Case outcomes of a methodology based on Matzinger's Danger Model

Gar Hildenbrand
Dir Epidemiology - Gerson Research Organization
Epidemiologist — St Andrews Clinic, Playas de Tijuana, BC

Lecture and PowerPoint presentation
to the American Academy of Anti-Aging Medicine
Fellowship in Integrative Cancer Therapy
April 11, 2013
Orlando, Florida
Fellowship Dir: Mark Rosenberg, M.D.

Gerson Research Organization
7807 Artesian Rd
San Diego CA 92127

http://www.gerson-research.org
garhildenbrand.com
email: ghildenbrand@earthlink.net

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Mark Rosenberg: Please welcome Gar Hildenbrand.

Gar Hildenbrand: I was looking at the criteria of what you were expected to pick up from this particular module, and I decided that my ratio was 1 out of 18, and that's because what we're doing is pretty far outside of the box. We're having a good time doing it. You'll notice that I'm paying homage to Polly Matzinger's Danger Model today. I'm doing so because Ephraim Fuchs and Polly Matzinger have offered us something that we have needed for awhile, which is an update to the self/nonself model of immunology which was, of course, advanced tremendously by Charlie Janeway who was the man who offered Polly a chance to move forward by asking her to publish in his journal. And what Christeene and I — Christeene is back there; she's my fellow epi — what we've done lately is partner with a long-time colleague in Playas de Tijuana, Mexico, who was not really a player in the alternative medicine scene; he was the guy they brought in to try to patch up the problems. Rafael Cedeño is a board-certified oncologist and oncological surgeon who recently came out of the closet as a supporter of Donato Perez Garcia's insulin potentiation, but not in the sense that it has been popularized as a set low dose, but rather as a means of delivering less chemotherapy with a good outcome, but not 10% or 5%, certainly more in the range of 20% to 40%.

I'll show you some cases, but first I wanted to take a little walk through Dr Matzinger's contribution, it's just so appealing logically, and then I want to give you a taste of what it looks like in an integrative methodological process to generate an outcome that might be a little bit — well — it's gratifying.

Case outcomes of a methodology based on Matzinger's Danger Model

Gar Hildenbrand
Epidemiology
Gerson Research Organization, San Diego CA
St Andrews Clinic, Playas de Tijuana, BC

Slide 1

Abstract

In 1996, Ephraim Fuchs and Polly Matzinger proposed a revolutionary model of immunity that expanded on Charlie Janeway's T-cell recognition of "not-self" pathogen-associated molecular patterns (PAMPs). In their "Danger Model," T cells are blind to tumors and their default setting for tumor antigens is "off."

Slide 2

Abstract (cont)

According to Danger, it is necessary to periodically wound a tumor in order to create damage-associated molecular patterns (DAMPs), "danger signals" that attract the attention of dendritic cells, which Matzinger has dubbed the body's "professional" antigen-presenting cells.

Slide 3

Abstract (cont)

With this knowledge arrives an understanding that the resulting immune response must be prolonged and enhanced. Constructing a supportive methodology around this mechanism minimizes exposure to cytotoxic drugs and other immunosuppressants, while maximizing anti-tumor responses. Novel roles are found for Coley Fluid and GM-CSF.

Slide 4

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So first, an important take-home is you can push the immune system — I am talking in the middle of the abstract and I apologize for that — I'm breaking the Linus Pauling principle, which is never say anything other than what you've got up there because it makes cognitive dissonance in people — but, literally, you can jam the patient into an accelerated lymphocytic cascade, you can kick the dendritic cells, you can make them co-stimulatory *in vivo* if you want, or *ex-vivo,* and administer by transfusion, but they are not going to see the tumor unless something happens — okay? — and that is the point of the Danger Model as it relates to oncology.

According to Danger, it is necessary to periodically wound a tumor in order to create damage-associated molecular patterns (DAMPs), "danger signals" that attract the attention of dendritic cells, which Matzinger herself dubbed the body's "professional" antigen-presenting cells. With this knowledge arrives an understanding that the resulting immune response must be prolonged and enhanced. Constructing a supportive methodology around this mechanism minimizes exposure to cytotoxic drugs and other immunosuppressants, while maximizing anti-tumor responses. Novel roles are found for Coley Fluid and GM-CSF.

I heard a lot of talk about Leukine from Dr Grace and Dr McKee and I appreciate that Leukine — and from Mark — that Leukine is overlooked. People grab the Neupogen when the absolute count goes low and they don't know that they're missing a treasure trove because GM-CSF, in my opinion, is an emergency-response cytokine that encourages an outpouring of all kinds of myeloid lineages from the bone marrow and, at the same time, primes tissue-resident dendritic cells rendering them co-stimulatory and sets you up for antigen presentation; but

you've got to have the antigens, and that's a little bit of what we're going to talk about here.

Early on — this is Ephraim and Polly talking — *Early on we stated that a new paradigm of immunology must take into account both the need to react to dangerous pathogens as well as to minimize the risk of dangerous autoimmunity.* We all know autoimmunity from the experiments made with, say targeted therapies.

Dr Grace: Yervoy.

Gar Hildenbrand: Exactly, where you end up without a thyroid or you end up with vitiligo, for example, or one of these unusual autoimmune conditions.

We favor the idea that the default response of the immune system is OFF, and that a 'danger' signal is required to activate dendritic cells to move to secondary lymphoid organs to alert T cells to ongoing tissue damage or distress. This would explain the need for adjuvant in the generation of immune responses to most soluble protein antigens, and would obviate the need for a mechanism — a hypothetical mechanism — *of suppressing immune responses to antigens released from cells dying physiologically*.

Is cancer dangerous to the immune system?
Ephraim J. Fuchs and Polly Matzinger
seminars in IMMUNOLOGY, Vol 8, 1996: pg 275

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Slide 5

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Slide 6

And I would point out that Charlie Janeway, before he passed away, untimely, was known to say often, to speak publicly and say often, that the dirty little secret of immunology is "adjuvant." "Adjuvant" as in Freund's, as in mineral oil, as in something noxious that pisses off dendritic cells; because if you don't do that, if you don't wound, you won't get a response because the immune system goes to wounds. Think about that. There is, without a doubt, there is pathogen-associated-molecular-pattern recognition on some of the toll-like receptors. There's no question about that. I love epidemiology — it lets you play in everybody's back yard, right? — But this is a particularly salient point, that beyond the evolutionarily-conserved pathogen-associated-molecular-pattern recognition of toll-like receptors, beyond that, there is an evolutionary-conserved mechanism which responds specifically to wounds in the organism wherever and whatever they are.

The factors most commonly associated with spontaneous regression — here we switch to cancer, per se — *as reported in the literature have been concurrent acute bacterial infections, administration of bacterial vaccines, or the removal of at least some of the tumor or its metastases*.

In each case, I would urge you to see these as tissue-wounding events so that we don't ask, "What is it about the

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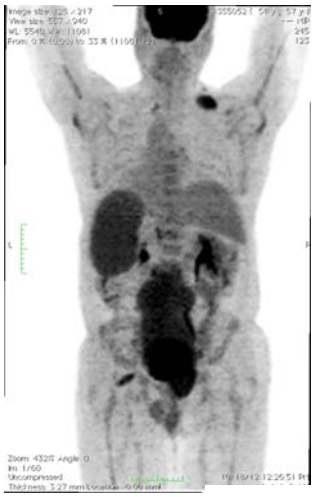
Slide 7

George M, 58-year-old man with 2nd recurrence of mantle-cell lymphoma after 8-year history of the disease. This Oct 18, 2012 MIP reveals many foci of FDG avidity, notably 2 nodes in the infrahyoid neck with greatest diameters of 1 cm and 1.5 cm, an external iliac node of 2.2 cm, a soft-tissue mass on the right shoulder of 4 x 2.5 cm, pronounced splenomegaly measuring 5.5 x 17 x 18 cm, and a huge retroperitoneal mantle of 6 x 11 x 21 cm.



Slide 8

bacteria that can have an anti-tumor effect?" We ask, "What is it about the immune system's response to the bacterial damage caused to the cells in which bacteria are infecting that leads to the kind of an immune response that can actually clear tumors?"

We are going to start with George M. Let's see if I can put an arrow on this guy and animate him. Are you going to turn, George? (Difficulty with animation program). Oh, we didn't put my AV clip in. Okay. Well, I'm sorry. He's supposed to turn. You're supposed to see that the mantle, here, that's in his abdomen and behind his intestine, in front of his spine, is a gargantuan, thick mantle. You can kind of get that anyway. You can certainly see that the spleen, where the cursor is resting right now – the spleen has been taken. So what we have here is definitely a couple of nodes in the infrahyoid neck, greatest diameters of 1 cm and 1.5 cm, an external iliac node of 2.2 cm, a soft-tissue shoulder mass of 4 x 2.5 cm, pronounced splenomegaly 5.5 x 17 x 18 cm, and a huge retroperitoneal mantle of 6 x 11 x 21 cm. This is the second relapse of a mantle-cell for this man.

And this is the protocol that he was going to get, many of you already know it, and some of you probably don't. He was going to get … cycle one was going to be maxi-CHOP without rituximab; cycle two was going to be rituximab and high-dose cytarabine; three was going to be rituximab and maxi CHOP; fourth was rituximab with high-dose cytarabine; and then R + maxiCHOP; and then R + high-dose cytarabine and additional rituximab day nine. And then consolidate with carmustine, etoposide, cytarabine, and melphalan followed by a peripheral blood stem cell transplant. The duration of treatment was going to be 9 months. And this is the chart (see slides 10-14), we'll just sort of breeze through it here.

Protocol proposed by MSKCC

Cycle1: maxiCHOP – no rituximab

Cycle2: R + High Dose Cytarabine

Cycle3: R + maxiCHOP

Cycle4: R + High Dose Cytarabine

Cycle5: R + maxiCHOP

Cycle6: R + High Dose Cytarabine + additional rituximab Day 9

Consolidate with carmustine, etoposide, cytarabine, and melphalan followed by a peripheral blood stem cell transplant

Duration: 9 months

Slide 9

Protocol proposed by MSKCC

Cycle 1 : MaxiCHOP (note no rituximab* on cycle 1)

Day (s)	Drugs	Dose	Route	Comments
1	**Cyclophosphamide**	1200mg/m^2	IV	Slow IV bolus injection over at least 15 minutes into the side-arm of a fast running Sodium Chloride 0.9% Infusion or in 250ml 0.9% Sodium Chloride over 30 minutes
1	**Doxorubicin**	75mg/m^2	IV	Slow IV bolus injection over 3-5
				minutes into the side-arm of a fast running Sodium Chloride 0.9% Infusion
1	**Vincristine**	1.4mg/m^2 (max 2mg)	IV	Infused in 50 minibag Sodium Chloride 0.9% over 5-10 mins
1 to 5	**Prednisolone****	100mg	Oral Days 1 to 5	Take in the mornings, swallowed whole with food. Give steroid card

*Rituximab is omitted from cycle one but given on day 1 of all other cycles – This avoids problems at presentation with high white cell counts.

Slide 10

Cycles 2 and 4: R- High Dose Cytarabine

Day (s)	*Drugs*	*Dose*	*Route*	*Comments*
1	**Rituximab**	375mg/m^2	IV	*See Rituximab protocol for details regarding pre-medication, infusion speed, observation during infusion and management of infusion related reactions*
1to 2	**Cytarabine**	3g/m^2 BD***	IV	IV infusion over 3 hours – give every 12 hours for four doses

Slide 11

Cycles 3 and 5 R-MaxiCHOP

1	**Rituximab**	375mg/m^2	IV	*See Rituximab protocol for details regarding pre-medication, infusion rate, observations and management of infusional related reactions*
1	**Cyclophosphamide**	1200mg/m2	IV	*Slow IV bolus injection over at least 15 minutes into the side-arm of a fast running Sodium Chloride 0.9% Infusion or in 250ml 0.9% Sodium Chloride over 30 minutes*
1	**Doxorubicin**	75mg/m2	IV	*Slow IV bolus injection over 3-5 minutes into the side-arm of a fast running Sodium Chloride 0.9% Infusion*
1	**Vincristine**	1.4mg/m2 (max 2mg)	IV	*Infused in 50 minibag Sodium Chloride 0.9% over 5-10 mins*
1to 5	**Prednisolone****	100mg	Oral Days 1 to 5	*Take in the mornings, swallowed whole with food. Give steroid card*

**Day 1 Prednisolone is given 1 hour before the start of the rituximab infusion (mandatory for the 90 min infusion)

Slide 12

Cycle 6: R- High dose cytarabine + rituximab on day 9

Day (s)	*Drugs*	*Dose*	*Route*	*Comments*
1	**Rituximab**	375mg/m^2	IV	*See Rituximab protocol for details regarding pre-medication, infusion speed, observation during infusion and management of infusion related reactions*
1to 2	**Cytarabine**	3g/m^2 BD***	IV	IV infusion over 3 hours – give every 12 hours for four doses
9	**Rituximab****	375mg/m^2	IV	*See Rituximab protocol for details regarding pre-medication, infusion speed, observation during infusion and management of infusion related reactions*

****An extra dose of Rituximab is given D9 cycle 6 post high dose Cytarabine as part of the in vivo purging prior to stem cell harvest.

