Thank you very much. I am going to talk about immune-mediated toxicity from a new class of therapies.
I would be remiss at an FDA-sponsored meeting if I didn’t note that I am an employee and shareholder of Bristol-Myers Squibb.
Historically, there have been three pillars for anti-cancer treatment: radiation, chemotherapy, and surgery. There is a new class of agents which you will start hearing about, or you may have started over the past couple of years, and that is immuno-oncology, which is harnessing the patient's own immune system to fight disease. This is a very exciting area. It is new; you are going to hear much more about it because there are more of this class of drugs. But one of the issues is, as was just pointed out, these drugs are intended to activate the immune system to attack the patient’s own tumor. Consequently, there is the risk that the patient will have immune-mediated toxicity.
There are some potential non-exclusive mechanisms why a patient who receives an immuno-oncology agent can develop an immune-mediated toxicity. The immune therapy could disrupt local or systemic homeostasis. The I-O agent could induce priming of a new T cell response to a self-antigen. And perhaps even the immune system can induce a supraphysiologic response to commensal flora, for example, in the gut or in the skin and this could lead to bystander damage.
Immuno-oncology agents can actually induce immune-mediated toxicity in almost any organ in the body, including the liver. And I know this is a liver meeting but I am going to focus mostly on the GI tract and the GI toxicities, and I will discuss why but I would like to come back to the liver towards the end.
The drug that I am going to focus on for the rest of the presentation is ipilimumab. This is a monoclonal antibody that is being used to treat advanced melanoma, and it targets CTLA-4. And the reason I am going to focus on ipilimumab, or ipi for short, is because it is one of the I-O agents with which we have had the most experience. We have had it for 15 years in the clinic and treated over 10,000 patients in clinical trials. And now there is a growing experience in the post-marketing use for advanced melanoma.

T cell activation typically requires two signals. The first signal is provided by the T cell receptor recognizing the target antigen in the context of an MHC molecule on an antigen-presenting cell. The second signal is provided by CD28, which binds to CD80 or CD86. Both of these signals are required for the T cell to be activated. And CD28 is called the co-stimulatory signal.

1. O’Day S, Hodi FS, McDermott D, et al. A phase III, randomized, double-blind, multicenter study comparing monotherapy with ipilimumab or gp100 peptide vaccine and the combination in patients with previously treated, unresectable stage III or IV melanoma. Presented at: Annual Meeting of the American Society of Clinical Oncology; June 4-8, 2010; Chicago, IL.
CTLA-4 is normally expressed in T cells but it resides in vesicles within the T cell. And upon strong T cell activation, these vesicles fuse to the membrane surface, releasing CTLA-4, which migrates to the T-cell antigen-presenting cell synapse. And because CTLA-4 has a much higher affinity for CD80 and 86, it can actually out-compete CD28. And this turns off the co-stimulatory signal, thus down-regulating the T cell.

Ipilimumab, the trade name is Yervoy, works by specifically binding and blocking CTLA-4 on the surface of T cells, thus restoring CD28 co-stimulatory signal. CTLA-4 was discovered in 1988 by a French group and for the first five or six years, it was not really clear how important CTLA-4 was. And in fact, people initially, erroneously thought that CTLA-4 was another co-stimulatory receptor. It wasn't until 1995 that two groups deleted CTLA-4 in mice, showing an incredibly striking phenotype of death by three weeks due to massive lympho-proliferation in multiple organs. And this includes spectacularly, the pancreas and the heart. Interestingly, the phenotype of this immuno proliferation does not match the organs that we see typically in patients treated with anti-CTLA-4. Another interesting, unfortunate fact is that in adult wild type mice blockade of CTLA-4 by an antibody does not recapitulate the immune pathology that we see in patients, for the most part. We can exacerbate chemically-induced colitis but we have been unable to really use mice or even cynomolgus monkeys as test cases for understanding the pathophysiology of anti-CTLA-4 toxicity in patients.
This is a summary of the immune-mediated toxicity that we observed with ipilimumab or Yervoy. This is from the USPI and it is from the pivotal phase 3 trial. It is a table showing the incidence of severe to fatal immune-mediated toxicity. And you can see 15 percent of all patients who received ipilimumab developed some form of severe to fatal immune-mediated toxicity. The most frequent is enterocolitis but other organs involved included dermatitis, hepatotoxicity and, interestingly, endocrinopathy, among others.

Now, one question arises why do only 15 percent of patients develop clinically significant toxicity? It is not clear. Why do some patients develop enterocolitis, whereas others develop hepatitis? Not clear. And the other interesting fact is that we tend not to see syndromes. We don’t see ipilimumab-induced SLE. We don’t see ipilimumab-induced rheumatoid arthritis. They tend to be organ-specific inflammation.
Now, this is a summary of three key questions which faced us in the early development of ipilimumab but it can really be applied and probably will be applied to all new I-O therapies that are being developed.

