary to cancer or cancer treatment?

MR. KRINSKY: Object to the form; scope and incomplete hypothetical.

THE WITNESS: So it would very much depend on the cancer patient, and so it's difficult to give you one answer for what a POSA would have recommended to a cancer patient, and the -- I'll stop there.

BY MS. SPIRES:

Q Are there cancer patients for which a POSA would have recommended that patients take vitamin supplements to address nutritional deficiencies?

MR. KRINSKY: Objection; scope.

THE WITNESS: So in general, you are

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facing a conundrum with the cancer patient.

You want them to eat well enough that they maintain their immune function and they don't lose muscle mass; and at the same time, you don't want to feed the cancer so that it grows faster.

And so an oncologist has to balance those two countervailing problems and try to come up at the optimum how much -- so again, it would have been recommended a patient eat well to maintain their health and their immune function and their body mass but not so well that they help the tumor to grow.

And so an oncologist would have modulated and a nutritionist would have modulated their recommendations depending on the exact circumstances of the patient.

BY MS. SPIRES:

Q But there would be instances then, depending on the circumstances of the patient, where an oncologist would recommend vitamin supplementation to address nutritional

deficiencies?

A Would have recommended that the patient eat a diet that contains the recommended intake of nutrients and not supplementing above the recommended intake.

Q When you say that "would have recommended the patient eat a diet that contains the recommended intake of nutrients," could that also be through vitamin supplementation?

A So "vitamin supplementation" is a very broad term. If you mean a multivitamin, like a one-a-day and that type, it might include taking that. But again, it would only be so in a patient for which that makes sense.

If you were going to use a treatment to block a specific vitamin's action, you wouldn't be recommending that they take more of that vitamin because it's the antidote to your treatment.

So for a patient where that isn't a consideration, then keeping them as healthy as possible in terms of their immune function and their muscle mass, et cetera, would say, eat

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enough to do that.

(Exhibit 1053, Excerpts from 1999 Physician's Desk Reference for Nonprescription Drugs and Dietary Supplements, was marked for identification.)

MS. SPIRES: I am marking as Exhibit 1053 -- this is in both proceedings, that exhibit number -- the 1999 edition of the Physician's Desk Reference for Nonprescription Drugs and Dietary Supplements, or a portion of that book.

BY MS. SPIRES:

Q Have you seen the nonprescription version of the PDR before?

A I have.

Q This is something that a POSA would rely on; correct?

A It is something that a POSA would consider as part of the body of information that they would weigh, yes.

Q And if you turn to the page that's numbered at the top right 403 -- I think it's

the page.

A I see. I just want to read through the whole thing to make sure I understand what I'm being handed here, because I haven't seen it before in this form.

Q These are excerpts, obviously, since the book is more than 843 pages.

A I understand. I'm just reading through what the excepts you handed me are.

Okay.

Q If you look at the page that has the number 403 at the top right.

A I am.

Q And if you look about halfway down on the left column it says, "Cancer, nutrients deficiencies secondary to."

Do you see that?

A Yes.

Q And it says, "Cancer may be treated with chemotherapeutic agents. The following products may be recommended for relief of nutrients deficiency."

Do you see that?

A I do.

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Q And then there's a list of basically multivitamins following that.

A Yes.

Q There is not any warning here about limiting the amount of B12 in the vitamins listed, is there?

MR. KRINSKY: I'd like to just object both to this line of questioning and to this exhibit to the extent there's no -- it hasn't been established that this is prior art; and also scope.

BY MS. SPIRES:

Q Are there any warnings at all with respect to the list of vitamins that this PDR says may be recommended for relief of nutrient deficiencies secondary to cancer?

A In this little section that you're drawing my attention to, they do not have a warning about B12.

Q If you turn over two pages to the page that is labeled 842 at the top -- are you there?

A Yes.

Q If you look at the bottom right you

see, "Slow Fe with folic acid."

A Yes.

Q And that is a supplement that includes 160 mgs of iron and 400 micrograms of folic acid; correct?

A Let me look -- under "Formula"?

Q Yes.

A 50 milligrams of elemental iron and 400 micrograms of folic acid; correct.

Q And then under "Dosage" it says to take 1 to 2 tablets a day or as recommended by a physician, and a maximum of two tablets daily; is that correct?

A Yes.

Q And then there is a warning that follows this product, isn't there?

A There is a warning.

Q And this warning says, about halfway through, "The intake of folic acid from all sources should be limited to 1,000 micrograms per day to prevent the masking of vitamin B12 deficiencies"; is that correct?

A It contains that line.

Q There are no warnings here about

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folic acid with respect to cancer here, are there?

A I don't see one.

Q Now, if we turn back, flipping back a page or two to the page numbered 831.

A Yes.

Q If you look under the product on the left-hand column, Tri-B.

A I'm sorry. My copy is missing the corner of that page that says Tri-B, so I can't really read it.

Q Can I see your copy?

That is the same as my copy, and I can read it just fine.

A I'm sorry. There's something before "Gems."

Q We're not on that one. If you go -- it's the one after that, it's Tri-B.

A Tri-B. Okay.

Q You can read that portion; right?

MR. KRINSKY: Objection. Which portion are we talking about, Counsel?

MS. SPIRES: Tri-B. It's in the -- midway, about a quarter of the way down,

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left-hand column.

MR. KRINSKY: Cut off in the margin.

THE WITNESS: Okay. What was your question? I can't remember it.

BY MS. SPIRES:

Q This says Tri-B is a product that contains three B vitamins, B12, B6 and folic acid; correct?

A Yes.

Q And it says that this product can help maintain normal blood level of homocysteine; correct?

MR. KRINSKY: Objection to the form; mischaracterizes the document.

THE WITNESS: Could you say that again. I missed something.

BY MS. SPIRES:

Q This says that this product can help maintain normal blood levels of homocysteine; is that correct?

A That's what they say, yes.

Q And there's no warning listed with respect to this product containing vitamins B12, B6 and folic acid, is there?

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A That's correct.

Q If a patient has a folate deficiency, how long does a patient need to receive folic acid supplementation to replace the patient's folate levels?

MR. KRINSKY: Object to the form of the question, scope and incomplete hypothetical.

THE WITNESS: Could you say that again.

BY MS. SPIRES:

Q If a patient has folate deficiency, how long does a patient need to receive folic acid supplementation to replace the patient's -- replete the patient's folate levels?

MR. KRINSKY: Object to the form of the question; scope and incomplete hypothetical.

THE WITNESS: So it's very hard to tell you an exact answer to that because it depends on how depleted the person's folate pools are, what are the reasons for being folate-deficient and how you -- what

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type of treatment you're giving.

So the amount of time it takes to rehabilitate a patient who has organ dysfunction and signs and symptoms of folate deficiency could vary depending on all of those factors.

BY MS. SPIRES:

Q If you were giving a cancer patient 1 mg of folic acid a day, would this be sufficient to replete the patient's folate levels, say, within a week?

MR. KRINSKY: Object to the form of the question; asked and answered, incomplete hypothetical, and scope.

THE WITNESS: So again, the same problem that I have, is that I have no idea how depleted the patient is; I have no idea of how you're giving the folate. And in a cancer patient, I don't have the understanding of the history of the cancer and what's been done to the patient that an oncologist would have. So I can't tell you that answer.

BY MS. SPIRES:

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Q And is your answer the same for B12 deficiency, that you wouldn't know how long after a B12 supplementation a patient's B12 levels would be repleted?

MR. KRINSKY: Same objections.

THE WITNESS: So again, I don't know how you're going to give the B12. I don't know how depleted the patient was. And without that information, it's hard to tell you how long it's going to take to rehabilitate them.

BY MS. SPIRES:

Q Say that you're giving the B12 intramuscularly and the patient is presenting with a homocysteine of 10 micromolar.

MR. KRINSKY: Object to the form of the question, asked and answered, lack of foundation, and scope.

THE WITNESS: So you're stipulating that you're going to give it intramuscularly; that the patient has a homocysteine of 10 micromolar.

I still don't know what its B12 -- what the patient's B12 status is because

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homocysteine may not reflect B12 status. MMA would, for instance, or having a -- whatever.

So I can't tell you exactly how long it would take to rehabilitate the person.

BY MS. SPIRES:

Q Do you have a rough idea?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: Just -- to tell you exactly is something that would require that you understand those other factors.

BY MS. SPIRES:

Q Is that something that an oncologist looking at a patient and having the patient's medical records would understand?

A Again, I don't know what is in those medical records and whether they've done -- I mean, if you have a measurement of their B12 status, et cetera, et cetera.

So it depends what's in there, what you could say in terms of treatment.

Q If an oncologist has a patient's MMA level, would an oncologist be able to formulate

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a regimen to replete the patient's B12 levels and know how long that regimen should take?

MR. KRINSKY: Objection; foundation, scope.

THE WITNESS: So I'm not an oncologist. So I defer to Dr. Chabner, who is an oncologist, could tell you what they could do.

BY MS. SPIRES:

Q And is that the same answer for a folate deficiency?

MR. KRINSKY: Same objections.

THE WITNESS: Same answer.

BY MS. SPIRES:

Q If an elevated homocysteine level is caused by a folate deficiency and the folate is repleted, how long after its repletion will the homocysteine level return to baseline?

MR. KRINSKY: Object to the form of the question; incomplete hypothetical.

THE WITNESS: It's a similar problem. But so you're saying if methionine synthase activity is restored to normal and you're predicating that the

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homocysteine was only elevated because the methionine synthase, then if, again, the backlog of homocysteine to be handled is modest, it would take a relatively short amount of time to process that homocysteine and methylate it. Hours, days.

BY MS. SPIRES:

Q By "days," you mean one, two days?

A Hours. I don't know. Again, you can't give a precise answer because, as I said, there are some pieces of information missing.

But with your predicated pieces, and that the homocysteine backup is low, modest, then that should be handleable in hours to days. I don't know how many days.

Q What did you consider to be a modest homocysteine backup?

A You know, methionine synthase can convert so much homocysteine per hour. And so you tell me how much, I can calculate how much it could convert per hour, and that's what I'm saying.

I don't know -- so given that it has

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a certain capacity, it can convert so much per hour. And if the amount there falls within that range of what it could convert in a few hours, then it's a few hours. If it's a few days, it takes a few days. If it's a huge amount, it might take more than that, again, because it can only do so much per hour.