Slide 13

Number and cycle frequency

6 cycles are given in total with alternating R-maxiCHOP (no rituximab on cycle 1) and high dose Cytarabine every 3 weeks. This is should be consolidated with BEAM PBSCT. Stem cells can be mobilised off the back of a cycle 6 of R-HD-Cytarabine.

BEAM = carmustine, etoposide, cytarabine, and melphalan
PBSCT = peripheral blood stem cell transplant

MSKCC oncologist estimated 9 months of treatment to completion of protocol. The protocol was presented as having a 25% mortality rate.

Slide 14

Sample protocol for immune-centered management

- Lab-guided periodic priming with GM-CSF
- Lab/clinical-guided stimulation with Coley Fluid
- Tumor sampling for chemosensitivity testing and lysate
- IPT (20-40% dose) chemo at 1-week interval (or p.r.n.)
- IPT amydalin, 6 grams, twice weekly
- Daily ex-vivo hyperthermia
- IV chelation (EDTA, Vit C, Multi vit/min, Hartmann
- IV H_2O_2 with DMSO
- Modified Gerson diet therapy with coffee enemas

Slide 15

The patient didn't want to do it, and he didn't want to do it because they told him that 25% of the people weren't going to make it through the protocol. They do six cycles and then, off the back of cycle six, you can mobilize and harvest stem cells and prepare for the peripheral-blood stem-cell transplant.

Okay, what we did instead: We used labs, we used objective data, as well as the clinical picture to decide when to prime again with GM-CSF. We weren't quite as aggressive as you might have been. We didn't use it daily; we used it more at weekly or at a 10-day interval. We're kind of following the effector cycle, life cycle, the effector life cycle, somewhere in the neighborhood of 10 days to two weeks on the outside, if we don't re-costimulate. The lab and clinically guided stimulation with Coley Fluid was a key component of management for us. This is something that … Coley is kind of a neat material. I'm still rooting for

Don MacAdam and Professor Hoption-Cann to get their GMP facility built so that they can make this stuff according to spec for Denmark, because Henrik Schmidt is ready to do a lymphoma-melanoma trial of the stuff over there. I personally don't think that the methodology of the trial is going to generate the most robust outcomes that one would hope to see, because I don't think Coley is a good stand-alone material. In fact, I've come to the opinion that there are no stand-alone materials; it's a both-and world. I mean, there's just no such thing as a magic bullet.

Fellowship participant: What is the Coley Fluid?

Gar Hildenbrand: Coley Fluid. The back story on the Coley Fluid is where Polly Matzinger came up with her observation regarding bacterial infections and what she uses as an example of how immunology can be used in cancer to cause the immune system to clear tumors. The back story is that in 1866, Wilhelm Busch a professor, an eminent professor of surgery and medicine, observed in a patient he had operated, a head-and-neck sarcomata patient who had seven tumors — he had to take one off the neck for mechanical purposes — he observed that when the tumor bed became infected with an erysipelas, which is now known to be group A Strep, β-hemolytic Strep, or Streptococcus pyogenes, that during the six weeks of immune responsive and reactive period where the woman was spiking fevers again and again, that she absorbed the remainder of the tumors and Dr Matzinger likes to point out that, you know, Coley injected his noxious material into the tumor stroma and thereby created a wound that alerted the body's professional antigen-presenting cells, drew them into the wound and we had a cross-presentation of antigen and therefore a clearing not only of the malignant cells but of the enormous quantity and variety of cells in the micro-environment that are not malignant but are simply collaborative cells mostly from the innate immune system.

IPT, oh — *Tumor sampling for chemosensitivity testing* — we didn't use Dr Weisenthal's — we used Dr Nagourney's method of sampling for chemosensitivity and resistance. But the first thing we did with George was we asked him if we could have the tumor off his shoulder, the big friable mass that the surgeon thought could come off very easily. We sent it to three labs: the pathologist, Bob Nagourney at Rational Therapeutics and to our own laboratory chief, Lucio, who made a whole-tumor lysate out of it. And the whole-tumor lysate was really an effort to construct a component of the methodology that would at least pay basic recognition to what Busch saw in 1866. We developed a protocol of injecting the Coley and creating a big fat wheal and then injecting the tumor-lysate into the Coley wheal, which is about as close as we could get to the infection in the tumor bed where there was all this detritus and antigen shed from the tumor, which was just there for the immune system to pick it up.

IPT (20-40% dose) chemo at 1-week interval (or p.r.n.) as could be tolerated by the patient. I need to point out that the patients, when you begin to do these integrative methodologies, and when you begin to get that immune system to really respond, you spend a lot of time doing basic supportive treatment because they go into reactions that make Mike Lotze's and Steve Rosenberg's IL-2 days look pretty mild. But the crazy thing about reacting to an immune response is it's like a woman who's had a baby and she was in transition with her feet in the stirrups screaming, "I hate you, you're parents weren't married, and I'm never going to do this

again — you did this to me," and two weeks later she's looking at the window display of baby booties and cute little clothes and she's saying, "I think he needs a sister now; what do you think?" And the patient has been through these raging immune responses and several days later they will say, you know, are we going to do that again? I think I need that again. And — yes?

Christeene Hildenbrand: You should tell them what happens to pain medicines.

Gar Hildenbrand: The pain? Oh, this is — people always want to know, what are you going to do to objectively to monitor the response of the tumor? We say we're not going to do anything objectively because you came in on ten Vicodin a day and morphine for breakthrough, so what we're going to do is we're going to use pain relief as a surrogate endpoint, and that way you can hold off on urging us to get this repeated scan done so soon because we want to, you know, we want to give a little time to the immune system to do something that's meaningful so you'll be impressed, so you'll be a happy person when you see the outcome of the scan. And pain management is really satisfactory.

The *IPT amygdalin*: Now this is one that … I put it in here and I said, "What are these guys going to think?" But, I have a little something on it later on, and I'll try to explain what I think is happening. All I can tell you is that when you do this with amygdalin, that a gastric cancer patient will grab for the stomach; that a colon cancer patient will blanche and have to rush off to the bathroom and pass a ton of mucus. You see physical, clear physical responses that just simply are not there when somebody gets a 6-gram bolus of amygdalin intravenously and there's been no preparation of the host. If you don't prepare the host, if you don't do something for the host, you're not going to get the response. And frankly I think there's something to this. This is not original with us, we didn't dream it up; it was in place in Dr Cedeño's practice when we arrived and partnered with him.

Daily ex-vivo hyperthermia — these are simple mechanisms — I'll talk about them later. We use a short-wave diathermy device, and Mag Ray, and little sauna tent. No big deal.

Fellowship participant: You're heating the blood outside the body?

Gar Hildenbrand: No, we're not doing *ex-vivo* hyperthermia of the blood; no, this is just applying heat to the body, to the corpus, and it's just to raise the core temperature a little bit — help the body to raise its core temperature; it's trying to. When we give Coley and people are doing hyperthermia with it they shake and shiver a little less — because the body doesn't have to go through all that to get warm.

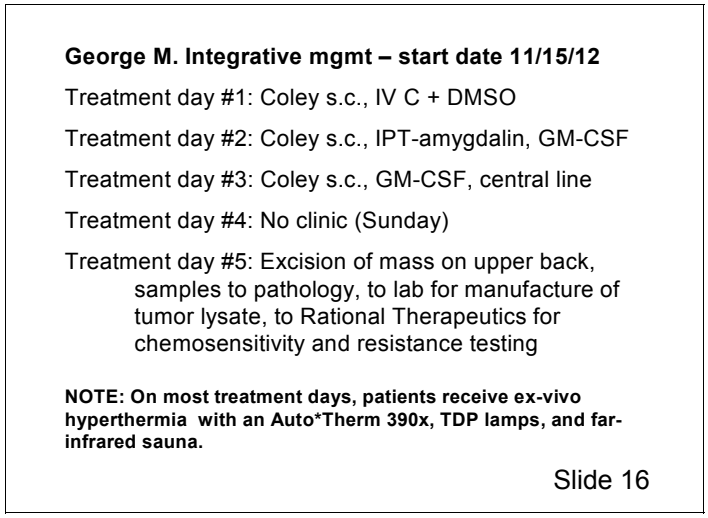
IV chelation with EDTA, Vitamin C, multivitamins and Hartmann — I don't need to go into that.

IV H_2O_2 with DMSO – Again, I mention it because it's a component of the clinic's treatments. We're doing the epidemiological thing right now which is collecting the data and wondering what is it doing, can we tease that out if it does anything, we don't know.

Modified Gerson diet therapy with coffee enemas — We've got enough historical data, we've got roughly 5,000 charts from four different Gerson facilities spanning a course of, well from 1977 to …

Christeene Hildenbrand: Four Gerson and one Issels.

Gar Hildenbrand: One Issels, yeah, right, one Issels. More on that later but actually more on



Gerson tomorrow because tomorrow we'll present a cohort of seven FIGO Stage III, mostly IIIC ovarian patients

Christeene Hildenbrand: All. All.

Gar Hildenbrand: Optimally debulked. Oh, they're all IIIC? Okay. You'll have to tell me why. Is that because of peritoneal washings?

Christeene Hildenbrand: Yes. [Editors note: I was wrong, because I don't have primary-source validation for the washings in Aurora L's case.]

Gar Hildenbrand: Okay. There's been a little stage migration in FIGO Stage IIIC and it now includes peritoneal washings positive for malignant cells. I'll be presenting those because those women are out none of them less than five years and some of them many — decades.

Christeene Hildenbrand: 12 to 38 years.

Gar Hildenbrand: Optimally debulked followed by the diet therapy, followed by raging fevers and no special anti-malignant or cytotoxic treatments, so we're relatively convinced that Gerson's diet therapy, maybe because of its gut pumping with coffee enemas and castor oil by mouth and so on, actually tricked the innate immune system into activating.

Okay, so on treatment day 1, — this is just for example — we gave Coley sub-cu; we gave Vitamin C with DMSO. On treatment day 2, Coley sub-cu, IPT-amygdalin, GM-CSF. Treatment day 3, Coley sub-cu., GM-CSF, central line. Treatment day 4, no clinic, it was a Sunday. Treatment day 5, excision of mass on the upper back.

Fellowship participant: Do they have fever from each day of Coley?

Gar Hildenbrand: No, not at first, because we're titrating slowly. We're starting low, we're not wanting necessarily to tip over the fruit cart; we're just wanting to begin to ask the bone marrow to wake up.

Fellowship participant: It's killed Strep A and Serratia marcescens?

Gar Hildenbrand: That's right. They're co-cultured because there was an observation back in the early 1900s that co-culturing Strep with Serratia, which was at that time thought to be non-pathogenic, actually brought out the virulent properties of the Strep A. And so, absolutely. Obviously, the guy knows his Coley.

Christeene Hildenbrand: The GM-CSF — wasn't that half and half — that wasn't a full dose both days?

Gar Hildenbrand: Oh, that's right. We did 200 mcg per injection of GM-CSF. We were going slow with the guy because of this gigantic disease in his gut. Our experience is that if you come in like a fighter pilot on a patient with bulky disease and you trip that immune system, the chances are you are going to trigger a bunch of host-cell-antigen cross presentation and end up with massive autoimmunity, you're going to have neutrophils swarming, you're going to just deplete the host, and the host is going to die. You're going to kill them. It's very hard to shut it off unless maybe you came in

George M. ***(cont)***

Treatment day #6: Levaquin, IV nutrients, chelation

Treatment day #7: IPT-amygdalin, Coley s.c.