Q Do you know how much methionine synthase can do per hour?

A No. I mean, again, that would vary person to person.

Q And is the answer the same for B12? That if you took the same assumptions but substituted B12 for folate deficiency, that it would take hours to days to return to normal homocysteine levels after repleting the patient with B12?

MR. KRINSKY: Object to the form; scope, incomplete hypothetical.

THE WITNESS: So again both folate, methylfolate and B12 are needed to activate methionine synthase. So the same consideration occurs. B12 just needs to be there so methionine synthase can be

active. Methylfolate needs to be there so methionine synthase has something to work with to convert homocysteine to methionine.

And then the speed at which methionine synthase can convert homocysteine then depends on the total amount of methionine synthase a person has, and then you could calculate roughly how long it might take.

BY MS. SPIRES:

Q So the -- because B12 is dealing with the same methionine synthase, the calculation is still based on how much conversion methionine synthase can do in an hour; is that correct?

A Yes, so that once you make methionine synthase capable of acting, then -- and you -- the rate at which homocysteine can be processed depends on how much of it is there and how much homocysteine it can convert every minute or hour or whatever. There's a set activity per amount of protein.

Q For a modest homocysteine backup, if

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the issue is a B12 deficiency, once B12 has been repleted, we're again looking at hours or days before the homocysteine level returns to baseline?

A Again, it depends on many things, but it's possible it could be in that time frame.

Q Folic acid and B12 are both B vitamins; correct?

A They are classified in the -- as B vitamins.

Q And as B vitamins, both folic acid and B12 are water-soluble; correct?

A They are.

Q And that means that for both folic acid and B12, the body takes what it needs and then flushes the excess away in urine; correct?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: That's not exactly true, but -- because folate can be stored as the polyglutamyl form. But in principle, once you're -- any excess can be excreted in the urine because the

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kidney filters it and can get rid of extra for those two vitamins.

But they are -- they have storage forms that are stored for a while.

BY MS. SPIRES:

Q So for B12, any excess can be excreted by the urine; correct?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: So the -- as I said, that B12 can be stored, bound to binding proteins; but the kidney does have the capacity to filter B12 and pee it out in the urine.

BY MS. SPIRES:

Q And the kidney would filter this -- any B12 that is not stored and bound in the system; correct?

MR. KRINSKY: Objection; scope, asked and answered.

THE WITNESS: So -- the kidney does filter the blood and would excrete some, not all of the B12 that's not bound in the blood.

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BY MS. SPIRES:

Q And the kidney's excretion of excess folic acid and B12 happens on a daily basis; correct?

A Yes.

Let me just reword carefully. You said "excess." Not all excess is going out that way, but some of the folate in B12 is being dumped in the kidneys.

Q Approximately how much of the folate in B12 would be dumped in the kidneys?

A I don't know.

MR. KRINSKY: Object -- give me time to object please, Doctor.

THE WITNESS: I apologize.

I have -- I can't tell you the exact proportion.

BY MS. SPIRES:

Q Is the proportion of folate in B12 being dumped in the kidneys -- does this correlate to the amount of intake of folic acid in B12?

MR. KRINSKY: Object to the form; scope, and incomplete hypothetical.

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THE WITNESS: I'm trying to remember the question now. Say it again, I'm sorry.

BY MS. SPIRES:

Q Does the proportion of folic acid in B12 that are dumped in the kidneys, does that correlate to the amount of intake of folic acid in B12?

MR. KRINSKY: Same objections.

THE WITNESS: So only folic acid and B12 present unbound in the blood are accessible to the kidney. So dietary intake for much of its curve doesn't overload the blood with folic acid and free folic acid and B12 and, at perhaps very high intakes, does, and then excretes more.

So the answer to your question is that it isn't a linear relationship between diet and what you excrete but that at very high levels of dietary intake, you will excrete more.

BY MS. SPIRES:

Q Earlier we were talking about

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betaine deficiency.

A Yes.

Q What are some of the symptoms of betaine deficiency other than elevated homocysteine levels?

A So I was talking about low dietary betaine intake to be precise, not betaine deficiency.

And at low -- and betaine is made from another nutrient, choline. That choline has a dietary requirement; betaine doesn't.

Low choline results in liver damage and perhaps muscle damage that reverses when you give it back.

Low betaine presents mainly with an elevated homocysteine. And that's mainly shown in animal models.

Q When you say it's mainly shown in animal models, does that mean that there is not human clinical data regarding low betaine presenting with elevated homocysteine?

A As I said, in 1999 there wasn't available information as to the food composition for betaine. That only became

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available in the 2000s. So you couldn't calculate intake, so you couldn't do a study in which you asked does low betaine intake correlate with high homocysteine.

So before 1999, it was mechanistically reasonable but unproven in human studies because you couldn't -- didn't know how to -- didn't have the information to conduct that study.

Q So in 1999, when a POSA sees presentation of high homocysteine and is checking through the potential causes, would betaine be on that list or no?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: So, yes, because mechanistically there's two parallel pathways for methylating homocysteine, one that uses methylfolate as a substrate and one that uses betaine as a substrate.

So theoretically, both pathways could be responsible -- a defect in both pathways could be responsible for high homocysteine.

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BY MS. SPIRES:

Q And low betaine intake, how is that treated, in 1999?

A Again, nobody would have been able to know if you had low betaine intake because we didn't know what was in foods until the middle 2000s.

At that point, I could have looked at your food intake and said, you know, You're not eating any of this nutrient. But right now at, 1999, that was an impossible question to answer, because we had no idea what was in food.

Q So in 1999, a POSA seeing a patient presenting with high homocysteine levels would not attempt to treat the patient with betaine supplementation; is that correct?

MR. KRINSKY: Objection; mischaracterizes testimony.

You can answer.

THE WITNESS: No, so I didn't say that. What I said was, is that they wouldn't have known that a patient was low in choline.

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However, based on the knowledge of the biochemical pathways that remove homocysteine, betaine would have been reasonable, as reasonable as folic acid, to try to lower homocysteine.

BY MS. SPIRES:

Q And in trying to lower homocysteine by addressing the betaine pathway, how would a POSA treat the patient?

MR. KRINSKY: Objection; foundation, object to the form.

THE WITNESS: So, it very much depends on the patient.

So let's -- let's take the predicate that we have a patient that we're going to use an antifolate on. Then the patient -- it would be sensible to say, rather than giving the antidote to the antifolate, which would be B12 and folate, we could treat with betaine and B6 to try to use the pathways that are not involving folate or releasing folate from its methyl form, and lower homocysteine without creating the situation of having to administer the

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antidote to the poison we are trying to kill the cancer cells with.

So there betaine, grams, vitamin B6 to try to get those two pathways that can remove homocysteine without using the methionine synthase pathway would be a reasonable physician's treatment option.

BY MS. SPIRES:

Q It's true that there are certain doses at which a POSA would know that supplemental folic acid is not going to compete with an antifolate in a sufficient manner to affect the efficacy of the antidote; correct?

MR. KRINSKY: Object to the form of the question; incomplete hypothetical and scope.

THE WITNESS: I don't agree that that's correct. An antifolate is they are competing with folate. The more folate you put in, the less effective that competition is. You're treating a person who has an exposure to folate from their diet anyway, and so everything you're adding is over and above that amount.

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And at some point, you end up getting to the tipping point where the cancer cells aren't completely eliminated by your antifolate and survive the dose. And exactly what that tipping point is is unpredictable.

BY MS. SPIRES:

Q But a POSA would know that there is a tipping point below which an amount of folic acid supplementation is not going to compromise an antifolate's efficacy; correct?

MR. KRINSKY: Objection; mischaracterizes testimony.

THE WITNESS: So again, a POSA would know that every molecule of folic acid given competes with the antifolate; and exactly when adding that one more molecule or several more molecules ends up making the last cancer cell that you have to get rid of survive is unknown and unpredictable. And treating a cancer doesn't do any good if you let cancer survive, because they just come back.

And so again, it's unpredictable how

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much extra folate you can get away with before you get to the point where your treatment was less effective than it would be because you're reducing its efficacy by giving folate, because the whole point is to block folate.

BY MS. SPIRES:

Q Is it also true for B12 that there is this tipping point, one side of which a POSA is going to recognize that adding B12 is not going to affect efficacy of an antifolate?

MR. KRINSKY: Objection to form; mischaracterizes testimony.

THE WITNESS: So to understand the role of B12, what you have to understand is that B12 is working by making it possible to have methionine synthase active.

And so whether -- so any amount of -- if B12 is low enough to be limiting the activity of methionine synthase, then giving some frees up methionine synthase. And if folate was not limiting -- methylfolate -- that allows methylfolate

to be converted and make reduced folate, tetrahydrofolate, available to make DNA.

So, again, it's a very similar story that at some point you make enough B12 available to allow methionine synthase to be active and convert trapped methylfolate into tetrahydrofolate that can be used to make DNA and allow the cancer cell to survive.

So it's a similar story, that it's very difficult to predict how much methylfolate will get converted to tetrahydrofolate when you make methionine synthase more active.

So there is some point that is hard to predict that adding more B12 causes the cancer cells not to die.

BY MS. SPIRES:

Q Are you aware of how much B12 a person's body is able to absorb in a day?

MR. KRINSKY: Object to the form of the question.

THE WITNESS: It depends greatly on the person. There are some people who

absorb B12 poorly; they don't have a protein needed to absorb it. There are other people who absorb B12 well.

Some people can absorb B12 from a vitamin well but not from meat, where B12 is located, because it's bound to proteins and they have trouble pulling it off.

So the amount of B12, somebody can absorb in a day varies.

In addition, the B12 is lost into the gut in the bile and other places, and so you need to recirculate it and reabsorb it. So, again, that varies person to person how well they do that. So the exact amount a person absorbs in a day is hard to tell you.

BY MS. SPIRES:

Q Do you know the range for that amount?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: Yeah, you know, I can tell you what the -- how much is recommended be in the diet each day, but

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how much of that is absorbed depends -- varies widely.

It's a catalyst, so you don't need much to get to the right amount.

BY MS. SPIRES:

Q What are the symptoms of folate deficiency?

A The most common symptom of being deficient in folic acid is that your red cells don't get made properly and you become anemic because you can't divide cells. The same thing that folic acid is used to help cancer cells divide, it's also helping your red cells, bone marrow cells divide and make new red cells for you.

Q Are there any other symptoms of folic acid deficiency?

A Again, with severe folic acid deficiency, you get some abnormality in your white cells as well, neutropenia.

Not a neutropenia, but a -- a smaller number of white cells.

Q Are there any other symptoms of folate deficiency?

A Those are the major ones. I mean, the red cells are the ones that a clinician would notice.

Q What are the symptoms of B12 deficiency?

A An anemia and very -- so again, low red cell production and now white cells that are large.

Q Are there any other symptoms of B12 deficiency?

A Some people can develop a neuropathy, which means usually their longest nerves, the one going from the head to the foot, don't work well; and they get, first, abnormal sensation in the big toe, and then later on abnormal gait and motor problems related to having nerves not working well that are coming out of the brain.

Q Are there any other symptoms of B12 deficiency?

A Again, those are the major symptoms.

Q Aren't there neurological symptoms of B12 deficiency?

A I think I just said that. They're

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the nerves that -- the longest nerves go first, and then eventually you get more and more nerves involved. But first, usually it's the longest ones, going to the toe.

Q The neurological symptoms of B12 deficiency are irreversible; right?

A Sometimes they are, sometimes they're not.

And again, to be more complete, for both folic acid and B12, there can be vaguer brain symptoms, like depression and some cognitive function, MM -- related to the functioning of brain cells. But again, they're not the first presenting symptoms. That's anemia.

Q The first presenting symptom is anemia, you said?

A I mean, anemia is the common presentation for both B12 and folate.

Q For folic acid deficiency, how long would a person need to be -- have this deficiency before anemia presents?

MR. KRINSKY: Objection; scope, incomplete hypothetical.

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THE WITNESS: So the minimum time would be about 90 days, because that's the lifespan of the red cell. But again, it depends on how severe the deficiency is and whether the person is bleeding, whether there are other limiting things around.

But a red cell takes 90 days to make, and so it's usually three months, six months, in that range, that you could see your red cells declining.

BY MS. SPIRES:

Q And is that the same amount of time it tends to take for anemia to present with B12 deficiency?

MR. KRINSKY: Same objections.

THE WITNESS: So B12 usually takes longer, because you need so little of it to work that -- but again, it depends tremendously on whether you're able to reabsorb B12 or whether you can't absorb B12 at all, the rate at which these things happen.

BY MS. SPIRES:

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Q Roughly how long does it take before -- roughly how long would a patient need to have a B12 deficiency to present with neurological symptoms?

MR. KRINSKY: Same objections.

THE WITNESS: So again, it depends on many factors. But it -- most of the literature says this is a late onset, years of being deficient before you see neuropathy developing.

BY MS. SPIRES:

Q Is it ever the case that neuropathy is the first symptom noticed for B12 deficiency?

A I am not aware of neuropathy presenting as the first symptom in a patient that is being followed by a physician. It's possible that in patients who are not being seen for medical care, that they present -- they already have neuropathy at the time they present. But somebody getting medical care would have been -- would have seen the anemia long before.

MR. KRINSKY: Counsel, we've been

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going about an hour. Should we take a break soon?

MS. SPIRES: Sure. Go ahead.

MR. KRINSKY: Go off the record.

THE VIDEOGRAPHER: The time is 10:32. We're off the record.

(Recess taken from 10:32 a.m. to 10:49 a.m.)

THE VIDEOGRAPHER: Time is 10:49. We're on the record.

BY MS. SPIRES:

Q Dr. Zeisel, I think you mentioned that you know what the recommended daily dose is of various vitamins; is that correct?

A What the recommended daily intake is.

Q What's the recommended daily intake for folate?

A 400 micrograms a day.

Q What's the recommended daily intake for vitamin B12?

A 4 or 5 micrograms.

(Exhibit 1054, Article, "Neuropsychiatric Disorders Caused by

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Cobalamin Deficiency In the Absence of Anemia Or Macrocytosis," was marked for identification.)

BY MS. SPIRES:

Q I'm going to mark a document as Exhibit 1054. This is an article by John Lindenbaum and others entitled "Neuropsychiatric Disorders Caused by Cobalamin Deficiency In the Absence of Anemia or Macrocytosis."

Do you see that?

A Yes.

Q Do you know of John Lindenbaum?

A I probably have met him. And I certainly have met Sally Stabler, who is on this. I'm pretty sure I met John at some meeting.

Q Are you aware, is John Lindenbaum a respected researcher?

A He is.

Q And I assume Sally Stabler is a respected researcher as well?

A Yes.

Q What about Robert Allen? He is also

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on this list.

A Yes.

Q Is he also a respected researcher?

A He is.

Q If you look at the first paragraph -- and I'm going to apologize. I actually gave you my copy. Can I trade you? I'll write the exhibit number on here. Sorry for that. I'm writing "Exhibit 1054."

MR. KRINSKY: Counsel, do we want to just put an exhibit sticker on? We can just do another exhibit sticker.

MS. SPIRES: That works.

MR. KRINSKY: So we don't get them confused, do you want to cross out yours or something?

MS. SPIRES: Done.

BY MS. SPIRES:

Q Have you ever seen this reference before?

A I may have, but I haven't reviewed it recently, so if you give me a few minutes, I'll read through the article and see if I can recall the details of it before answering your

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questions.

Q Sure.

A (Witness reviews document.)

Q Have you had a chance to read this article?

A I did. Not thoroughly, but I think I can understand what it was about.

Q Do you agree with this article's conclusion that neuropsychiatric disorders due to cobalamin deficiency occur commonly in the absence of anemia or in elevated immune cell volume?

MR. KRINSKY: Objection to scope and form.

THE WITNESS: So this article is talking about a very specific portion of patients that have what's called pernicious anemia, and they're not the common form of having low B12. They exist, but they're not -- so this is a subset of all patients.

And what I see this article saying is, is that out of a bigger number of subjects -- and I can't recall; I'd have

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to go back and recall exactly how many -- they found 38 out of -- so a quarter, maybe, that presented with neurological symptoms in the absence of anemia.

So it's -- for people with pernicious anemia, it looks like a quarter might present this way.

But this is a tertiary hospital getting referred cases that are different than might be seen in the general public, so it may not be that high in a non- -- they're specialists in this field in the country.

BY MS. SPIRES:

Q Do you agree that it is possible for neuropsychiatric disorders to be the first symptoms appearing for B12 deficiency?

A For these patients, yes.

Q Isn't the threat of irreversible neuropsychiatric disorders the reason that doctors often treat B12 deficiency through intramuscular injection?

MR. KRINSKY: Objection; form, foundation, and scope.

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THE WITNESS: So your question was, the irreversible neurological damage, is it the reason for using an intramuscular dose; and it is not.

BY MS. SPIRES:

Q What's the reason for using an intramuscular dose?

A A patient who can't absorb an oral dose would get an intramuscular dose.

Q Is it common for B12 patients not to be able to absorb an oral dose?

A For patients with pernicious anemia, this subset of patients we've just been talking about, their problem is is they do not make a protein in the gut needed to intramuscular B12.

So they cannot absorb B12. And you treat people with that problem with intramuscular dose.

Q Are you aware of what the standard intramuscular B12 dose is?

MR. KRINSKY: Object to the form; foundation, scope.

THE WITNESS: In this -- I don't know what the standard was in 1999. In

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this paper, in 1998 -- in 1988, they are using -- let's see if I can find it in here.

I'm not sure I can find what dose they used in this amount at this time. So I can't tell you what -- it's somewhere in here but I haven't had time to note it. Perhaps you know where they tell you the dose in this paper.

BY MS. SPIRES:

Q I'm less concerned with --

A Okay.

Q -- this particular paper and more concerned with, as a practicing nutritionist, what you believe to be the standard intramuscular dose for vitamin B12.

A It varies. And it would have been -- you need about 4 micrograms. But you would have given a dose that is milligrams, probably at that time, as an intramuscular dose.

So that would be 10 to -- you know, or more times what the normal oral dose would have been, because it's to serve as a depot

that they can absorb from for a while.

And so it doesn't really matter how much you give intramuscularly; it's just how long it lasts before they lose it by not being able to reabsorb the amount that they excrete into their gut.

Q What do you mean when you say "it doesn't really matter how much you give intramuscularly"?

A So you only need a few micrograms. That's available from the intramuscular dose.

The problem is, for people who can't absorb it every day, they are secreting B12 into their intestine, and then they can't reabsorb it. So they rapidly run themselves down.

And so when you give an IM dose, you don't have to give it to them daily. You give them something more than the few micrograms they need so that they can draw on that dose that's sitting in the muscle that you stuck it in for a period of time.

And so a standard dose probably, you know, a milligram would have been enough to

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last them weeks before they run it down. A microgram being a millionth of a gram, and a milligram being a thousandth of a gram.

Q I'm sorry. I didn't catch the last part.

A A milligram is a thousandth of a gram; and a microgram is a millionth of a gram. So a milligram is a lot of micrograms.

Q And that dosing was true in 1999; correct?

A Yes, it would have been about that. I don't remember exactly what preparations you could order off the counter.

In the PDR you gave, Tri-B, which is an oral one, they were giving 400 micrograms, or about 100 times what the person really needs. But again, it's just giving a lot orally, hoping some gets in. But these people, they don't absorb it well.

Q Am I correct that in your declaration you testified that vitamin B12 deficiencies are not acute?

A What I testified to is that to develop the symptoms of vitamin B deficiency

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takes a long time. It takes a long time to run down your vitamin B12. And then when you do, it takes three or four months before the red cell production is tapering off.

And for the neurological symptoms, nobody knows their exact time course. But in 1999, it was believed they take a good time to develop, because they involve the wrapping of nerves in an insulator called sphingomyelin, myelin, and that that took a long time to develop problems and didn't occur in days or weeks but much longer periods than that.

Q If you look back at Exhibit 1054, this Lindenbaum article.

A Yeah.

Q Do you agree with its statement in the first paragraph after the abstract that neurological symptoms often result -- are the result of inappropriate therapy with folic acid?

MR. KRINSKY: Objection; scope. Objection to the form.

THE WITNESS: In the -- at that time, there was a theory that folic acid

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could exacerbate the appearance of symptoms with vitamin B12. That hasn't been a consistent finding since then, and so I -- in 1998 and 1999, I would have said it was a possibility that was still under consideration.