Treatment day #8: IV nutrients, Vit C, DMSO

Treatment day #9: Coley s.c., IPT-amygdalin

Email from Dr Cedeño Nov 23, 2012: Hi Gar, regarding George, I just checked on him and ***the tumor in the abdomen is shrinking about 20 to 25% already***. I just put the Coley s.c.

Treatment day #10: IV nutrients

Slide 17

with, say, possibly cyclophosphamide. Okay — the 3 laboratories — and the note here: *On most treatment days, patients receive ex-vivo hyperthermia* — and that's the name of the device, just for what its worth.

Treatment day 6, Levaquin, IV nutrients and chelation; and Levaquin was continued for seven days I think. IPT-amygdalin on day 7 and Coley sub-cu.

Fellowship participant: What was the cause? Did he have an incurrent infection or was that part of the protocol?

Christeene Hildenbrand: Surgery. Didn't they do the Levaquin because of the surgery?

Gar Hildenbrand: Yeah. He was operated. Post-surgical …

Question: Levaquin was for an infection or …?

Gar Hildenbrand: No, the Levaquin was to prevent an intercurrent infection postsurgically.

Fellowship participant: I would think you would want an intercurrent infection.

Gar Hildenbrand: Well, we like to chose the microbes, you know, we don't want volunteers because they don't all behave the same way. You know, if you look at the history of Streptococcus back when it was called erysipelas, the Faculty of Medicine in Paris in the 1830s would talk about it in terms of being able to trade this infection for that disease; if you want to get rid of CNS syphilis, let me give you erysipelas, and we'll cure your central nervous system syphilis.

So, on day 8, IV nutrients, vitamin C, DMSO. Day 9, Coley sub-cu, IPT-amygdalin, and an email that same day from Dr Cedeño: *Hi Gar, regarding George, I just checked on him and the tumor in the abdomen is shrinking about 20 to 25% already. I just put the Coley sub-cu.* Now this is a perfect example of "Danger", a place where I can take the springboard and talk about, "Why would that that happen?"

The patient's primed; we've used the Coley as a universal adjuvant; we've used GM-CSF as a way of priming tissue resident dendritic cells; and then we've used the surgeon's scalpel to create danger signals right at the site where the tumor was situated. And this translated from a surgery on the shoulder to a very substantial reduction in volume of the retroperitoneal mantle. And we confirmed it.

Christeene Hildenbrand: You should talk about his difficulty going to the bathroom, peeing.

Gar Hildenbrand: Yeah, he couldn't pee, and that cleared up right away after the response. And this was a silent response. This was not attended by major symptoms, this was a silent response. The lymphocytes just cleared the tumor. And we don't know, by the way, if it was CD-8 or CD-4 cells, I mean I wish we did, but who's got the budget?

Fellowship participant: Or macrophages or neutrophils.

Gar Hildenbrand: It could be neutrophils, of course, it could. I don't lean towards the macrophages, so much, but the neutrophils definitely. There's a story that hasn't been told there.

George M (cont)

Treatment day 11: Coley s.c. (Sunday – no other tx)

Treatment day 12: IPT-miniCHOP #1

Treatment day 13: Intratumoral Coley, IV inputs

Treatment day 14: IPT-amygdalin

Treatment day 15: Coley s.c. (104°F)

Treatment day 16: GM-CSF, IPT-amygdalin

Treatment day 17: GM-CSF, Coley s.c.

Treatment day 18: No treatment (Sunday)

Slide 18

George M. ***(cont)***

Summary of treatments in clinic

In total, there were 70 treatment days.

IPT-miniCHOP x 4 on treatment days 9, 24, 40 and 61

Coley x 28 (1 intratumoral, 27 s.c.)

GM-CSF x 6 on treatment days 2, 3, 16, 17, 58 and 59

Diet therapy and detox with coffee enemas throughout

On treatment day 36, Dr Cedeno texted: I DON'T FEEL THE TUMOR NEITHER THE SPLEEN …. WHAT TIME YOU'LL BE HERE?

Slide 19

Day 11, Coley sub-cu again, and day 12 IPT-miniCHOP, okay, it's the first day we did that. The IPT-mini-CHOP was really just two drugs with Solu-Medrol rather than daily prednisone. What's in that [CHOP]?

Dr Grace and other fellowship participants: Cytoxan, doxorubicin (Adriamycin), vincristine …

Gar Hildenbrand: And then the intratumoral Coley, IV inputs and the IPT-amygdalin, Coley and GM-CSF …

Dr McKee: You probably didn't have your Nagourney results back yet? Your Rational Therapeutics results back yet?

Gar Hildenbrand: No, we guessed and it turned out that they were right.

Dr McKee: So CHOP was good?

Gar Hildenbrand: CHOP was good. Right. And I've got some comparative cumulative dosing data that I'll show you in a minute to give you an idea of how much money you can save on chemotherapy if you do it this way.

Okay. *In total there were 70 treatment days. The IPT-miniCHOP was given 4 times on treatment days 9, 24, 40 and 61.Coley was given 28 times (once intratumorally and 27 times subcutaneously.* By the way…

Fellowship participant: Which tumor did you put it in?

Gar Hildenbrand: That was a lymph node, a clavicular … or cervical, a low-cervical-chain lymph. And, it was a little bit arbitrary that we did that. *GM-CSF was given 6 times on treatment days 2, 3, 16, 17, 58 and 59*. Each time we took 400 mcg and divided it into a two-day dose just to be gentle with the guy who was, you know, he was going through a lot. *Diet therapy and detox with coffee enemas were used throughout. On treatment day 36, Dr Cedeño texted me* — and the capital letters is not original with me, that's what he texted me, I mean, I always feel like people are shouting when it's all capital letters — *I DON'T FEEL THE TUMOR NEITHER THE SPLEEN …. WHAT TIME YOU'LL BE HERE?* That's Spanglish. And, he was blown away. He was happy.

Christeene Hildenbrand: He was sure, Dr Cedeño was sure we would have to take that spleen. He said, when this is all over we will have to take the spleen.

Gar Hildenbrand: Right. The mantle-cell expert thought the spleen would have to come out too — the mantle-cell trialist. And, you know, appreciate that, because that's the historical experience. You know, what we're trying to do is find a

new way forward and we're not walking

So — *Comparison of cumulative cytotoxic drug dosages*. The MSK proposed giving Cytoxan at 6.84 grams and we ended up suing a total of 2.74 grams, doxo was at 426 mg, comparatively we used 170 mg; vincristine was 7.98 mg versus 2 mg, it was very, very low; and the Ara-C was not used at all. So we saved 68.4 grams of Ara-C toxicity for the patient (slide 20, right).

And here's the outcome. We sent him home with the Coley and a tumor lysate and he kept injecting it, so we were able to stretch this out. We saw him in November; we had him November, December, January and we sent him home the end of January. He spent February and the first part of March at home. And in New York, a mantle-cell trialist is following him and ordered the PET/CT there. And you can see a nice resolution of that mantle. The patient is in great shape. He's an IT guy at a college in New Jersey and he's playing hardball with the intramural team there, you know, baseball, and running around the bases and having a wonderful time of it.

Here's just a look at the spleen (slide 22, right). I put this in here because of the spleen is, as you can see — it's all lit up, here, and it's — not only has the splenomegaly resolved, the FDG avidity is gone as well. And there was a hot nodule protruding from the spleen that's not caught in this image, but that was one of the first things to disappear and that's why Cedeño was so surprised not to be able to palpate the spleen.

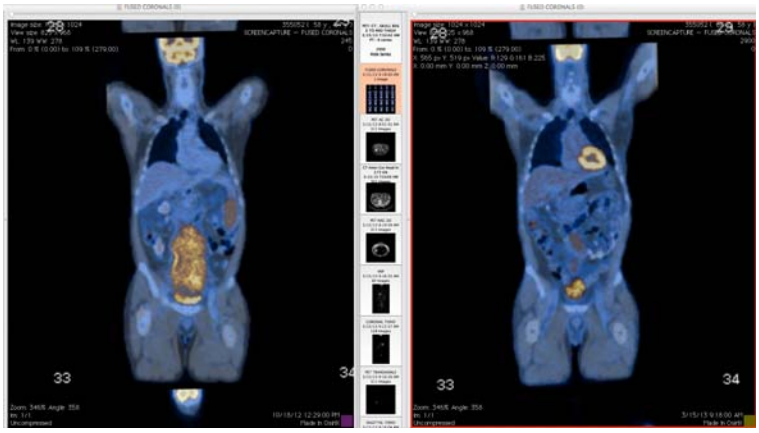
Comparison of cumulative cytotoxic drug dosages

Drug	MSKCC	St Andrews Clinic	
CTX	6.84 grams	2.74 grams	(40%)
DOX	426 mg	170 mg	(40%)
VCR	7.98 mg	2 mg	(25%)
ARA-C	68.4 grams	0 grams	(0%)

Slide 21

George M. Comparison of PET/CTs

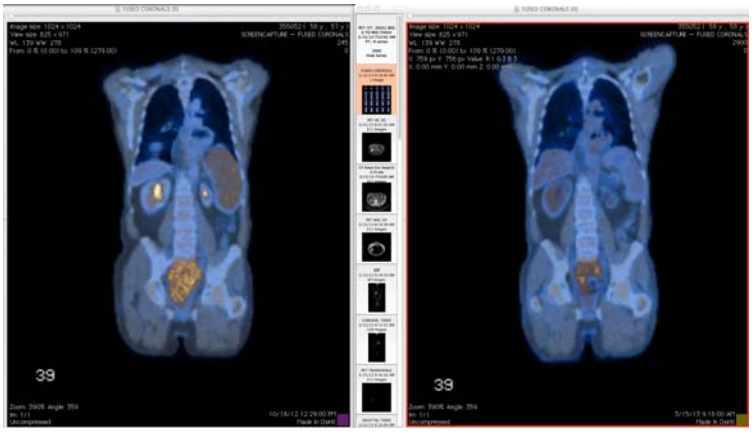
Oct 18, 2012 **March 15, 2013**

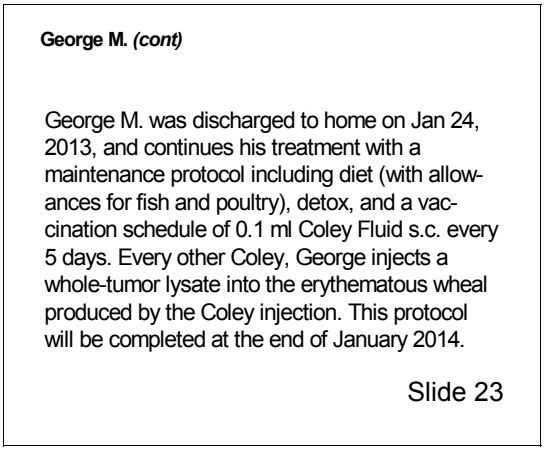


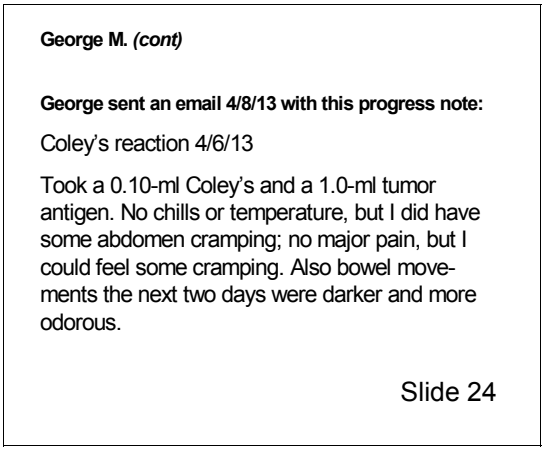
Slide 22

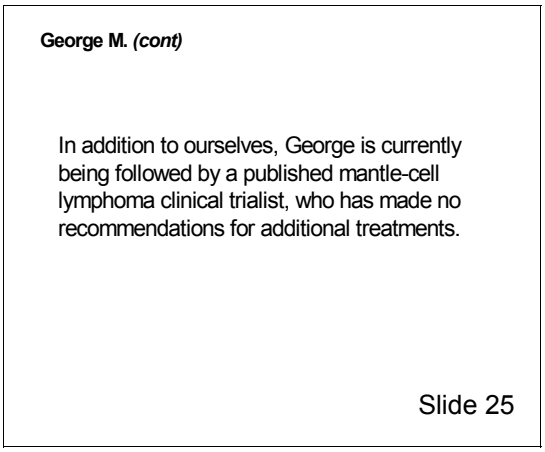
George M. Comparison of PET/CTs

Oct 18, 2012 **March 15, 2013**









George was discharged to home on Jan 24, 2013, and continues his treatment with a maintenance protocol including diet (with allowances for fish and poultry). You figure, you're putting people through fevers, they're going to go through their amino acid reserves, so, you know, veganism is not actually most people's preference when you have to do a lot of vegetable protein powders and legumes and things like that to be able to keep up. That way it's easier to just say go ahead and have some fish and chicken if you want to, and eggs of course, and dairy. Detox, and a vaccination schedule of 0.1 ml Coley Fluid sub-cu every 5 days. Every other Coley, he injects a whole-tumor lysate into the erythematous wheal produced by the Coley injection. This protocol will be completed at the end of January 2014. In other words we gave him enough to go a year at home. And, thus far it's going smashingly.