BY MS. SPIRES:

Q And the possibility that was under consideration in 1999 was that if a person is given folate supplementation when they have a B12 deficiency, that the folate ends up masking the B12 symptoms and so the B12 deficiency goes untreated; is that correct?

MR. KRINSKY: Object to the form; mischaracterizes testimony.

THE WITNESS: So again, we're talking two different things.

So the masking concept is that usually the physician, the flag, the red flag the physician sees in a patient is anemia. Giving folate, folic acid, to a B12 patient can help to correct the anemia without correcting the underlying B12 problem. And that meant that a physician

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had to be more alert and not assume that, because they gave folate and it got better, that this was folate deficiency.

And if you'll notice in this paper, all but one of the subjects had very elevated MMA. MMA is the other enzyme that B12 is needed for, and it's a very sensitive marker for B12 deficiency.

So in these patients, for example, having very high MMA present, a good physician would have said, Even though the anemia went away, I am still not going to miss that this is B12, because of the MMA.

And so masking was, when the physician wrongly concluded that this was folate deficiency because folate treated the anemia, without going on and doing the other standard test in -- of whether this is B12 or folate by looking at MMA.

BY MS. SPIRES:

Q But even MMA misses a portion of the patients that are B12-deficient; correct?

A It does. But I don't believe it does in people with pernicious anemia, which

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are the patients that are being studied by this group here.

I believe all of the -- from quickly reading this, all of the patients they isolated who had neurological symptoms had pernicious anemia; and in them, MMA, I don't believe, misses a part of the population.

Q What about in the general population?

A Perhaps 5 percent of people with low B12 do not have abnormal MMA.

Q I'll hand you what has been marked as Exhibit 1017.

Have you seen this article before?

A I have. Let me just review it to make sure I remember it properly.

Q Sure.

(Witness reviews document.)

A Yes. What's the question?

Q Do you agree with the statement in the last paragraph before the conclusion section that says that "In patients with cobalamin deficiency in about 10 percent, only the homocysteine value is high"?

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MR. KRINSKY: Object to the form.

Counselor, where are you? I'm sorry.

Object to the form; scope.

THE WITNESS: So they do say that in their experience, that whoever they're citing -- they don't tell me who it is. But in their knowledge base, they believe that only -- that as many as 10 percent of people with high homocysteine and low cobalamin may also have -- may not have an MMA that's elevated.

BY MS. SPIRES:

Q In light of the masking issue, how would a POSA in 1999 address this 10 percent of B12-deficient patients that can't be detected by MMA?

MR. KRINSKY: Object to the form; foundation and scope, and incomplete hypothetical.

THE WITNESS: So, again, it depends on what kind of patient a POSA would be dealing with.

A patient who was going to get an

antifolate, you wouldn't to give the antidote before treatment anyhow. And then after treatment and the drug has been effective, you would give the folate and you would give B12 as well as the folate if you had a clinical reason to.

In a patient who comes in and you're going to do something else with but not treat them with an antifolate, and you weighed the pros and cons of risk/benefit and decided that it was appropriate to treat with folate, it would be appropriate to have given B12 with that treatment of -- let's say --

And if you notice, the homocysteines in the Lindenbaum paper are all 200, 100, 80, 70; not 10. And those were high enough, if you had somebody come in with that and you weren't going to be treating them with an antifolate and they didn't have cancer, you might give them both treatments.

So it's a "depends" question: Depends on who you're talking about and

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clinical judgment.

BY MS. SPIRES:

Q But for patients where it makes sense, you would give -- if you are going to treat the patient with folate, you would also treat the patient with B12 to account for potential masking?

MR. KRINSKY: Object to the form; asked and answered.

THE WITNESS: Yes, for a -- the clinical judgment, I would give folate and B12, because I would have made a judgment in that specific patient situation that the folate and B12 weren't going to have a -- counteract another intervention that I was going to be giving.

If I can add to that answer, I should say that the US government, in '98, mandated folate fortification; and specifically, prior to the date of 1999, came to the conclusion that masking was not sufficiently likely that B12 needed to be added to the fortification.

There was a lot of scientific

panels, a large number of experts on both sides giving information. And in the end, the expert conclusion around fortification of the food supply was that there was not sufficient risk of giving folate without B12 to come to the conclusion that it was reasonable to also mandate B12.

So this was a question that, in 1999 and the year before -- '98, '96, '97 -- when the position policy was being developed, was very actively being discussed; and the definitive conclusion was you don't need to give both together.

BY MS. SPIRES:

Q Isn't that in part because of the low levels of folate being added to the food supply?

A So the food supply, they were adding 400 micrograms daily intake; and that would be enough to mask B12 under these theoretical conditions that they bring up that you might mask B12.

So giving adequate folate is enough to let the anemia get -- go away; and you don't

have to give twice adequate or five times adequate to get that effect.

So the amount that people would take in the food supply, remember, is above what they might have been taking before with the fortification. So that took them above or at least to adequate intake if not one and a half times adequate intake, whatever.

And so I think, you know, that the overall consensus of science in that time was, it's fine to fortify.

And I think in pernicious anemia patients with this data, you might have said, I'll give them B12 as well. But pernicious anemia is rare enough that it isn't the standard intervention, as evidenced by the government's decision to only mandate the fortification with folate and not B12.

Q And how long did the discussion go on with the government regarding whether or not to add B12 to the food supply as well?

A So I can't tell you exactly. But since the implementation started in '99, I'm sure the regulations came out in '95, '96, if I

remember correctly. And there would have been a lot of discussion, probably years before that of coming to that conclusion.

And then after it came out, there still remained a group of nutrition and medical scientists who argued you should do it. And I think that the conversation kept on for a few more years after that.

Now, less common, but every once in a while somebody comes to a meeting and makes a presentation that why not put B12 in; it doesn't cost much and here is my reason for doing that.

So I think it's persisted for a decade; but probably was a 10-year conversation around the time of the policy, because it was a big policy to give everybody in this country extra folic acid.

Q I believe you say in your declaration that administering vitamin B12 to a person can release folate caught in a methyl trap; is that correct?

A Yes. Let me just pull up my declaration so I get the wording exactly.

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Yes. What was is the question now?

Q How common is this methyl trap issue in patients receiving pemetrexed?

MR. KRINSKY: Object to the form of the question; scope.

THE WITNESS: So in patients receiving pemetrexed, I don't know how common it is.

In -- so again, the methyltetrahydrofolate is a form of folate, and it has no way to return -- and it can't be used to make DNA.

So the folate piece of it can be recycled into tetrahydrofolate, which then can be used in the pathways to make DNA. And the only way it can be recycled, if methionine synthase is working.

So if I give a drug that blocks methionine synthase or I give -- I have somebody who has low B12 so that methionine synthase doesn't work well, I end up building up behind this metabolic roadblock, methyltetrahydrofolate.

And how much you build up is hard to

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predict, how much an individual patient might have built up. Because, again, it's like the earlier story of removing homocysteine: Every time you remove homocysteine, you use a methyltetrahydrofolate; but how fast that's going and what the backlog is not easily predicted.

And so you could have, in anybody who has methionine synthase not working well -- let's say it's low B12 -- they would build up methylfolate. And then if you give them B12, make methionine synthase work, the methylfolate now starts removing homocysteine. And as we said, that's a period of hours to days that it's starting to work lowering the homocysteine; and at the same time, it's releasing active -- the form of folate needed to build DNA, which is what you're trying to block with your drug, pemetrexed.

BY MS. SPIRES:

Q So I think you said that methyl trap

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was a concern in patients with low B12; is that correct?

MR. KRINSKY: Object to the form; mischaracterizes testimony, asked and answered.

THE WITNESS: So I believe what I said is any situation in which methionine synthase isn't working properly, one of the reasons being that it doesn't have the B12 it needs, will build up methyltetrahydrofolate.

Another -- there are many other reasons.

BY MS. SPIRES:

Q What are the other reasons?

A If I gave you an anesthetic, nitrous oxide, your methionine synthase stops working and you build up methylfolate, would be another example of the problem that you could have.

So in any case, if it is due to relatively less B12 than you need to get optimal, maximal methionine synthase activity, then giving B12 breaks that roadblock and allows the methylfolate to get out of its

methyl form and get converted in.

So the trap is really that it's trapped as the methyltetrahydrofolate form and has no way to get out if methionine synthase isn't working to do it for it.

Q Is your concern about the methyl trap primarily concern for B12-deficient patients?

A So "B12-deficient" has a very specific clinical meaning. But having low enough B12 so that methionine synthase isn't working as fast as it could, we could call that deficient; and in that case, that's what I'm saying.

But what I mean in my statement is, is that if you could amp up the activity or the amount of methionine synthase by making B12 available, then it will work.

B12, in a published paper before 1999, also apparently increases the production of the methionine synthase protein to make absolutely more protein available. So in addition to being a required catalyst for working, it appears that it may also be

important for expressing the gene or making the protein that gives you a little more capacity by having more of the protein available.

So B12 could theoretically, and realistically, increase the amount of flow of methyltetrahydrofolate into tetrahydrofolate, making more available to make DNA and making your -- making the antidote for the treatment that you're making -- that you're giving pemetrexed, which is trying to stop DNA synthesis by blocking the availability of those folates.

Q See if I can get it in the correct language.

Am I correct that a methyl trap is a concern for -- primarily for patients with B12 that is low enough to impair methionine synthase?

A B12 that's low enough to provide less-than-maximal methionine synthase activity.

Q Then that's a correct statement, with your modification?

A Again, it's one of the reasons that you'd worry about the trap. As I said, nitrous

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oxide anesthesia you worry about the trap as well, but that's not pertinent to this specific question.

Q And you'd know that you'd given nitrous oxide anesthesia to a patient.

A Yes. Again, you may -- you should know it, but maybe you didn't know it went on in the operating room; right?

Q Other than B12 and nitrous oxide anesthesia, are there any other patients -- or B12-deficient or low-B12 patients and nitrous-oxide-receiving patients, are there any other patients for which you'd be concerned about a methyl trap issue?

A Those are the -- B12 are the main -- having less B12 than you need would be the major concern. Nitrous oxide, you would know, as you say. So I don't think there are a lot of other reasons to worry about giving B12.

But then again, I wouldn't give B12 -- I'm sorry -- releasing the methyl trap, I wouldn't give B12 unless I thought they were low in B12. And if I think they're low in B12, then I have to think they've got methylfolate

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trapped behind that low B12, because methionine synthase must not be working optimally, or else they'd have adequate B12.

Q If you have a patient that is B12-deficient, has a methyl trap issue, and you supplement with B12, how long does it take for the folate to be released from the methyl trap?

MR. KRINSKY: Object to the form; incomplete hypothetical, scope.

THE WITNESS: So you're asking me, if I unblock methionine synthase by making B12 available, how much -- my answer is the exact same, how fast would homocysteine go down.

It depends on the capacity of the methionine synthase. It does so many molecules per hour; and every molecule of homocysteine it's doing, it's converting a methyltetrahydrofolate to that. So the rate of lowering homocysteine tells you the rate of release of the methyltetrahydrofolate.

And so you're going to release at the same rate and, as I indicated before,

it's hard to predict that but it could be hours to days to release your trapped pool, depending how big it is and how much methionine synthase activity that you have.

BY MS. SPIRES:

Q So when the homocysteine has stopped going down, that means that the methionine synthase has stopped -- or -- strike that.

So when the homocysteine stopped going down, that means that folate has stopped being released from the methyl trap; is that correct?

A So I wish it were as simple as that. But as I said earlier, a level of homocysteine -- think about a bathtub. The level of water in your bathtub is dependent on how fast it goes down the drain and how fast it's coming in.

So homocysteine going down the drain is what we're talking about, but the level might not reflect that, if the water coming in rates are changing; right?

So measuring the level of

homocysteine, or water in your bathtub, you can guess at what the rate of its falling out the drain is, but you can't know it without knowing the rate of addition of water to the bathtub.

So homocysteine might not continue dropping, because the rate of production is increasing for some reason; or the other pathways for getting rid of it are changing their rate. So it makes it even more complicated. It's not a bathtub with one drain but a bathtub with two drains, and you're only interested in one of the drains. The other drain is that cystathionine beta synthase outlet.

So it's a complicated equation. But for every homocysteine reduced by methionine synthase, there's a methyltetrahydrofolate being converted to tetrahydrofolate. So they are highly coordinated, but exactly what level means compared to the rate of methionine synthase I can't tell you precisely. I can only guess at it.

Q You said there's a one-to-one correspondence between the folate being

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released from methyl trap and the pemetrexed decreasing; is that right?

A I don't -- I don't think I said that. What I said was, I believe, that pemetrexed is trying to act to block the use of reduced tetrahydrofolate to form thymidine, let's say, in thymidylate synthase.

It has to compete with reduced folates. And every time I release reduced folates from a place where -- essentially makes new reduced folates available to compete, I am increasing competition. And so I naturally decrease the efficacy. How much I decrease the efficacy depends on how much I change the competition.

Q My question was about the relationship between the folate being released from the methyl trap and the homocysteine.

A You asked me pemetrexed.

Q Then I misspoke. I meant to say homocysteine.

A Okay.

Q So to my original -- well, what I intended my original question to be --

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A I might have heard pemetrexed.

MR. KRINSKY: Counsel, you said pemetrexed.

THE WITNESS: I thought you said pemetrexed.

MS. SPIRES: That's fine.

THE WITNESS: I might have misheard you.

MR. PERLMAN: She can ask a new question. That's okay.

THE WITNESS: Fine by me. I'll use the same answer.

BY MS. SPIRES:

Q All right. So going to what I intended my original question to be: If I understand correctly, when you release the -- when a methyl group is released -- strike that.

When a folate is released from the methyl trap, then the homocysteine level goes down; correct?

A So, again, I tried to answer that. The level isn't the same thing.

One homocysteine is converted to methionine and gotten rid of every time one

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methyltetrahydrofolate is converted to tetrahydrofolate. That could change the level, assuming nothing else changes; but it might not change the level, because it depends on those other drains and inputs that I talked about.

So if we say one homocysteine is used up for every folate that is freed up from the trap, then I am happy with that statement.

MS. SPIRES: I think we need to take a break to change the tape.

THE WITNESS: Okay.

THE VIDEOGRAPHER: Time is 11:42. We're off the record.

(Recess taken from 11:42 a.m. to 11:52 a.m.)

THE VIDEOGRAPHER: Time is 11:52. We're on the record.

BY MS. SPIRES:

Q Dr. Zeisel, would a POSA in 1999 have been concerned about administering B12 to pemetrexed patients because of this methyl trap issue?

A So a POSA in 1999 would have understood that pemetrexed works by opposing

the action of folic acid, of folate; and would understand that giving B12 had the potential to release a folate that doesn't interfere with pemetrexed and convert it into a form that can interfere with pemetrexed. And a POSA would have said, in the absence of a compelling reason to give B12, a POSA would not have done so, because you're giving essentially the antidote to the treatment that you're giving.

So why would anybody give the antidote to a poison at the same time they give it? It would have been something they wouldn't have done; and strongly, they would have said, This isn't worth the risk.

Q And the concern from a POSA in 1999 about giving B12 to a pemetrexed patient is because the pemetrexed patients might be B12-deficient, and thus, making the methyl trap a relevant issue; is that correct?

MR. KRINSKY: Object to the form; mischaracterizes testimony, asked and answered.

THE WITNESS: So I have not enough B12 to maximally run methionine synthase,

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and therefore, might have backed up a pool of trapped folate that it would release to compete with pemetrexed, would be the precise way I'd answer your question.

BY MS. SPIRES:

Q And a POSA would want to minimize the amount of useable folate in a pemetrexed patient system. Is that your testimony?

MR. KRINSKY: Object to the form. You can answer.

THE WITNESS: So would have wanted to minimize the reduced forms of folate that can compete with pemetrexed, would be the exact answer I'd give you.

BY MS. SPIRES:

Q Wasn't it your testimony in your declaration that a POSA reading the Niyakiza would have believed that vitamin B12 deficiency was not a cause of the elevated homocysteine?

A Do you have a specific part of my testimony that -- my declaration that you're referring to?

Q Paragraph 70, for instance.

A 70?

Q Yes.

A Can you repeat your question again so I get it exactly.

Q Wasn't your testimony in your declaration that a POSA reading Niyakiza would have believed that vitamin B12 was not a cause of the elevated homocysteine seen in Niyakiza?

A What I specifically say is that vitamin B12 deficiency was not related to the pemetrexed-induced toxicity. Throughout my piece, I have said that there are numbers of reasons that homocysteine could be elevated, B12 or low B12 being one of them.

However, in the presence of normal MMA, I believe that Niyakiza does not support the conclusion that B12 is -- B12 deficiency is the cause. It just says that, at least in this group of patients, I can't say it is and I can't rule out it isn't.

But with the absence of MMA being elevated, I don't have any positive evidence to say that that abstract, or Zervos, says that there's something wrong with B12.

Q I want you to assume that a POSA in

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1999 decided, against your advice, to give a pemetrexed patient folic acid. Is there anything a POSA could do to minimize any potential negative effects of the folic acid?

MR. KRINSKY: Object to the form; scope, incomplete hypothetical.

THE WITNESS: The key problem is that the effect of folate or B12 is unpredictable; that it would depend where you are on that tipping point for killing all the cancer cells.

You can't increase the dose, because there were off-target toxicities of pemetrexed, hurting the kidney, for instance. So if you decrease the efficacy of the equivalent dose, you didn't have the option of saying, Well, I'll just give more pemetrexed, because there were known problems with going higher that had nothing to do with the methionine synthase/folate trap, folate in competition with pemetrexed. It's a different mechanism for those.

And so it's very hard to say that

you could have mitigated it, since you can't really understand or predict how -- whether you're right at that threshold point, tipping point, a little before it, a little after it. And so I think, the best choice is not to give it. And the POSA shouldn't give it because of the unpredictability of the response to it and the negative effects it might have on the efficacy of the tumor, killing.

BY MS. SPIRES:

Q You said that you couldn't increase the dose of pemetrexed; correct?

A Again, I'm not an oncologist, but -- and I would defer to Dr. Chabner.

But I have -- I am aware that one of the toxicities, the renal toxicity, is apparently not related to the antifolate effect of pemetrexed. But for more meat and detail in that answer, I'd have to defer you to Dr. Chabner, who is an oncologist and who has considered this very carefully.

Q In your view, in 1999, if a POSA had the ability to either increase the dose of

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pemetrexed or give additional doses of pemetrexed at the expense of losing some of the efficacy of pemetrexed, is this something that a POSA would consider?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: Again, I'd say an oncologist would be able to give a better answer and a more knowledgeable answer about dose adjust and treatment of cancers, because it depends on what cancer and what side effects and many other factors.

So I couldn't tell you whether a POSA would choose to increase dose or increase the time, change the timing of delivery.

BY MS. SPIRES:

Q I think at the beginning of the deposition you mentioned that you worked with oncologists on dosing, scheduling, that sort of thing for clinical trials. Is that right?

A Yes.

Q What was your role with respect to

the dose and scheduling for those clinical trials?

A Again, in those studies, there was a protocol. And the oncologist took advice but made the final decision about any adjustment in the stated protocol that was based on clinical judgment and knowledge of the cancer and the patient.

So I, as a nutrition-consultant member of the research group, give advice. The final decision laid with the person responsible for the treatment of the cancer and who -- and so that's the oncologist, who took the advice, weighed it, and decided what was reasonable or not.

And so I was not the final word in the structure of the dosing. I was just a contributor of information.

And again, in the research protocol, you come up with the ideal treatment plan; but in real life, the clinician has to adjust the treatment plan based on the realities of the patient.

Q In your experience, do oncologists

you're working with typically defer to a nutritionist's advice in developing a clinical protocol?

A So I think "defer" is the right word. I think they weigh the consultant's advice against their knowledge and other people's advice and come up with a conclusion. Sometimes they implement the consultant's advice, and sometimes they say, I don't think this is the best thing to do.

In designing the research ideal protocol, there you're dealing with an ideal protocol, and there's more give and take in the discussion of what it might look like. But when dealing with the patient and adjusting that protocol to the realities of life, they would take the information -- not defer, but respect the information and make a decision based on their best assessment.

Q It was genistein, is that the --

A Genistein.

Q What drug is that?