This last progress note, I just typed in. George sent this: *Coley's reaction April 6th—Took a 0.10-ml Coley's and a 1.0-ml tumor antigen* — He calls it tumor antigen. He could never get it into his head that there is a difference between antigen and lysate. An antigen is the result of a search for the Holy Grail that probably didn't work and the lysate is an acknowledgment that this entire slurry is important to us and — *No chills or temperature* — this is a new trend for George. He's not reacting anymore – he says, *but I did have some abdomen cramping; no major pain, but I could feel some cramping. Also bowel movements the next two days were darker and more odorous.* And this is what it looks like on the tapering end of things. We are hopeful. We're going to keep following. I present that as a very reasonable response to an integrated management that allows you to be very modest with your cytotoxic inputs and which is watching out for the patient in a number of ways that are not currently of much clinical meaning in the general community treating cancers.

In addition to ourselves, George is currently being followed by a published mantle-cell lymphoma clinical trialist, who has made no recommendations for additional treatments.

Now Carol, I want to talk about this case a little bit before we go to mechanisms just to show that a similar, very similar protocol can be used in a very, very different cancer. The mantle-cell lymphoma is its own bag of spiders, but Carol came in for ablation of a metastasizing poorly-differentiated invasive ductal carcinoma. In January, an ultrasound revealed the mass and a core biopsy found it to be

poorly-differentiated invasive-ductal carcinoma. The modified Bloom-Richardson-Nottingham grade 8/9, ER(-), PR(-), and ominously, Her2/neu 3+. This woman would not stay home, so she ended up in the Mexican practice and she wanted to get a good result. And I don't have to tell you that Herceptin is kind of pricey, so nobody is going to be really buying it out of pocket. So we had to ask, "What can we do rather than that?"

Carol C, 66. Ablation of a metastasizing poorly differentiated invasive ductal carcinoma

- 1/10/12 Ultrasound: Irregular mass with spiculated margins = 12 x 13 x 14 mm L breast 7:00.
- 1/16/12 Core Bx: Poorly differentiated invasive ductal carcinoma, Nottingham grade 8/9, ER(-), PR(-), Her-2/neu 3+.

Slide 26

She began Dr Gerson's diet therapy at home and — oh, this is something that — I want to point this out. This is something you'll see with virtually any type of immunotherapy — *at first, her mass became smaller; then the mass grew rapidly with increasing axillary lymphadenopathy* — let me explain. Polly Matzinger is… Christeene and I have spent a bunch of time with her in the garden behind the NIAID lab, closing Harry's Tap and Grill, which is permanently closed now, sadly; and she tells stories that are wonderful stories. For example, a friend of hers who's a co-researcher at NIH went in for a physical and had a routine AP and lateral chest done, and a tumor was found in her upper left lobe. It was biopsied, with a needle of course, and she was referred to a surgeon; the surgeon asked for a formal study. So, by the time the formal study was done, the tumor had shrunk by 60%, and Polly said, "See, that's Danger." She was on a natural high, her immune system was flowing, not ebbing, and the needle punctured the tumor, the wound attracted the immune system, and the response of the immune system was to clear a chunk of tumor.

Carol C *(cont)*

- Began Gerson's diet therapy at home and, at first, her mass became smaller; then the mass grew rapidly with increasing axillary lymphadenopathy.
- 7/5/12 Came to Mexico to add Coley, Gramal, IPT-Ctx with intent to shrink mass for surgical resection.

Slide 27

Carol started the Gerson diet therapy. This is something we have seen over and over again and which maps a trend in the follow-up charts, which is that Gerson had a real immunotherapy. If you don't believe that, you look at the tuberculosis literature, and if you read German you're really well off, because it's a rich, rich oeuvre. And it's evident that this man, who was a division director of the department of tuberculosis at the University of Munich, one of the top medical universities of the world, really had interdisciplinary help, and he really had an immunotherapy that could smoke out tuberculosis that was already cavernous and making cavities in the lungs bilaterally and *cured* it — and cured it. So, one can accept that this is an immunotherapy. "Why does it stop working?" is the question.

Why would immunotherapy start and then stop working? And the answer becomes just fiendishly simple; it's incredibly simple. It's a good diet and detox program. It addresses what 's happening around the tumor, which is often quite messy, a little bit like what a pyogenic granuloma will do in its immediate vicinity; it would be locally damaging. So you heal that. You get the immune system to go in and it clears all that up and then where are your danger signals? Then you end up with a healthy patient and a healthy tumor, because there's no additional wounding of the tumor then there is no

Carol C *(cont)* Slide 28

Exam Date: 01/10/2012
US BREAST LEFT
CPT:76645 Accession#:5169593

FINDINGS:

- There is an irregular mass with spiculated margins measuring 21 x 13 x 14 mm seen in the left breast at 7 o'clock.
- This finding correlates to the palpable mass. Numerous satellite masses are present in the 7-8:00 radius. The largest at 8:00 measures 12 x 13 x 8 mm. There is an abnormal axillary lymph node present as well.

Carol C *(cont)* Slide 29

Collected: 1/16/2012

Final Diagnosis:

BREAST, LEFT, LOWER INNER QUADRANT, CORE BIOPSY:

TUMOR TYPE: Invasive ductal

NOTTINGHAM GRADE: Poorly-differentiated TOTAL SCORE: 8/9

ESTROGEN RECEPTOR: NOT PRESENT

PROGESTERONE RECEPTOR: NOT PRESENT

HER2/NEU BY IHC: 3+ (OVER-EXPRESSED)

Carol C *(cont)*

At the time of admission for immunotherapy, July 5, 2012, Carol's left-breast tumor had grown to 7 x 6.3 cm with bulky left-axillary adenopathy.

At 110 pounds, 5'8'', she had lost a great deal of weight and was started on supplemental proteins and Coley Fluid. She had 33 Coley injections during her 4.5-month stay in Playas de Tijuana. She was enormously reactive and often went days between injections. This was especially true after each IPT-chemo.

Slide 30

additional immune response. There's no more antigen presentation. So, what might have been a mystery looking at it through Charlie Janeway's eyes becomes a clear picture looking at it through Polly Matzinger's and Ephraim Fuchs' eyes; that you get rid of the danger signals, you get rid of the wounding, you essentially heal the mess that is the tumor microenvironment and you set the stage for rapid progression of the disease in a healthy host, which seems ironic, but it's the way it is.

7/5/12 Came to Mexico to add Coley, Gramal, IPT-Ctx with intent to shrink mass for surgical resection. This one was never presented to her that we thought we could get it to go away entirely because we didn't know, and Her2/neu scares the socks off of us, it just does.

Ultrasound finding was a — in January, 2012 — was a 21 x 13 x 14 mm mass seen in the left breast at 7 o'clock and it correlated with palpable mass and there were numerous satellite masses present in the 7-8:00 radius. The largest at 8:00 was 12 x 13 x 8 mm and there was an abnormal axillary lymph node present as well. By the time we saw her, it was much, much, much larger. Final diagnosis: breast, left, lower inner quadrant, core biopsy: Invasive ductal, poorly differentiated, total score 8/9. No estrogen receptor, no progesterone receptor.

At the time of admission for immunotherapy, July 5, 2012, Carol's left-breast tumor had grown to 7 x 6.3 cm with bulky left-axillary adenopathy. At 110 pounds, 5'8'', she had lost a great deal of weight and was started on supplemental proteins and Coley Fluid — I want to tell you that this woman is a 65-year-old woman who revealed to us that, and you can find a record of it, she ran the Iditarod. She canoed, she kayaked 900 miles by herself, solo, toward the Arctic in Alaska and then she did the Iditarod. And when we asked her about it she said, "Well, you know, first you gotta build the sled."

This is no shrinking violet; this is a tough, tough woman, and she was emaciated when we first saw her; she was just gaunt. During her stay, she had 33 Coley injections — she was with us for four-and-a-half months because, as we were discussing with Mark, if oncology isn't outpatient, what is it? You can't keep people in a facility for that length of time and get anything done. She hung out in Playas at hostels. She was enormously reactive and often went days — I would even say sometimes weeks — between injections. This was especially true after each IPT-chemo. What we saw

was a pattern where you give the chemo on day one and on day two you follow with Gramal and day three you follow with Coley and on day four they get the symptoms, and they're in hell on days four and five.

Fellowship participant: What's Gramal?

Gar Hildenbrand: Oh, Gramal, sorry, GM-CSF, the Latin presentation, right, so in the U.S., Leukine, and in Latin America it's Gramal.

Christeene Hildenbrand: Hildy, do you remember what she received in the chemotherapy? I don't remember what she got.

Gar Hildenbrand: Yeah, it was 5-FU, it's on a slide here later, too.

Christeene Hildenbrand: Okay, just as long as you cover it.

Gar Hildenbrand: One of the drugs was 5-FU. Let's see, treatment consisted of the following: The diet — continue her Gerson — but we told her, look, batch your juices in the morning. You're going to have to come to the clinic anyway and there is no point being a slave to the juice machine. It's insane; we keep whole blood on the shelf in the fridge for three days for transfusion; why would juice die in 8 hours? So just make your juices in a batch, get away from the juicer, and come to the clinic and then walk around Playas, do something to entertain yourself. Frequent Coley Fluid, subcutaneous and progressing to intratumoral. Now I do think that the preferred route, if you can get to the tumor, the preferred route with Coley fluid is intratumoral. GM-CSF was p.r.n; excision en bloc of axillary nodes for chemosensitivity/resistance testing and construction of a whole-tumor lysate. So again you've got the theme that we're trying to recreate the Busch observation.

Yes, this is what we used: Vinorelbine and 5-fluorouracil. And she had 4 administrations only. And, in fact, the tumor responded only on the first three, so we abandoned it. Definitive surgery with lumpectomy and reconstruction. At the time of surgery, no satellite lesions were found, and the primary tumor had diminished to 2.3 cm in greatest dimension. She was discharged to home Nov 15, 2012, after a month of wound cleaning and maintenance therapy with Coley Fluid and tumor lysate injected into the Coley wheal.

Carol C *(cont)*

Treatment consisted of the following: Slide 31

- Continue diet therapy and detox with coffee enemas
- Begin frequent Coley Fluid subcutaneous, progressing to intratumoral.
- GM-CSF p.r.n.
- Excision en bloc of axillary nodes for chemosensitivity/resistance testing and construction of a whole-tumor lysate.

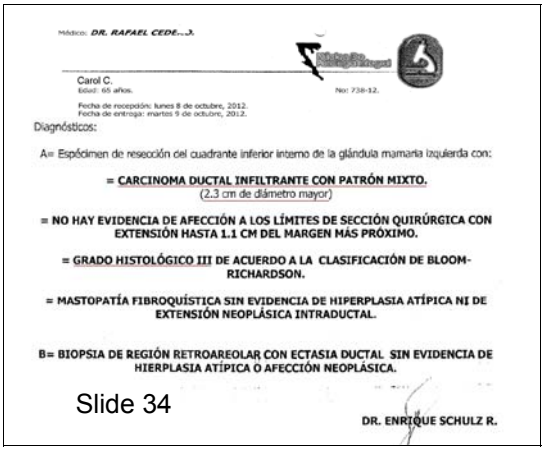
Carol C *(cont)* Slide 32

- IPT-chemo with vinorelbine and 5 fluorouracil times 4 administrations only.
- Definitive surgery with lumpectomy and reconstruction. At the time of surgery, no satellite lesions were found, and the primary tumor had diminished to 2.3 cm in greatest dimension.
- She was discharged to home Nov 15, 2012, after a month of wound cleaning and maintenance therapy with Coley Fluid and tumor lysate injected into the Coley wheal.

Carol C *(cont)*

She continues to date, April 8, 2013, with her diet, detox, and vaccines, and is arranging for a follow-up MRI. There are no signs of returning pathology, and the symptoms evoked by her vaccines diminish with every weekly injection. She recently reported that for the last 3 weeks she has suffered no limb soreness or other signs or symptoms that were typical of vaccine reaction during the early months of her treatment.

Slide 33



Médico: DR. RAFAEL CEDE...J.

Carol C.
Edad: 65 años. No: 738-12.

Fecha de recepción: lunes 8 de octubre, 2012.
Fecha de entrega: martes 9 de octubre, 2012.

Diagnósticos:

A= Espécimen de resección del cuadrante inferior interno de la glándula mamaria izquierda con:

= CARCINOMA DUCTAL INFILTRANTE CON PATRÓN MIXTO.
(2.3 cm de diámetro mayor)

= NO HAY EVIDENCIA DE AFECCIÓN A LOS LÍMITES DE SECCIÓN QUIRÚRGICA CON EXTENSIÓN HASTA 1.1 CM DEL MARGEN MÁS PRÓXIMO.

= GRADO HISTOLÓGICO III DE ACUERDO A LA CLASIFICACIÓN DE BLOOM-RICHARDSON.

= MASTOPATÍA FIBROQUÍSTICA SIN EVIDENCIA DE HIPERPLASIA ATÍPICA NI DE EXTENSIÓN NEOPLÁSICA INTRADUCTAL.

B= BIOPSIA DE REGIÓN RETROAREOLAR CON ECTASIA DUCTAL SIN EVIDENCIA DE HIERPLASIA ATÍPICA O AFECCIÓN NEOPLÁSICA.

DR. ENRIQUE SCHULZ R.

Slide 34

Tumor Ablation:
Effects on systemic and local anti-tumor immunity and on other tumor-microenvironment interactions
Yona Keisara, Ed, Dept Clin Microbiol and Immunol, Tel Aviv Univ; Pub Springer, Heidelberg, 2013, pg 1.

An understanding of the interactions between cytotoxic therapies and the immune system and the tumor microenvironment is crucial for the rational development of combination treatments of immunotherapy with conventional or targeted therapies to achieve a synergistic antitumor effect and improved treatment outcomes.

Slide 35

Tumor Ablation ***(cont)***

When we consider the immune response to a tumor that has escaped immune surveillance and destruction, the antitumor immune response can involve components of the innate immune system as well as the humoral (antibody-mediated) and cellular (T-cell mediated) arms of the adaptive immune system. However, CD8+ cytotoxic lymphocytes are generally considered the most effective of the anti-tumor immune responses …

Slide 36

Tumor Ablation ***(cont)***

There are six steps postulated for an effective antitumor CD8+ T-cell response:

a. Tumor antigens must be present;

b. Antigens must be seen as 'dangerous' and acquired by professional antigen presenting cells (APCs);

c. Tumor-specific CD8+ T-cells recognize antigens and respond by proliferation;

Slide37

She continues to date, April 8, 2013, with her diet, detox, and vaccines, and is arranging for a follow-up MRI. There are no signs of returning pathology, and the symptoms evoked by her vaccines diminish with every weekly injection. She recently reported that for at least 3 weeks she has suffered no limb soreness or other signs or symptoms that were typical of vaccination reaction during the early months of her treatment.

This is our pathologist Dr Schultz (slide 34) having a look at the 2.3-cm mass that was taken in the lumpectomy and conservative surgery. You can see, it was still a grade 3 histologically, what was left in the tumor; but it was a good resection, it was clean; okay.

Now, some of the back story, because some of you will — I know that Bill and Dwight will be familiar with a bunch of this stuff, but some of the rest of you may not.

Effects on systemic and local anti-tumor immunity and on other tumor-microenvironment interactions — this is a monograph called *Tumor Ablation* — it comes out of Australia; it is a lovely monograph, it's brand new this year. Yona Keisara did a great job as Editor and Chief. *An understanding of the interactions between cytotoxic therapies and the immune system and the tumor microenvironment is crucial for the rational development of combination treatments of immunotherapy with conventional or targeted therapies to achieve a synergistic antitumor effect and improved treatment outcomes* — improved treatment outcomes. *When we consider the immune response to a tumor that has escaped immune surveillance and destruction, the antitumor immune response can involve components of the innate immune system as well as the humoral (antibody-mediated) and cellular (T-cell mediated) arms of the adaptive immune system. However, CD8+ cytotoxic lymphocytes are generally considered the most effective of the anti-tumor immune responses* — that's if you talk to Steve Rosenberg, for sure.

There are six steps — this is continuing *Tumor Ablation*, and this is in sync with the Danger Model – *six steps postulated for an effective antitumor CD8+ T-cell response*: *(a) Tumor antigens* have to *be present* – that doesn't

mean *present*, it means *(b) Tumor antigens must be seen as 'dangerous'* — Well, it isn't the antigens themselves; you've got to blow up some tumor cells and get some of that ATP and mitochondria out from behind those membranes, get even some mitochondrial DNA or RNA out from behind triple membranes and into the blood stream because that's where the dendritic cells will respond to it. I don't know if any of you are familiar with the history of trying to give ATP to increase energy, back during the '40s, but people almost died because it's such a potent danger signal that the immune system goes completely nuts when you put ATP into the blood stream.

Tumor Ablation *(cont)*

d. Circulating CD8+ T-cells must reach the tumor

e. CD8+ T-cells must overcome immune suppressive signals within the tumor microenvironment;

f. And memory cells should be generated for a durable response.

Slide 38

So the antigens themselves are not dangerous, and I would say that this is a misunderstanding. You know, you take a — you grow a flu vaccine in an egg and you get the antigen out of that — that antigen is not going to cause immunization by itself. Unless it is mixed with an adjuvant it will not be presented. So, *(c) Tumor-specific CD8+ T-cells recognize antigens and respond by proliferation.* That's a little bit of a misstatement; I would say that they accept the antigen and then, if they are costimulated by the B7 molecule of a DC that's been matured, *then* they become effectors and *then* they will work to clear a tumor. *(d) Circulating CD8+ T-cells must reach the tumor* — yeah, and they can do that because a tumor is a blood hog and there's great vascularization, so there's no trouble getting any immune cell into the tumor. *(e) CD8+ T-cells must overcome immune suppressive signals within the tumor microenvironment* — to that I say amen. Oftentimes, it is necessary to delete lymphocytes and that's a trick that Coley does that I'll talk about in a moment. *(f) And memory cells should be generated for a durable response.* This is, this "*f*" is, in a nutshell, why we do whole-tumor lysate directly injected into a Coley erythema; it's that you are not going to get memory cells unless you repeatedly induce this response. Memory cells don't just happen, you know; it's not like gravy happens when you cook the meat or something. That's *The Odd Couple*, Neil Simon, right?

CD4 cells can be more efficient at tumor rejection than CD8 cells
Perez-Diez A, Joncker NT, Choi K, Chan WFN, Anderson CC, Lantz O, and Matzinger P. *Blood*, 15 June 2007 Vol 109, No 12, pg 5353

A quarter of a century ago, when tumor antigens were first beginning to be characterized, tumor immunotherapists dreamed of activating CTL against the very protein that made a tumor tumorigenic (eg, mutated ras, hormone receptors or growth factor receptors, viral proteins, etc). The idea was that, should a tumor cell "escape" from the immune response by losing that particular protein, it would, in the process, lose its tumorigenicity.

Slide 39

CD4 cells can be more efficient at tumor rejection than CD8 cells — I just had to throw this in here, this is Polly Matzinger and colleagues; actually, they were all post-docs in the T-cell Tolerance and Memory Section at NIAID.

A quarter of a century ago, when tumor antigens were first beginning to be characterized, tumor immunotherapists dreamed of activating CTL (cytotoxic Lymphocytes) *against the very protein that made a tumor tumorigenic (e.g., mutated ras, hormone receptors or growth factor receptors, viral proteins, etc). The idea was that, should a tumor cell*

CD4 cells can be more efficient at tumor rejection than CD8 cells
Perez-Diez A, Joncker NT, Choi K, Chan WFN, Anderson CC, Lantz O, and Matzinger P. *Blood*, 15 June 2007 Vol 109, No 12, pg 5353

But it soon became clear that tumors could instead escape from CTL by losing MHC class I molecules while retaining the critical oncogenic proteins that maintain the tumor state—and the dream faded. The finding that CD4 T cells can efficiently induce tumor clearance using indirect measures that do not require MHC expression by the tumor cell suggests that it might be possible to resuscitate that early goal by designing strategies to elicit CD4 T cell responses against the tumorigenic proteins.

Slide 40

CD4 cells can be more efficient at tumor rejection than CD8 cells
Perez-Diez A, Joncker NT, Choi K, Chan WFN, Anderson CC, Lantz O, and Matzinger P. *Blood*, 15 June 2007 Vol 109, No 12, pg 5353

Although we do not know how often CD4 cells will turn out to be more efficient than CD8 cells, we suggest that this is an understudied area. Characterization of the conditions in which CD4 effector cells are, or are not, successful might help to predict which clinical trials could take full advantage of them and allow CD4 effector cells to become an additional tool in the treatment of cancer

Slide 41

"escape" from the immune response by losing that particular protein, it would, in the process, lose its tumorigenicity. But it soon became clear that tumors could instead escape from CTL by losing MHC (major histocompatibility) *class I molecules while retaining the critical oncogenic proteins that maintain the tumor state — and the dream faded* — I love the way Polly writes — *the dream faded* — this is a science paper, I love it. *The finding that CD4 T cells can efficiently induce tumor clearance using indirect measures that do not require MHC expression by the tumor cell suggests that it might be possible to resuscitate that early goal by designing strategies to elicit CD4-T-cell responses against the tumorigenic proteins.* And I leave that to you guys who are resourceful. I haven't figured anything out yet but keep looking.

Although we do not know how often CD4 cells will turn out to be more efficient than CD8 cells, we suggest that this is an understudied area. Characterization of the conditions in which CD4 effector cells are, or are not, successful might help to predict which clinical trials could take full advantage of them and allow CD4 effector cells to become an additional tool in the treatment of cancer. Okay.

***Update:* Turning the Heat on Cancer**
DeNardo GL, DeNardo SJ. *Cancer Biother Radiopharm.* 23(6): 671–679.

Slide 42

The use of elevated temperature, hyperthermia, is not a new treatment for cancer. Hippocrates was aware of the potential of heat to cure or shrink tumors. Tumor shrinkage after a high fever due to an infection was reported in 1866.*

*Busch W. Über den Einfluss, welchen heftigere Erysipelin zuweilig auf organisierte Neubildungen ausuben. Verhandlungen des naturhistorischen Vereines der preussischen Rheinlande und Westphalens. 1866;23:28.

Heat. *The use of elevated temperature, hyperthermia, is not a new treatment for cancer. Hippocrates was aware of the potential of heat to cure or shrink tumors. Tumor shrinkage after a high fever due to an infection was reported in 1866.* And, of course, this is the original Busch observation. He published a paper in 1866 and another one in 1867 with the same observation in the second patient. And then in 1868, he published one in which he wounded the tumor and put the patient in what was now known as a "dangerous bed" because the hospital ward had Strep in the woodwork so that anybody with a wound who was put in a dangerous bed in a dangerous ward was pretty much insured of getting an erysipelas.

***Update:* Turning the Heat on Cancer**
DeNardo GL, DeNardo SJ. *Cancer Biother Radiopharm.* 2008 December; 23(6): 671–679.