A So genistein is a molecule derived from soybean that inhibits cancer growth and

induces cancer cell suicide. And the National Cancer Institute was considering developing it as a therapy for prostate cancer at the time. And our work was designed to ask whether it has toxicity; specifically, does it damage DNA.

Q So if you were looking at toxicity, was this in Phase 1 trials?

A Yes.

Q And when were you working on these Phase 1 trials?

A I'd have to go back and look at my resume for when the papers were published, and I was working on it for a few years before those papers were published.

It was sometime in the '90s. I just don't remember the exact date. Might have been early 2000s, you know -- I just don't have the exact date for exactly when we did it.

Q Did you conduct Phase 2 trials for that drug as well?

A No, we did not do Phase 2 trials on that drug.

Q What was the purpose of the Phase 1 trials that you conducted?

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A So the Phase 1 trials were conducted to ask whether dose escalation, what was the maximally tolerated dose, first in a healthy person, and then, 2, in a person with prostate cancer.

And it was a standard dose escalation designed at what point did we start to see toxicity. And we used a rating scale used by the National Cancer Institute that had a series of tests that related to the function of different organs -- kidney, brain, bone, heart, et cetera.

And when we reached a dose at which we developed abnormal -- grade of toxicity rated from 1 to 4. When we got to Grade 2, that stopped the escalation of the dose. And so we came up with, in the first two studies, on what's the useable dose that the NCI might use.

And one of our findings was that there was a significant amount of DNA damage that the drug was inducing, which meant that it might cause mutations in genes if you used it. And so that played into the NCI's decision as

to whether to continue it on into a therapeutic trial or not.

Q You mentioned that you conducted two studies with this drug; is that right?

A So Phase 1 was using healthy humans who did not have cancer. And that's because the genistein was connected to have modest toxicity, because people are exposed to it when they eat soy protein products. But the dosing went up higher, several-fold above what normal exposure might have been.

And then to do it in a group of men with prostate cancer, which is the group they intended to do the trial, to ask whether there was something about prostate cancer that might change the metabolism. And our studies measured all the metabolites formed, the pharmacokinetics in blood in both those groups.

Q Did you quantify efficacy in this Phase 1 trial with the prostate cancer patients?

A So like most Phase 1 trials, we are not powered to determined efficacy. But we did look at PSA levels of protein that is secreted

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by many prostate tumor cells and rises in people with prostate cancer and asked did we lower it. And we had a number of responders -- I don't remember the exact number out of the group -- that lowered their PSA.

So that was a preliminary indication that it might be efficacy, and that's good that a Phase 1 trial can provide that. But proof of efficacy requires a study with adequate numbers of subjects to rule out random chance and null hypothesis.

So we did not draw conclusions about efficacy. Just said it appears that there may be efficacy.

MS. SPIRES: I think we've gone our about half hour before, if you want to --

MR. KRINSKY: It's up to you.

MS. SPIRES: It's a good stopping point.

THE VIDEOGRAPHER: Time is 12:12. We're off the record.

(Luncheon recess taken from 12:12 p.m. to 1:06 p.m.)

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THE VIDEOGRAPHER: Time is 1:06. We're on the record.

BY MS. SPIRES:

Q Dr. Zeisel, are you familiar with the definition of a POSA in this matter?

A I am.

Q And do you believe that you are a POSA in this matter?

A I do.

Q And why is that?

A In my declaration -- let me get to that page -- the definition of a POSA on Item 16 is defined as a person of knowledge in oncology and a person with an understanding of how nutritional issues relate to the issues of chemotherapy agents. And in that regard, a POSA would have an understanding of the interrelationships between antifolates, folic acid pathway and pathways related to vitamin B12.

A POSA is a theoretical person, but I can represent a person with understanding of the interrelationships between antifolates, the folic acid pathways and pathways related to

vitamin B12.

Q The definition of a POSA in your declaration is more than just understanding oncology. It's a medical doctor who specializes in oncology; correct?

A So the definition is one set of understandings of the theoretical person -- that is, a person who has oncology experience, and specialization, and also has an understanding of the interrelationships between antifolates, folic acid pathways and pathways related to B12. And so I represent that piece of what a POSA would -- a theoretical POSA would understand.

Q So you represent -- so I have this clear, you represent a portion of a POSA's knowledge with respect to antifolates and their folic acid pathways and pathways related to B12, but you do not have the knowledge of a medical doctor who specializes in oncology; correct?

A So just to reword what you said I should agree to, I represent the portion of the POSA that -- which represents the understanding

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of 1-carbon metabolism, but I do not represent the POSA with expertise in the practice of oncology.

Q So you yourself are not a POSA, but you have a portion of the knowledge that a POSA would have; is that correct?

A So I understand it, a POSA could never be a person, because it's a theoretical concept.

And so the concept is there's a theoretical person who has knowledge of the entire literature around oncology and the entire literature around the interrelationships between antifolates, folic acid and pathways. And I am representing the theoretical person's understanding in those areas but, as you said, not their understanding in the practice of oncology, because I'm not an oncologist.

Q So in terms of the experience of a POSA, of being a medical doctor who specializes in oncology, that is a portion of a POSA that you do not represent; is that correct?

MR. KRINSKY: Objection; asked and answered.

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THE WITNESS: Yes. I mean, who specializes in medical oncology. I do not specialize in medical oncology.

BY MS. SPIRES:

Q And that's why, for certain questions today, you've said that you would defer to Dr. Chabner?

A That's right.

Q So Dr. Chabner is the POSA in this matter that represents at least the medical doctor specializing in oncology; correct?

A I'm not Dr. Chabner, so I can't say what his range of expertise is. But my expectation is is that he has excellent credentials as a medical oncologist and likely is representing that portion of a theoretical POSA's knowledge in medical oncology; and he has other knowledge as well, I'm sure.

Q Do you agree that when a patient is given folic acid, that the supplementation with folic acid will result in a reduction in homocysteine?

MR. KRINSKY: Object to the form; incomplete hypothetical.

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THE WITNESS: So you're asking me whether every patient given folic acid will lower their homocysteine.

BY MS. SPIRES:

Q Yes.

A And my answer is, they will not.

Q Why not?

A Because only a certain portion of the public, or people, has insufficient methyltetrahydrofolate to meet the needs of methylating homocysteine. And so only the portion for which they have inadequate methyltetrahydrofolate would giving folate or folic acid have an opportunity to improve their homocysteine. Giving more to somebody who already has more than enough will make no difference.

Q Are you aware of any literature stating that giving folic acid to a patient will reduce homocysteine in only those patients with a folic acid deficiency?

A It is obvious that if you have enough methyltetrahydrofolate to methylate homocysteine, having more

methyltetrahydrofolate will not make the reaction go faster because then methionine synthase becomes limiting. So only when you don't have enough to meet and drive optimal activity will it make a difference.

So I'm not aware of a paper that says that giving excess folic acid to a person already who has -- is driving methionine synthase need for folic acid adequately makes any difference.

Q If you give folic acid to a person who has enough methyltetrahydrofolate to methylate homocysteine, what happens?

A You get a little bit of extra methyltetrahydrofolate. You get folate being used for other uses, which might be to make formyltetrahydrofolate or methylenetetrahydrofolate, to make purines, pyrimidines, et cetera, RNA and DNA. And some of the extra, as we discussed later, gets peed out in your urine because the kidney excretes it.

Q You say that if you give folic acid to a person who has sufficient

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methyltetrahydrofolate, you get more methyltetrahydrofolate; is that correct?

A I think you get the folic acid metabolized to form the various forms of folate, and whatever else is available to the kidneys gets removed. So you would get more methyl-, formyl, methylene-, et cetera, tetrahydrofolate up to a point; and then you know, you pee it out.

Q And part of this getting other forms of tetrahydrofolate is through methionine synthase; is that correct?

MR. KRINSKY: Object to the form of the question.

THE WITNESS: So again, no. Methionine synthase is a -- we're predicating is already maximally active and converting; that the other forms of folate are being formed as di -- this is folic acid you're giving, so dihydrofolate reductase creates tetrahydrofolate; and then that is shunted by an enzyme that sends it either towards making DNA and RNA or towards methylation, and so it will

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distribute with that.

And so some part of it may go through methionine synthase that's active and become tetrahydrofolate again and used for other things. But much of it can -- is getting used, because of the excess folic acid you're giving, to make the other compounds without having to transit methionine synthase, because you're making tetrahydrofolate as a product of dihydrofolate reductase, DHFR.

BY MS. SPIRES:

Q Would a POSA in 1999 have been aware that giving a patient B12, B6 and folic acid would have a synergistic effect in terms of lowering homocysteine?

MR. KRINSKY: Object to the form of the question; incomplete hypothetical and scope.

THE WITNESS: I believe you're asking were there in the public domain papers and information that said that when you combine some of the B6, B12 and folate that you might get a bigger reduction in

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homocysteine than if you used any one alone. Is that right? And that was and is true for a couple of reasons.

One, a subportion of the population has high homocysteine due to low folate. Another subpopulation or population has it because of a B12 problem. Another has it because -- perhaps because of a B6 problem.

And so when you give all three, you are getting different people but each one having a different problem and so that, combined, you see a bigger effect.

In addition, there were a paper or so suggesting that beyond that, folate, B12 and B6 in a given individual could have a bigger effect, and that could be because they have multiple roadblocks in metabolism that you're overcoming when you put them together, as opposed to treat separately.

BY MS. SPIRES:

Q Would the synergistic effect of treating a patient with vitamin B12, B6 and

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folate allow a POSA in 1999 to treat the patient with less folic acid?

MR. KRINSKY: Object to the form of the question; incomplete hypothetical and vague.

THE WITNESS: Can you restate the question just one more time.

BY MS. SPIRES:

Q Would a POSA in 1999 -- strike that.

Would the synergistic effect of treating a patient with B12, B6 and folic acid allow a POSA in 1999 to treat a patient with less folic acid than if it were given on its own?

A So it would depend on the patient and what you're saying. But I could imagine an example where a patient is low in B12, and giving folate at a dose can only do so much, because then methionine synthase sits waiting for the B12 to catalyze it so could it work and that by making B12 available, you now allow that lower dose of folate to be used.

And so there you might have an example in a patient where it makes a

difference.

On the other hand, in a patient where you've given folate and it's optimized the methionine synthase, giving B12 won't let it do any more to optimize because the methionine synthase is maximally active. Has all the B12 and all the folate it needs. Giving more doesn't make it work any better.

So there are a number of situations for -- it depends, but there are some situations where you're correct, you could give less folate because the person's real problem is that something else is creating the roadblock that, once you get rid of it, the amount of folate you give them works very nicely.

Q Didn't you mention earlier that B12 might increase the amount of methionine synthase?

A It could be.

Q So that would also contribute to the synergistic effect, wouldn't it?

A It could.

Q So that would also allow for a

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reduction in the dose of folic acid but the same -- with the same effect on lowering homocysteine?

A That's true.

So, again, B12 and folate can be two sides of the same story activating methionine synthase and making, then, more tetrahydrofolate available while, at the same time, reducing homocysteine. And therefore, either approach would end up creating -- allowing the methyltetrahydrofolate to be converted and escape from being trapped behind poorly functioning methionine synthase.

And as you said, the B12 might be also kicking up the activity; thereby, even allowing more methyltetrahydrofolate to transit, convert to tetrahydrofolate while at the same time methylating homocysteine.

Q Are you familiar with what we have referred to as the EP-005 reference?

A I am.

Q And I believe you testified in your declaration that this regimen is intended to avoid the potential negative effects of

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elevated homocysteine; is that correct?

A Could you give me a copy of the EP-005, just so I'm talking with it in front of me.

Q Sure.

I'm handing you what's been previously marked as Exhibit 1010.

A Okay.

(Witness reviews document.)

Now, I'm sorry. Give me the question, once again.

Q I believe you testified that the EP-005 regimen is intended to avoid the potential negative effects that long-term elevation of homocysteine can have on the vascular system. Is that right?

A Yes. This patent application defined high homocysteine as above 16.3; and they said for people with homocysteines higher than 16.3, long-term exposure can be associated with increased heart disease, and they propose a mixture of vitamins, B6, folic acid and B12 as a treatment.

Q So in the EP-005, they're addressing

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three of the four causes of elevated homocysteine that you mentioned earlier: B6, B12 and folate; is that right?

A Yes. Obviously, low B6, low folic and low B12.

Q Right.

So that my question is clear, since it wasn't, for the EP-005, they're addressing three of the four causes of elevated homocysteine that you mentioned earlier, which are low B6, low B12 and low folate; is that right?

A That's correct, yes.

Q Going back to your declaration, I think you state that because administering vitamin B12 can increase the conversion of 5-methyltetrahydrofolate to other forms of folate and so increase the amount of available folate for making DNA, a POSA would have been concerned that administering vitamin B12 pretreatment to a patient with cancer would likewise increase tumor growth; is that correct?

A I'm just going to state what you

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said to make sure I understood it.

That because vitamin B12 can release methyltetrahydrofolate and allow it to be converted to tetrahydrofolate, it would make tetrahydrofolate available and prevent the action of an antifolate and feed a tumor's requirement for making DNA, because it's a rapidly dividing cell and has to make a lot of DNA and it would use the folate to do so.

Is that what you're -- what you're saying and that I'm restating?

Q Not quite. I'm talking about tumor growth specifically.

A Right. So what I'm saying is, is that for a tumor to grow, it has to make DNA. For it to make DNA, it has to make thymidine; it has to make purine.

If that tumor is growing rapidly and becomes limited in its ability to grow because folate needed to make DNA has become limiting, then giving B12 and releasing folate that couldn't be used to make DNA into a form that can be, or giving folic acid and allowing DNA to be made that way, would help a tumor grow.

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So I think I answered your question.

Q So that we're clear, your concerns about B12 are the same as your concerns about folic acid, that it has the potential to increase the amount of folate available for making DNA; is that right?

MR. KRINSKY: Object to the form; mischaracterizes testimony.

THE WITNESS: I am concerned that rapidly growing cells, like tumors, need folate and B12 to make DNA properly; and that if they were limited by folate and B12, because they couldn't get enough of a supply, making more available will help them grow.

So both folate and B12 can help a tumor grow if it's been held back by not having those two nutrients available in the right amount.

BY MS. SPIRES:

Q I believe in your declaration you stated that, as of 1999, there was at least one medical reference describing vitamin B12 and saying that it can give rise to a risk of

exacerbation of cancer progression; is that right?

A So in my declaration I say that there is a mechanistic understanding of why tumors need folate, and specifically the forms of folate that are formed when B12 and folate are given.

And there is a -- in Vidal, a statement that goes along with that mechanistic understanding, suggesting that giving B12 had a risk of helping tumors grow. And again, I've given the explanation in my last answer for how it could help a tumor grow.

Q Is Vidal the reference that you were referring to in your declaration when you say there is at least one medical reference describing --

A Can you tell me what section I'm saying that so I can read that exactly. I know I talk about Vidal.

Q Paragraph 47.

A Yes, I even cite Vidal at the end of that sentence.

Q And Vidal does not provide any

clinical data; correct?

A Again, a clear mechanism based on basic understanding of what tumor cells need to divide is well-established. And all Vidal says is that based on, whatever, that and other information, they would recommend to a -- not "they" -- the company and the regulatory agency in France -- required a warning that this could be problematical on the product insert.

And they -- at least the mechanism and the direction of the warning agree, and therefore, my statement is that that there's a reason to avoid it; and an understanding of the biology and the fact that somebody has a reference -- a recommendation in the same direction just slightly strengthens that evidence.

Q But my question was a little more specific. And it was: Vidal itself, does it provide any clinical data to support its statement regarding B12 exacerbating cancer progression?

MR. KRINSKY: Object to the form.

Do you want to show him Vidal, Counsel?

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MS. SPIRES: I'm happy to if you want to see it.

THE WITNESS: Sure, I'll be glad to.

BY MS. SPIRES:

Q There are two Vidal exhibits that you cite to. I'll give you both of them.

A Thank you.

Q The first has been previously marked as Exhibit 2059. That's the 1999 Vidal reference. And then the 1998 Vidal reference is Exhibit 2124.

(Witness reviews document.)

A So your question was do they provide clinical data.

They provide conclusions that are based on clinical data, but they don't provide the clinical data that they base their conclusion upon in these references.

Q Are you aware of any studies related to humans showing that B12 supplementation can release folate and cause cancer growth?

A So that's a compound question. There are studies that -- and again, you asked about B12; is that correct?

Q Yes.

A That B12 can activate, release -- convert methyltetrahydrofolate to tetrahydrofolate. And I -- I'm trying to think whether I recall whether there's a study that says that it increases cancer growth.

There is a study by Farber, who is one of the pioneers in chemotherapy, that concluded that in a type of cancer -- leukemia -- giving more folate increased cancer growth, mass; and that's the study I recall most clearly.

Q But Farber didn't give B12.

A I'm sorry. That's folate. You're right. I'm sorry. He was giving folic acid.

And so, again, people -- I don't recall of a clinical study in which they did a randomized control trial with tumor growth or a clinical study in which they gave B12 and asked whether B12 or no B12, there was a difference in tumor growth rate.

In cell culture, I'm sure there are such studies, but I can't name one for you right now. I'd have to go back and look and

refresh my memory.

Q You mentioned the Farber study. Do you recall the antifolate at issue in that study?

A I think I cited it. So could I see a copy of the cite?

Q If you cited it, we should have it.

I'm handing you what's been previously marked as Exhibit 2042.

(Witness reviews document.)

A So it looks like they're using pteroyltrigluatmic acid, diopoteron, and pteroylaspartic acid -- P-T-E-R-O-Y-L, and then aspartic, A-S-P-A-R-T-I-C; and same pteroyl and diglutamic D-I-G-L-U-T-A-M-I-C, are the two treatments.

And these were early stage antifolates that were in use at the time of this study.

Q The doses of folic acid given in Farber far exceeded the recommended daily allowance for folic acid; correct?

A Yes.

Q And the folic acid given in Farber

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was given in a liver extract; is that correct?

A Yes.

Q So it was folic acid plus some other kind of unknown things that were in the liver extract?

A That's right.

Q And this Farber article is from 1948; correct?

A That's right.

Q Are you aware of any clinical data since 1948 showing that folic acid has promoted tumor growth?

MR. KRINSKY: Objection; scope.

THE WITNESS: Yes, in the specific area of treating with an antifolate, there have been articles -- I'm just trying to remember if tumor growth was a measured property rather than tumor toxicity or tumor size.

So I just -- I don't know if there is a paper where tumor growth was the marker and they did a control trial with and without folic acid.

BY MS. SPIRES:

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Q I think you state in your declaration that vitamin B12 deficiencies which lead to clinical symptoms, such anemia and neuropathy, are rare; is that correct?

A In my declaration?

Q Yes.

A Do you know where I said that?

Q Paragraph 33.

(Witness reviews document.)

A So what I've said is that vitamin B12 deficiencies which lead to clinical symptoms -- anemia and neuropathy -- are rare, in part because we're very good at recycling the vitamin B12 so we need extremely small amounts every day; and the stores last a good piece of time, unless there's something wrong with us.

Q Are vitamin B12 deficiencies with or without clinical symptoms also rare?

MR. KRINSKY: Object to the form.

THE WITNESS: So you have to differentiate between having low vitamin C [sic] levels that I could measure versus having low enough vitamin C to become

deficient and develop abnormal organ function, which presents then as anemia, neuropathy, something has gone wrong.

So deficiency is being low enough that the enzymes that depend on B12 -- methionine synthase and MMA -- aren't working properly. And, that's different from having low B12 but still having methionine synthase and methyl melamine CoA synthase abnormally functioning.

So -- so then my answer would be that being low enough to have abnormal MMA, which is the sensitive one, is relatively rare compared to the number of people who don't have that problem.

BY MS. SPIRES:

Q Do you have an idea of approximately what percentage of people in the general population have B12 that's low enough to, as you say, have abnormal MMA?

A I don't know an exact number. But, you know, some percentage. It depends on population, age, a bunch of other factors.

But in the elderly, you know, more;

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and in young people, very few are B12 deficient. But in India, more are because they don't eat meat products. So in that population of vegans, B12 can be more common.

Q So low levels of B12 is more common in, you said, the elderly and in vegetarians and vegans; is that correct?

A Yes.

Q Are there any other groups of people you can think of that have low vitamin B12 levels?

A We talked about pernicious anemia, which is relatively rare, but again, a population who can't absorb B12 properly.