The effects of heat on cancer cells are well-known. Cell death from exposure to heat is a function of both the intensity of the applied heat and the time of exposure. Cells die at high dose-time combinations by necrosis. For milder exposure conditions, cells undergo apoptosis. Sublethal heat insufficient to cause cell death sensitizes cancer cells to radiation and many drugs.

Slide 43

But you know, I just want to riff on this for just a second. Yeah, it was a high fever, so what? Is the fever what's doing it? There are a lot of people, you know, who are pushing the Oncotherm 3000 and literally sending patients home burned, with this idea that heat, alone, can start a complex immune cascade that can clear systemic tumors, and I'm not a believer. I believe that those machines are interesting and very utilitarian but we're reaching beyond our limitations oftentimes, and I don't think it's heat, *per se*, but there are some very cool things that happen with heat shock proteins and antigen presentation, so let's look at that a little bit.

The effects of heat on cancer cells are well-known. Cell death from exposure to heat is a function of both the intensity of the applied heat and the time of exposure. Cells die at high-dose-time combinations by necrosis. For milder exposure conditions, cells undergo apoptosis. Sublethal heat insufficient to cause cell death sensitizes cancer cells to radiation and many drugs. I'm with that, totally.

We use shortwave diathermy and far infrared, TDP far infrared heat lamps and far infrared sauna. So these are modest attempts. And all we're really wanting to do is mobilize antigens. We're just cooking them a little bit while we're helping to heat the patient. These are simple things that can be done.

And, by the way, we cast the Gerson diet therapy as a do-it-yourself. There is no way in the world that's a medical specialty, *per se*. It took a medical genius to sort it out, but this is a do-it-yourselfer. Gerson, himself used to have the wife who dragged the old farmer husband into the office by the earlobe — he didn't want to be there — and she says, "Look, lupus is eating a hole through his cheek," lupus vulgaris, tuberculosis of the skin. And Gerson would realize that talking to her was going to be more profitable than talking with him, so he'd say, "Now look, you do this." And he'd write it on a piece of paper, give it to her, and say, "Come see me in a month." Who needs to be hospitalized for that when the house-frau can do it, right? Really, a woman who runs the kitchen.

Ex-vivo hyperthermia

- Shortwave diathermy
- TDP far infrared heat lamps
- Far infrared sauna

Slide 44

Christeene Hildenbrand: I've noticed with the patients when they have a lot of pain, like Diann, the warmth of the sauna and the warmth of these machines helps her relax.

Gar Hildenbrand: That's a great point. We should probably add that to the prospective database and trap that data, pain management by just gently applying external heat. It's in your nursing manual.

What we're looking at is what was known during the Golden

Auto*Therm 390x

Radiofrequency shortwave diathermy – **Mettler Electronics**

Slide 45

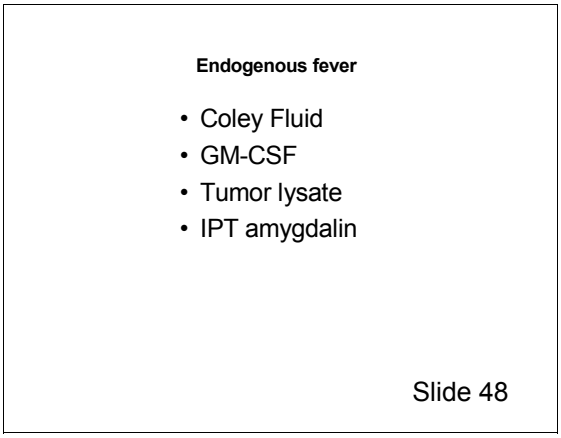
TDP far infrared heating lamp

FDA 510 (K): K020851

Slide 46

Portable far-infrared sauna

Slide 47



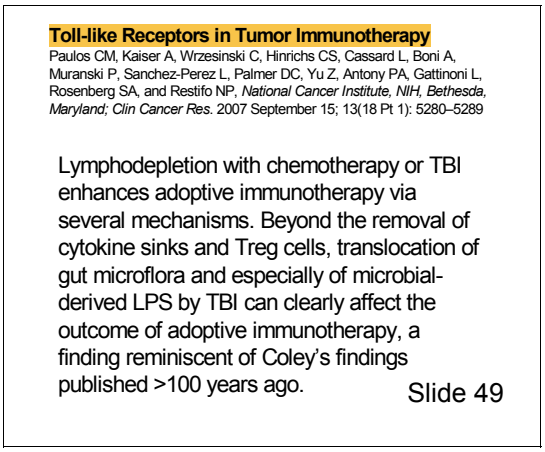
Age of German Medicine as the spa effect. People are only going there for clay and herbals and saunas and then suddenly they're raging with fever and they're having a full blown immune response. And what is that? It's that these general measures applied consistently and in an integrated way can lead to something much greater than, you know, the sum of the parts is greater than the parts themselves.

Yeah. Endogenous fever. We do appreciate the exogenous fever, but we make fever with Coley fluid, GM-CSF contributes to that. On rare occasions GM-CSF can produce a fever on application or shortly after. Tumor lysate can lead to a fever; when its applied with the Coley can lead to a higher fever; and the IPT-amygdaline can lead to fever and that's just simply because it's presumably — it's killing tumor cells and making antigens available in a primed host.

Fellowship participant: What is IPT?

Gar Hildenbrand: IPT refers to insulin-potentiation therapy, but this is actually a misnomer. What we do is different from the published method of insulin potentiation in that we do not lower serum glucose first; we do not routinely use 3-4 chemotherapeutic drugs; we do not follow the published 5-10% dosage range; and we do not administer chemotherapy by IV push. Instead, only 1-2 drugs are selected; these are dosed in the 10-40% range; they are administered conventionally by IV; and when the serum concentration is nearing peak, insulin is administered by bolus into the central line. Blood glucose is allowed to drop to 50, at which point sugar levels are restored with IV and oral inputs.

This is a favorite observation of mine (slide 49) and I don't know if anyone else has made the observation, so I'm going to say we are probably the first to make the observation: *Lymphodepletion with chemotherapy or TBI enhances adoptive immunotherapy via several mechanisms* — TBI being total body irradiation — *Beyond the removal of cytokine sinks and Treg cells, translocation of gut microflora and especially of microbial-derived lipopolysaccharides by TBI can clearly affect the outcome of adoptive immunotherapy, a finding reminiscent of Coley's findings published >100 years ago.* That's from a paper called *Toll-like Receptors in Tumor Immunotherapy* which comes out of the NCI.



Christeene Hildenbrand: Hildy.

Gar Hildenbrand: Yep. I'm sorry.

Dr Grace: This sort of marriage was, I think, reported in the last two weeks; the use of rituximab to enhance, you know, is the humoral response to the tumor masquerading the T-cell elimination? And they found that giving rituximab enhanced the clearance of the solid tumor.

Gar Hildenbrand: That sounds very good.

Dr Grace: This was just recently reported. Using rituximab as a way of reducing the humoral response that may be interfering with the immune potentiation.

Gar Hildenbrand: Right, and we tend to characterize that reduction as lymphodepletion now, but it may be more than lymph.

Dr Grace: Well, you know, rituximab is a great way to get rid of, lymphodepletion, of B cells and this was shown that, I forget the article, but it was in the last two weeks, I read, obviously as I get older I get forgetful …

Gar Hildenbrand: It's very logical, yeah, it's very logical. The other one you can use is cyclophosphamide, we did that because it predictably does that.

Lymphodepletion by Coley Fluid

An April 5, 2013, review (by Christeene Hildenbrand) of logs and labs of 109 mostly-advanced-stage historical Coley IV patients was pulled randomly. Of 98 patients (who received 258 Coley IV administrations), 46 patients (who received a cumulative 118 Coley IV) were assessable. Of their 118 Coley IV treatments, 90 treatments were assessable for changes in CBC and differential by comparison of pre- and post-Coley labs.

Slide 50

An April 5, 2013, review (by Christeene Hildenbrand) of logs and labs of 109 mostly-advanced-stage historical Coley IV patients was pulled randomly — this was the Coley fluid that was built in Guatemala City by a microbiologist down there who had been hired by Wayne Martin in 1996. Wayne used to be a chemist at Purdue, yeah?

Dr Dwight McKee: Is this the same as Coley's toxin?

Gar Hildenbrand: Yes. It's the same. Coley's toxins, yeah, it's just that they used the old recipe. Wayne Martin, the guy who used to be a chemist at Purdue and he used to make Coley for the doctors at Purdue in the 30s. He just became very, very unhappy with the fact that Helen Nauts and Lloyd Old were staging one after another good clinical trials with good outcomes and they couldn't get any traction because they didn't know, and in fact, the corporate world of the biotechs didn't really know, that the reason FDA kept saying "no" to this, to developing this vaccine, was that in … Well, this is a fun story …

Remember in 1972 when biologicals moved from NIAID to FDA? NIAID had always had biologicals. The immunology lab at NIAID, they had biologicals since 1906, when the Pure Food and Drug Act was passed. There were some chummy sort of clubby relationships between vaccine manufacturers and some of the ensconced bureaucracy of NIAID and this resulted in two back-to-back scandals having to do with flu vaccine that was about as potent as water. Vaccines were being distributed over a course of more than a decade and, when this came to light, Congress was just furious and yanked biologicals from NIAID and gave them to FDA. FDA panicked and froze all therapeutic vaccines, allowing only preventive vaccines to continue on the market; stopped one that Lilly had, which was an intravenous Strep A vaccine for rheumatoid arthritis. It was probably a damn good idea. And when, you know, when doctors and patients and family went and beseeched Commissioner Donald Kennedy for some avenue of redress and appeal, Kennedy said you don't have standing for an appeal. People don't have standing. Only a FDA-licensed corporation has standing for an appeal. You know, for you libertarians out there, chew on that one. Only a

corporation has standing for an appeal. So, there was nothing could be done about that vaccine and for five years the therapeutic vaccine industry was halted. In 1979, Kennedy announced that he had suspicions and concerns that repeated exposures to GAS would result in delayed adverse events that could show up decades later. And a ban was put in place for GAS or any part of GAS or even the supernatant from the culture of GAS.

Fellowship participant: What is GAS?

Gar Hildenbrand: Oh. Group A Strep.

Fellowship participant: Oh. Group A Strep.

Gar Hildenbrand: Even the supernatant was forbidden — I should move this (microphone), I've hit it twice — and that ban essentially poisoned the well. Every time a corporation or biotech would go there and say, well you know, it looks pretty good, and Lloyd Old … I mean … it's Ludwig — Ludwig's huge — and they're doing this Coley stuff, and it looks pretty good, we'd like to get in on it; and the FDA would just say, "don't go there. It's no use."

Dr Dwight McKee: But they left Staphage Lysate 'til much, much later.

Gar Hildenbrand: They did but it's been relegated to veterinary medicine now.

Dr Dwight McKee: Why?

Gar Hildenbrand: I don't know; the institution had a memory, right?

Christeene Hildenbrand: They gave up — Delmont gave up in 2009 because I …

Gar Hildenbrand: Delmont in Swarthmore, PA, put up a valiant fight — they fought for two decades at least …

Christeene Hildenbrand: More than thirty years.

Gar Hildenbrand: … to try to get — to stay in human medicine, and FDA wouldn't budge. I just say this — that the agency has a memory and the memory exists independently of any of the individuals in it. It's some sort of supernatural phenomenon, I'm sure. At any rate, in 1979 it banned Group A Strep and any component of Group A Strep from interstate commerce, which is FDA's bailiwick. It was not until July of 2006 that that ban was quietly lifted with a notice in the pages of the Federal Register which read simply, we don't know why they banned it but we can characterize it completely now, so we know there's no risk. So it's off the list. So, right now, if somebody were to get the brilliant idea to go ahead and develop the only vaccine of its type — a whole bacterial lysate, two species of microbes — it would be a good time. Here's a way that you could handily bring forward something that's not really a drug, but which is a trigger for an evolutionarily-conserved, complex defense mechanism that has dealt with this microbe for thousands and thousands of years. So, it's deep inside of us and it may be that that, in itself, is part of the reason that it evokes a response that can help clear tumors because, think of this, if you will, Streptococcus pyogenes is a commensal in us all of us. It lives in our oropharynx in small numbers. It's a freakin' commensal. So how does our body take it out of a virulent phase and put it back into balance and homeostasis? And that's really the question we ask about tumors too, because these were once stem cells, these were once macrophages, these were once platelets that were on our side. How do we get them back to our side? How do we get them to stop that group be-

havior? At any rate …

Christeene Hildenbrand: Hildy? In Coley fluid, there's nothing else other than the S marcescens and the ..?

Gar Hildenbrand: Serratia marcescens and Streptococcus pyogenes; 8 to 1 is the ratio of Serratia to Strep, or the Serratia ratio, which I really love saying. The Serratia ratio is 8 to 1, and there is a little bit of thymol in it as a preservative, it's just a thyme oil derivative, as a preservative.

Fellowship participant: What is it?

Gar Hildenbrand: Thymol. T-h-y-m-o-l. And, of course, I'm referring to MBVax Bioscience in Canada which is the little bitty start up biotech that is trying valiantly to get up the hill — the little engine that thinks it can.

…Of 98 patients (who received 258 Coley IV administrations), 46 patients (who received a cumulative 118 Coley IV) were assessable. Of their 118 Coley IV treatments, 90 treatments were assessable for changes in CBC and differential by comparison of pre- and post-Coley labs.

Pre- and post-Coley labs demonstrated a distinct shift to the left with deletion of lymphocytes following 57 of those 90 treatments (63%). An additional 7 labs showed bandemia without lymphodepletion. And *In summary, 40 patients (87%) of 46 (that were assessable) achieved bandemia with lymphodepletion, while 6 (13%) did not.* I say that's pretty cool. And that's what our body does when it's frightened by bacteria. The response to bacteria is that it deletes its lymphocyte population, probably under the biological assumption that we don't need these guys right now, they were for something else. So we need guys who are appropriate to do something new, something that deals with this immediate threat.

Lymphodepletion by Coley Fluid

Pre- and post-Coley labs demonstrated a distinct shift to the left with deletion of lymphocytes following 57 of those 90 treatments (63%). An additional 7 labs showed bandemia without lymphodepletion.

In summary, 40 patients (87%) of 46 achieved bandemia with lymphodepletion, while 6 (13%) did not.

Slide 51

Granulocyte-macrophage stimulating factor (GM-CSF) increases circulating dendritic cells but does not abrogate suppression of adaptive cellular immunity in patients with metastatic colorectal cancer receiving chemotherapy.
Martinez M, Ono N, Planutiene M, Planutis K, Nelson EL, Holcombe RF. *Cancer Cell International* 2012;12:2

One of the most significant findings from this clinical trial was the increase in the number of circulating myeloid DCs (Lineage-, MHC high). These increased in approximately 75% of subjects across all cycles. In subsequent cycles of chemotherapy and GM-CSF, approximately 50% of the subjects experienced increased DC numbers over the initial cycle.

Slide 52

GM-CSF. This is from Cancer Cell International. *One of the most significant findings from this clinical trial was the increase in the number of circulating myeloid DCs (Lineage-, MHC high). These increased in approximately 75% of subjects across all cycles. In subsequent cycles of chemotherapy and GM-CSF, approximately 50% of the subjects experienced increased DC numbers over the initial cycle.* You are probably aware of studies that have demonstrated clearly that survival after stem cell transplant is a function of the dendritic cell population and the more you have, the better off you are. And one could argue that GM-CSF is way cool, because not only does it mature and make costimulatory tissue-resident dendritic-cell populations, which are in the gazillions of cells

GM-CSF *(cont)*

Dendritic cells are potent antigen presenting cells and play a pivotal role in the induction of immune responses. Dendritic cells have been directly implicated in anti-tumor immune responses. The increase in circulating DCs, likely a consequence of GM-CSF administration, may contribute to the anti-tumor effects of chemotherapy administered in the adjuvant or metastatic setting.

Slide 53

throughout the body, but it also opens up that bone marrow compartment to flood the circulation with myeloid precursors; and the signaling environment created by GM-CSF is such that monocytes coming out of the bone marrow are going to preferentially differentiate into dendritic cells because of the signaling environment, because that cytokine itself is an emergency-response cytokine.

Dendritic cells are potent antigen presenting cells and play a pivotal role in the induction of immune responses. Dendritic cells have been directly implicated in anti-tumor immune responses. The increase in circulating DCs, likely a consequence of GM-CSF administration, may contribute to the anti-tumor effects of chemotherapy administered in the adjuvant or metastatic setting. And I think that Bill was seeing exactly that. You can give GM-CSF every day and some of your patients are going benefit from it tremendously. And we found that you don't have to give it every day if you stretch the integration of your protocol a little bit;

Systemic effects of local radiotherapy
Formenti SC and Demaria S.
Dept Rad Onc, NYU Langone Medical Center and NYU Cancer Institute
Lancet Oncol. 2009 July ; 10(7): 718–726.

The use of cytokines promoting the growth and differentiation of DC such as granulocyte-macrophage colony-stimulating factor (GM-CSF) is one of the most promising approaches in cancer immunotherapy [63,64]. The rationale is that GM-CSF increases the mobilization, differentiation, and function of DC [65,66] resulting in potential reversal of the host's immune tolerance its own tumor-associated antigens.

Slide 54

Oh, yeah. Wu, et al, *In vivo vaccination with tumor cell lysate plus CpG.* This one, I wanted to pull this out just because, you know, when you have a good clinical response, sometimes you have to work backward in the literature and see, did anyone else have this phenomenon under their observation? And we found that already in 2007, in the *Journal of Immunotherapy*, *In vivo vaccination with tumor cell lysate plus CpG* – CpG, that's a non-methylated pathogen-associated molecular pattern that you see in Gram-positive microbes like Strep and, in fact, it was one of the candidates for the immune-stimulating capacity of Coley fluid. And Lloyd Old spent his last years trying to develop a commercial CpG adjuvant, but this, essentially, this is very similar to what we're doing making a Coley wheal and injecting whole tumor lysate. These guys said, *We investigated the therapeutic efficacy of in vivo DC activation, by directly administering glioma cell lysate with CpG oligodeoxy-nucleotides (CpG/lysate), in glioma-bearing mice. Subcutaneous vaccination with CpG/lysate induced a significant increase (P<0.05) in the number of total T cells and activated DCs in lymph nodes draining the vaccination site as compared to mice treated with CpG or tumor lysate alone*. But wait, it gets better.

In vivo vaccination with tumor cell lysate plus CpG oligodeoxynucleotides eradicates murine glioblastoma.
Wu A, Oh S, Gharagozlou S, Vedi RN, Ericson K, Low WC, Chen W, Ohlfest JR. *J Immunother*. 2007 Nov-Dec;30(8):789-97.

We investigated the therapeutic efficacy of in vivo DC activation, by directly administering glioma cell lysate with CpG oligodeoxy-nucleotides (CpG/lysate), in glioma-bearing mice. Subcutaneous vaccination with CpG/lysate induced a significant increase (P<0.05) in the number of total T cells and activated DCs in lymph nodes draining the vaccination site as compared to mice treated with CpG or tumor lysate alone.

Slide 55

(Slide 56, next page) *Mice vaccinated with CpG/lysate exhibited over 2 times greater median survival than mice in the control groups (P<0.05). Up to 55% of mice vaccinated with CpG/lysate were rendered tumor-free as assessed by survival and bioluminescent imaging.*

Now, that really got my attention and the reason it did is because unlike sepsis, which can't be studied in mice, mice with their own tumors can be reliably studied for immune responses. So it's a model we can use, although we should move to larger animals, frankly; you know, Arabian horses get melanoma, for example; dogs get sarcomas; you know, these are a lot more elegant models to study than murine models. So we'll probably be doing that.

In vivo vaccination with tumor cell lysate plus CpG ligodeoxynucleotides eradicates murine glioblastoma.
Wu A, Oh S, Gharagozlou S, Vedi RN, Ericson K, Low WC, Chen W, Ohlfest JR. *J Immunother*. 2007 Nov-Dec;30(8):789-97.

Mice vaccinated with CpG/lysate exhibited over 2 times greater median survival than mice in the control groups (P<0.05). Up to 55% of mice vaccinated with CpG/lysate were rendered tumor-free as assessed by survival and bioluminescent imaging.

Slide 56

Adjuvants for Enhancing the Immunogenicity of Whole Tumor Cell Vaccines. Whole tumor cell lysates can serve as excellent multivalent vaccines for priming tumor-specific CD8++ and CD4++ T cells … adjuvants, such as Toll-like receptor agonists (e.g., CpG, MPLA and Polyl:C), and cytokines (e.g., granulocyte-macrophage colony stimulating factor), have also been investigated. I just have to say that line of research is a great line of research. I don't know whether they can make it into a true translational model. It would be nice if it were done.

Adjuvants for Enhancing the Immunogenicity of Whole Tumor Cell Vaccines
Chiang C, Kandalaft LE, Coukos G. *International Reviews of Immunology*, Volume 30, Numbers 2-3, May 2011 , pp. 150-182(33)

Whole tumor cell lysates can serve as excellent multivalent vaccines for priming tumor-specific CD8++ and CD4++ T cells … adjuvants, such as Toll-like receptor agonists (e.g., CpG, MPLA and Polyl:C), and cytokines (e.g., granulocyte-macrophage colony stimulating factor), have also been investigated.

Slide 57

Now, my guilty pleasure. This comes from Hammersmith Hospital, the Royal Postgraduate Medical School, the University of London in 1994: *Amygdalin has been used, ineffectively, as an anti-cancer treatment under the name Laetrile. Its poor activity was due to the slow, untargeted release of the toxic moiety, cyanide.* In their paper they suggested why not just develop a monoclonal antibody to deliver beta-glucosidase to tumor cells because glucosidase would cleave this into benzaldehide and cyanide and, preferentially inside tumor cells, you'd have a metabolic poison that would be competitive with other cytotoxics and good for the host. But tumors don't constitutively express beta-glucosidase, but they do express beta-glucuronidase, and it tends to be on the outside, the membrane of the tumor, cells which means that you actually can, if you tweak the beta-glucuronidase expression, you can get amygdalin cleaved in the interstices of tumors so you can still have a whale of a lot of cyanide in the interstices of the tumor, which is probably pretty good.

Targeting enzymes for cancer therapy: old enzymes in new roles M.P. Deonarain & A.A. Epenetos. Tumour Targeting Laboratory, ICRF Oncology Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 OHS, UK. *Br. J. Cancer* I994;70:786-794

Amygdalin has been used, ineffectively, as an anti-cancer treatment under the name Laetrile. Its poor activity was due to the slow, untargeted release of the toxic moiety, cyanide.

Slide 58

Yeah, I forgot that I did this slide (slide 59, next page). *Most cancer cells differ from normal cells in that they show higher beta-glucuronidase activity and lower pH of their cytoplasm.* The lower pH of the cytoplasm of the cancer cell is due to the fact that it's enormously inefficient in making ATP out of sugar; it cleaves it with an enzyme which byproduct is lactic acid. So, you've just got this

A minimal toxicity approach to cancer therapy: possible role of beta-glucuronidase
Rubin DM, Rubin EJ. Med Hypotheses.1980;Jan;6(1):85-92.

Most cancer cells differ from normal cells in that they show higher beta-glucuronidase activity and lower pH of their cytoplasm.

Slide 59

slow drainage.

(Slide 60)

The probable mechanism for IPT-amygdalin: Below is demonstrated the cleavage of amygdalin by beta-glucuronidase, which expression in cancer cells is induced by glucose loading – right? Because it's there to metabolize glucose, so if a tumor comes into glucose, what does it do? It expresses its glucose-cleaving enzyme, so this … *might be accomplished by insulin-potentiation therapy).* Because when you lower the blood sugar, it's got to go somewhere, right? It's going into the cells that have the most insulin receptors; that would be the cancer cells, which as we all know probably have more insulin receptors and more glucose receptors, ontologically, as a whole. *The resulting benzaldehide and cyanide (a metabolic poison) are released into the tumor interstices.* So, that is my guilty pleasure; but I think laetrile can be used; I just think it takes a significantly different approach, but I don't think it's that difficult in the clinic; it can be done. Anybody that tries it, if you get a response, please let us know; we'd love to hear your data — you know — if you do try it. It doesn't hurt anybody, but it does hurt tumors.