Q Are there any others?

A I think that's most people.

Q Vitamin B12 deficiencies are more common in patients undergoing chemotherapy; isn't that true?

MR. KRINSKY: Object to the form; scope.

THE WITNESS: I don't know that to be the case. It may be that for some type of chemotherapy that has been reported;

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but I'm not aware that it's generally that people getting chemotherapy have low B12.

MS. SPIRES: Could we take a quick break?

MR. KRINSKY: Sure.

THE VIDEOGRAPHER: Time is 1:50. We're off the record.

(Recess taken from 1:50 p.m. to 2:08 p.m.)

THE VIDEOGRAPHER: Time is 2:08. We're on the record.

BY MS. SPIRES:

Q Dr. Zeisel, you'd agree that prior to June of 1999 it was known that administering a folic acid can reduce a patient's level of homocysteine; correct?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: I think what I agreed is, is that for a patient who is -- doesn't have enough folate for methionine synthase to be active and that patient has high homocysteine, giving folate would allow the methylation of the homocysteine

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to lower it.

And again, not the level, but the amount, levels, how they change depends on many factors that have to do with other outlet pathways and rates of formation of homocysteine.

BY MS. SPIRES:

Q And you'd agree that prior to June of 1999, it was believed that giving folic acid to a pregnant woman or a woman wanting to become pregnant would increase the woman's level of folate and so possibly reduce the incidence of neural tube defects; is that correct?

MR. KRINSKY: Objection; scope.

THE WITNESS: That isn't something that I commented on in my declaration.

However, the idea was, is that for women who had low folate status, providing folic acid during pregnancy might bring their folate status up to normal; and that there was something about low folate status that increased the risk for having a baby with a birth defect.

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BY MS. SPIRES:

Q And prior to June of 1999, what other reasons would a POSA have to administer folic acid to a patient?

MR. KRINSKY: Object to the form of the question; incomplete hypothetical and scope.

THE WITNESS: So one of the reasons that a POSA might treat a patient with folic acid would be that they had an anemia that was consistent with the anemia that would be caused by low folic acid. Not all anemias, because it would have been a difference in the red cell size that would have said folic acid, and then might have been some other tests.

But if you had a patient who looked like they had anemia due to low folic acid, again, you might want to bring their folic acid towards normal and they weren't being treated with some treatment like an antifolate to treat a cancer.

BY MS. SPIRES:

Q Anything else?

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MR. KRINSKY: Same objections.

THE WITNESS: Without thinking about it carefully, I'm not thinking of other reasons that you'd give folic acid at the moment. Anemia would be the presenting symptom.

BY MS. SPIRES:

Q In forming the opinions in your declaration, did you consider the differences in the folic acid receptors between normal cells and cancer cells?

MR. KRINSKY: Objection; foundation.

THE WITNESS: I did not believe that, generally for cancer cells, there was enough difference in the folic acid receptors that it would change the observation that an antifolate depends on competing with folate and giving the antidote to folate -- to antifolate, which would be folater B12, would then somehow be recommended.

So I can't think of a case where the difference in their transporters or whatever would make a big difference in

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the recommendation that I make in my expert report, that you would not pretreat a patient being treated with pemetrexed with folater B12. You could wait until the treatment was over to take care of low folate B12 and still maintain maximum potential for killing cells by not competing during the time you're treating with the tumor -- treating with the pemetrexed.

BY MS. SPIRES:

Q I wasn't clear from your answer. Is the difference between the folic acid receptors for cancer cells and healthy cells something that you considered prior to drafting your declaration?

MR. KRINSKY: Objection; asked and answered.

THE WITNESS: Again, what I said is I didn't consider it as being important.

BY MS. SPIRES:

Q Did you consider differences in the catabolism between normal and cancer cells when coming to the conclusions drafting your

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declaration?

MR. KRINSKY: Objection; foundation, scope.

THE WITNESS: I certainly considered that cancer cells growing faster had some differences in their needs and metabolism for folates and transport of folates. And again, that only made it -- did not change the overall opinion that giving the antidote to the poison you're trying to kill the cancer cells with would not have been something a POSA would have done.

BY MS. SPIRES:

Q So you say you did consider the differences in the catabolism.

A In the metabolism; metabolism being a bigger subset than catabolism, but catabolism being part of it.

Q And did you discuss this in your declaration?

A No, because, again, I concluded that it did not impact on the fact that whatever the state of the tumor cell, you were depending on interfering with folic acid metabolism for the

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action of pemetrexed, and anything you did to make folate available only interfered with the mechanism whereby pemetrexed would work; and so you would not do it.

Q Dr. Zeisel, you testified about this subject matter a year or two or three back in Indiana; correct?

A I did.

Q And you were deposed in that case?

A Yes.

Q And you were under oath when you were deposed in that case?

A Yes.

Q Do you stand by the testimony that you gave in that deposition?

A Yes.

MS. SPIRES: Marking as Exhibit 1055 your deposition transcript from that Teva litigation.

(Exhibit 1055, Deposition of S. Zeisel, 4-17-13, Eli Lilly v. Teva, was marked for identification.)

MR. KRINSKY: I'd just like to object to the admission of his entire

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deposition from the prior case. I don't think it's appropriate to somehow incorporate this testimony by reference. I think it's hearsay, and I just don't think this is an appropriate way to introduce a deposition into an IPR proceeding.

BY MS. SPIRES:

Q And Dr. Zeisel, you also testified at trial in the Teva litigation a few years ago.

A Yes, I did.

Q And you realized you were under oath in giving that testimony at trial?

A Yes, I did.

Q And you stand by your testimony given at trial?

A Yes, I do.

(Exhibit 1056, Trial testimony of S. Zeisel, 8-28-13, Eli Lilly v. Teva, was marked for identification.)

BY MS. SPIRES:

Q I'm introducing as Exhibit 1056 your testimony at trial in the Teva litigation.

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MR. KRINSKY: And once again, I would object to the admission of this exhibit in this proceeding. I don't believe that it's appropriate to take trial testimony of a witness who is testifying in this proceeding and just admit it wholesale as an exhibit to his cross-examination. I think it's hearsay.

MS. SPIRES: Dr. Zeisel, thank you for your time. I don't have any further questions, but your counsel may.

MR. KRINSKY: Let's take a break and go off the record, please.

THE VIDEOGRAPHER: Time is 2:18. We're off the record.

(Recess taken from 2:18 p.m. to 2:41 p.m.)

THE VIDEOGRAPHER: Time is 2:41. We're on the record.

EXAMINATION

BY MR. KRINSKY:

Q Doctor, do you remember over the course of today a series of questions about different strategies for lowering homocysteine?

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A I do.

Q Would a desire to lower homocysteine levels give the person of ordinary skill in the art a reason to pretreat patients who are about to receive pemetrexed chemotherapy with either vitamin B12 or folic acid or both?

A No. The -- we talked about strategies for lowering homocysteine. Homocysteine's effect take a long time to occur.

And a POSA would have known that you could wait until after the chemotherapy is completed to address lowering homocysteine; and would not have wanted to lower homocysteine because both B12 and folate, in order to lower homocysteine, have to make more folate -- reduced folate, THF -- available, and that would compete with your antifolate and be an antidote.

And so you would not do it. It would have been really unprecedented to think about it, because I don't think there was a single publication where any cancer patient, before getting pemetrexed, had gotten B12.

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And so it just wouldn't have been done. You would have waited.

Q And do you recall also recall being asked some questions about a potential synergistic effect on lowering homocysteine between folic acid and vitamin B12 and I believe it was vitamin B6?

A Yes.

Q Would any potential synergy between folic acid and vitamin B12 or among folic acid and B12 and B6 have given the person of ordinary skill a reason to pretreat patients who are going to receive pemetrexed chemotherapy with any of those vitamins?

MS. SPIRES: Object to form.

THE WITNESS: So to the contrary, if there were a synergy that worked by making methionine synthase more effective at converting methyltetrahydrofolate to tetrahydrofolate, thereby lowering homocysteine more, you would have more of a problem with creating more tetrahydrofolate to compete with your antifolate. And, therefore, if there was

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a synergy, you'd have more reason not to consider pretreating and every reason to wait to treat the homocysteine, which has long-term chronic effects and not short-term effects.

In fact, just to be clear, none of the evidence suggests that homocysteine -- high homocysteine themselves are causing the toxicity. They were just markers of something wrong in folate 1-carbon metabolism. And lowering homocysteine would have done nothing to lower your toxicity. It would have just -- it isn't a problem. The problem was something else in 1-carbon metabolism that made you more sensitive to the pemetrexed because probably your folate competition to pemetrexed was lower.

MR. KRINSKY: Thank you, Dr. Zeisel. I have no further questions at this time.

MS. SPIRES: We don't have any further questions either. You're free to go.

THE WITNESS: Thank you.

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MR. KRINSKY: Thank you, Doctor.

THE VIDEOGRAPHER: Time is 2:45. We're off the record.

(Time noted: 2:45 p.m.)

_____

STEVEN H. ZEISEL, M.D., PH.D.

Subscribed and sworn to before me

this _____ day of _____, 20 ____.

_____

CERTIFICATE OF SHORTHAND REPORTER

I, Gail Inghram Verbano, Registered Diplomate Reporter, Certified Realtime Reporter, Certified Shorthand Reporter (CA), and Notary Public in and for the District of Columbia, the officer before whom the foregoing proceedings were taken, do hereby certify:

That STEVEN H. ZEISEL, M.D., PH.D., the witness whose deposition is hereinbefore set forth, was duly sworn by me and that such deposition is a true record of the testimony given by such witness.

I further certify that I am not related to any of the parties to this action by blood or marriage; and that I am in no way interested in the outcome of this matter.

IN WITNESS WHEREOF, I have hereunto set my hand this 28th day of November, 2016.

_____

Gail Inghram Verbano, RDR, CRR, CLR
CA-CSR No. 8635

----------I N D E X----------

----------E X H I B I T S----------

PREVIOUSLY MARKED EXHIBITS REFERENCED:

ERRATA SHEET

Case Name:

Dep. Date

Dep. Name:

CORRECTIONS:

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Signature of Deponent

SUBSCRIBED AND SWORN BEFORE ME

THIS_____DAY OF _____, 20____.

_____

(Notary Public) MY COMMISSIONS EXPIRES: _____