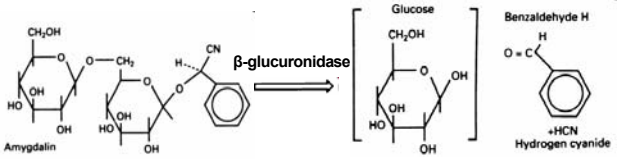
Chemoimmunotherapy — this is the last slide I'll put up here — *reengineering tumor immunity* — this is out of Sidney Kimmel and Johns Hopkins — *It is clear that strategically integrating immune-based therapies with standard cancer treatment modalities, in particular chemotherapy drugs, has the potential to reengineer the overall host milieu and the local tumor microenvironment to disrupt pathways of immune tolerance and suppression.*

So I'll stop here. We've had a bunch or running comments all the way through; if you're satisfied with what you've added, or if you have additional questions, now's the time.

Dr Dwight McKee: I have an interesting historical anecdote. In 1996 or 7, I had recently finished my fellowship at Scripps and was practicing in San Diego. A patient in her fourth relapse of ovarian

Probable mechanism for IPT-amygdalin

Below is demonstrated the cleavage of amygdalin by beta-glucuronidase, which expression in cancer cells is induced by glucose loading (such as might be accomplished by insulin-potentiation therapy). The resulting benzaldehyde and cyanide (a metabolic poison) are released into the tumor interstices.



Slide 60

Chemoimmunotherapy: reengineering tumor immunity
Gang Chen and Leisha A. Emens
The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University
Cancer Immunol Immunother. 2013 February ; 62(2): 203–216.

It is clear that strategically integrating immune-based therapies with standard cancer treatment modalities, in particular chemotherapy drugs, has the potential to reengineer the overall host milieu and the local tumor microenvironment to disrupt pathways of immune tolerance and suppression.

Slide 61

cancer came to me. She had about an 8-month-pregnancy-worth of tumor and she had, the year before, or maybe six months before, been a patient at CHIPSA, and had had IV Coley's toxins and had profound hypotension requiring ICU therapy, and her tumor had not responded. But she came to me and I sent her to this surgeon at Scripps Memorial who was doing extraperitoneal resection and deep hyperthermic chemotherapy and I had him send tumor to Larry Weisenthal. He did it very quickly and we used that to guide the intraperitoneal hyperthermia. Weisenthal found that her ovarian cancer, very unusually, was exquisitely sensitive to interleukin-2, and I gave him the history of her exposure to Coley's toxins, and he felt that there was probably a relationship, why it was sensitive to IL-2. So, after her belly bath and her long recovery from a brutal surgery, I started giving her intraperitoneal IL-2. And then she went back to Northern California and her local oncologist continued to give her monthly intraperitoneal IL-2, and she's still alive and well.

Gar Hildenbrand: Excellent.

Christeene Hildenbrand: Is her name Sarah?

Dr Dwight McKee: Sarah Morgan.

Christeene Hildenbrand: I just found her, yeah. Yes, thank you.

Gar Hildenbrand: That's a good tumor registrar for you.

Christeene Hildenbrand: Is that Sarah? She's not [in the cohort we're presenting tomorrow] because she did have chemo – because she did receive chemo before she came in, and so that put her out of that cohort, right? Right.

Dr Dwight McKee: She had, basically, septic shock response.

Christeene Hildenbrand: Oh my God, her reaction was horrendous. She was in ICU for a couple of days.

Dr Dwight McKee: She was in ICU for a month after the surgery.

Christeene Hildenbrand: Oh, gosh.

Gar Hildenbrand: When wholesale experimentation with Coley Fluid began, it was de rigueur to give it IV and to, as soon as possible, get up to that target range of 102° Fahrenheit or 38.9° Celsius. And when you piece this story together in the rear view mirror, epidemiologically, it has revealed a number of terrible mistakes that can be made. And one of them is attempting treatment in a patient with bulky disease, and a patient who was still suppressed from pretreatment, so there are a number of reasons where, you know, to which one could attribute the complications and the difficulties she had. As I said earlier, you know, you take a patient with bulky disease, especially the liver or lung, and slam them into an immune response, you're liable to kill them, and there are certainly instances of that.

Christeene Hildenbrand: You also, did you talk about Diann and the platelets?

Gar Hildenbrand: I should mention that, yeah. One of the other utilitarian tricks we found with Coley Fluid, when we used in conjunction with GM-CSF and cyclophosphamide was to take a patient who had been to … she'd been to Boulder for a recurrent uterine adenocarcinoma. This was now a big bulky disease in the bottom of the pelvis and she'd been to Germany and British Columbia twice getting hyperthermia treatments and chemotherapy as well, and when she came in she couldn't make platelets. And Rafael Cedeño spoke to me on

the side, quietly, and said, we can't do IPT or anything with her because she's not making platelets, and she's not responded for nine days to prednisone. And I proposed that there's another mechanism for suppression of platelet production and that has to do with the regulatory environment that's been seized by the cancer and if you clear that, if you deplete the regulators, that you might be able to get the bone marrow and the megocaryocytes to come back to life. So, we gave low dose cyclophosphamide and Coley IV on top of a GM-CSF prime and, although it was difficult for her for a couple of days, within a week she was within normal limits on her platelets. And, of course, she did show the shift to the left, the depletion of lymphocytes and that sort of hectic bandemia that develops. It was quite impressive. So, I would point out that you can use the body's response to pathogenic bacteria in logical ways. And, you know, I don't think we have anything else on the market that is like the Coley, yet.

Dr Dwight McKee: You also mention bacterial translocation and I think that that is probably a part of what coffee enemas do.

Gar Hildenbrand: Absolutely.

Dr Dwight McKee: Because I had patients that did coffee enemas when they were neutropenic and they got septic… they caused translocation.

Gar Hildenbrand: When I said gut pumping, that's what I meant; that I think that Gerson's gut pumping was probably the immunotherapy.

Dr Dwight McKee: So you had LPS being presented on a daily basis.

Christeene Hildenbrand: Yes.

Gar Hildenbrand: Yes. Even studies of colonics with water will induce higher lymphocyte counts in peripheral circulation, for example, and some modest leukocytosis.

Fellowship participant: Could you comment on Hal Gunn's work? He's got other…

Dr Dwight McKee: Site-specific immunotherapy.

Fellowship participant: Yeah, site-specific immunotherapy.

Dr Dwight McKee: He uses killed Klebsiella for lung; he uses killed E coli for belly. He found that the bacteria that habitually infect the region that the tumor is, it doesn't matter where it originated, so Streptococcus for breast — he found a stronger effect when he used bacteria that were sort of native or homing pathogens for the area where the tumor is.

Gar Hildenbrand: Fascinating.

Dr Dwight McKee: He's up in Vancouver.

Gar Hildenbrand: Fascinating.

Dr Dwight McKee: He's clearly building on Coley's work.

Gar Hildenbrand: My answer would be, I'm interested.

Fellowship participant: He trying to commercialize that.

Dr Dwight McKee: Yeah, he is. He's got clinical trials going and Jeff White from NCI is involved.

Gar Hildenbrand: That's… it's very cool because for so long we've regarded microbes as being nothing but a pain in the neck — a problem with pathology; and we've never paused to ask what role did they have in our evolution. How much do we really rely on challenges from the microbes in our air and our water, our

dirt and our bodies and — you know — that first kiss when you fall in love and it makes you sicker than a dog, and you're riding the porcelain bus because you kissed a girl — you know.

(General laughter)

Fellowship participant: Speak for yourself.

Christeene Hildenbrand: He grew up in the 60s.

(Laughter)

Gar Hildenbrand: Richly presenting rich microenvironments; I think people are rich microenvironments. You know, actually, it depends upon your immune sensitivities, you know, you might want to ask, if you fall in love and kiss somebody you've never kissed before and you don't get sick, are you suppressed?

Fellowship participant: How often do you do insulin potentiation? Do you do it, like, once a day? How do you do it?

Gar Hildenbrand: Insulin potentiation? We do it three times a week. We do three times a week, and I've got to say, every time we do it, I'm reminded of Valter Longo at USC and his fasting to prevent tissue damage from the chemotherapy. I wonder, what are we doing when we give insulin potentiation therapy to, for example, insulin-like growth factor and what are we altering, but yeah, we only use it three times a week because we respect that it's a lot of work for the patients. And so the general schedule is on Monday we're going to do IPT-Amygdalin, on Wednesday, IPT-Chemo, and on Friday, IPT-Amygdalin. And then everyday is other inputs and hyperthermia and Sundays off for good behavior.

Dr Dwight McKee: There's an interesting thing developing as an offshoot of Carl June's group, you know, they engineered the CD-8 cells to kill CD-20 cells and wiped out pounds of CLL in refractory patients, and now they're giving, in ovarian cancer patients, they're making patient-specific dendritic cells, tumor-specific dendritic cell vaccines and then treating them until they either don't have a response or progress. And then they're taking out CD-8 cells and growing them up and reinfusing them and they're seeing responses.

Gar Hildenbrand: So they're loading tumors with CD-8s.

Dr Dwight McKee: Exactly. So the dendritic cells are educated with the CD-8s and they're taking them out and expanding them and putting them in and they're getting responses.

Gar Hildenbrand: Shades of Rosenberg's adoptive immunotherapy.

Dr Dwight McKee: Yeah, exactly.

Gar Hildenbrand: Very cool.

Dr Dwight McKee: It's expensive.

Christeene Hildenbrand: Yes it is, I know, the money's a big deal.

Gar Hildenbrand: A couple of colleagues and I introduced PUVA-photopheresis and extracorporeal-dendritic-cell culturing into the Tijuana area more than a decade ago and I'm trying to lead a move to abandon that because GM-CSF is so much cheaper and easier to use, and it's in-vivo and you get better results.

Christeene Hildenbrand: Well, especially in Mexico. Because in Mexico Gramal is only what? $150 - $300 a vial.

Gar Hildenbrand: $150 if you're the oncologist buying it from the biotech.

Comment: For 500 mcg?

Gar Hildenbrand: For 400 mcg. But it's a good price.

Dr Dwight McKee: Yeah, absolutely.

Christeene Hildenbrand: With the photopheresis it's — we were telling Mark — they're charging what the market will bear. And so they're all charging like $5000 a treatment in Mexico for photopheresis when it costs what? $1500 maximum to do it.

Gar Hildenbrand: That's after you pay everybody.

Christeene Hildenbrand: Yeah, after all the people are paid to do it. I mean, its way less expensive.

Gar Hildenbrand: You have to buy a Baxter bag, and you know a semi permeable plasma bag to culture the monocytes overnight and you're paying for the interleukin, and …

Dr Dwight McKee: And how well does it work?

Gar Hildenbrand: What's that?

Comment: How well does it work?

Christeene Hildenbrand: Oh, it works.

Gar Hildenbrand: Well, it works. When we first innovated it, we found if we sequenced so that people got Coley first and then they had the PUVA-photopheresis and dendritic cell transfusion, that we were looking at a population that was Karnofsky 60 or less — and because they're the ones who are going to die first, so we're just kind of looking at survival with quality — and we had a 40% — at one year — a 40% survivorship in that population using that technique when we were with the Issels project at Oasis. But, as I said, it's inordinately expensive, it's cumbersome, it involves orchestrating an entire team of interconsultation specialists, and it's a nightmare, you know — just to — logistically it's a nightmare. And it doesn't work as well as GM-CSF, it just doesn't. GM-CSF is better. So, I mean, so just think, we turned the corner and its funny, because a whole population of guys down in Tijuana are profiting from this, you know, gross participation in the PUVA-photopheresis, dendritic-cell market and …

Dr Dwight McKee: But are they using it in the same context? Or are they just doing it?

Gar Hildenbrand: Oh God, when these things get legs, you know, forget methodology. You know, all the rules go away, you know, because what you've got is an unsteady paradigm, you know, in a Kuhnian sense. What's called for is a return to rules, but nobody's asking what are the rules because they don't even know that the freakin' paradigm is unstable. They're just out there using this — they're selling it because it's high priced and because there are a lot of people in the participation. That's why they're selling it, which is not unlike a lot of medicine in the U.S. GM-CSF is cheaper and it works better. That's my bottom line. C'mon you guys, let me go.

(General laughter and applause)