First, can you design a management algorithm? Second, can you prevent the toxicity? And for Yervoy, the focus was really on GI because it was the most frequently severe and the most frequently fatal problem. And then finally, can you identify the mechanism of this toxicity? And that includes looking at the histology but also can we differentiate it from autoimmunity and from graft-versus-host disease?

Now, even when we started, we didn't fully expect to find a complete overlap with autoimmunity with Crohn’s or ulcerative because we know those are polygenic. They result from a gene environment interaction, probably. Whereas, with ipilimumab, we are specifically targeting a single pathway. But, nevertheless, we wanted to see if there was some overlap.
So, I am going to focus on those three. First, I will focus on the management algorithm. There was a lot of trepidation when ipilimumab was first administered to patients because, remember that the mice who had CTLA-4 deletion died at week three and there were thoughts about patients. It just was not really clear. Thankfully, the toxicities were manageable. And through trial and error, an algorithm was defined. 

First, recognition that these toxicities could be fatal and, therefore, the hallmark of the management algorithm was close monitoring. This is not a drug where you treat the patient and send them on a cruise for six weeks to come back. You really need to follow these patients closely. Toxicities that are severe to life-threatening require corticosteroids and drug interruption or discontinuation, based on the management algorithm. Thankfully, the majority of patients do respond to high-dose corticosteroids and the majority do have complete resolution, although not all. And through trial and error, at least for ipilimumab, we had identified potential secondary rescue medications. For enterocolitis, infliximab seems to do very well. And for hepatitis we used mycophenolic acid. Now, I have been giving presentations on ipi toxicity for about ten years and for oncologists I always have to spend five or ten minutes explaining why we never wanted to use infliximab for hepatitis. But I think in this audience, based on the earlier discussion, I don't think you need an explanation about why avoided infliximab for hepatitis.
Now, I am going to move on to how we could prevent the most severe toxicity. And what we came up with in discussions with IDD experts was the hypothesis that prophylactic oral budesonide could be used to reduce GI toxicity. And we chose oral budesonide because it has low systemic absorption. It is an oral corticosteroid and so we thought maybe this would dampen down the local immunity and not result in systemic immunosuppression.

Our primary endpoints, using the oncology CTCAE criteria was grade 2, which is essentially moderate to worse diarrhea. And we randomized patients in a one-to-one fashion to oral placebo versus budesonide in a double-blinded fashion and all patients received ipilimumab. Unfortunately, prophylactic budesonide did not prevent GI toxicity. And you can see here in this table 33 percent of the budesonide arm developed grade 2 or worse diarrhea compared to placebo. So, unfortunately, budesonide cannot be used prophylactically to prevent diarrhea.
Fortunately, in this study, we collected a series of biopsies and evaluations to try and characterize the pathophysiology GI toxicity. The first thing we did was pathology because I am a pathologist by training and we had all patients undergo endoscopy with biopsy one to two weeks after starting ipi. And we did one to two weeks because we really wanted to identify the incipient changes that were occurring in the gut, rather than waiting until patients had developed florid inflammation that was potentially secondary, rather than -- and that would obscure the primary pathology. One in four patients did have inflammation by histology. Similar numbers had inflammation by endoscopy. The histology included both acute inflammation and chronic inflammation. And there was no significant association between patients who had inflammation at biopsy and subsequent enterocolitis. We also had this reviewed by an expert gastropathologist who found that the histology did overlap, somewhat, with IBD but it is was distinct. For example, there was some overlap with ulcerative colitis from a histologic pattern but the location and the endoscopic findings did not really match what is typically seen with UC.

The hallmarks of Crohn’s disease were present in some patients but they were not consistently observed in all patients. And, interestingly, there was a distinct pathology from graph-versus-host disease. So, we could not clearly assign it to any of the classic buckets that previously existed. Just as a point here, I will take a second and little diversion to talk about terminology. We have actually gone through a whole series of
terms to describe this. In fact, when the drug first started, the term used was autoimmune toxicity. We then evolved into immune-related. And then finally, when working with the FDA, we actually came up with the term immune-mediated. And we actually moved away from calling these autoimmune toxicities, although they may very well be autoimmune toxicities, was that we found -- we were concerned that some of the doctors or the emergency room doctors who would have seen these patients from a secondary standpoint would confuse these with classic autoimmune toxicity that might treat them differently if they just got a report that this patient had autoimmune enterocolitis. So, we have actually moved away not from a mechanistic reason but just from a medical information to calling these toxicities immune-mediated.
We also collected fecal calprotectin in all of these patients at regular intervals. This is a neutrophil-derived protein that is shed in the stool and can be a marker of disease activity for inflammatory bowel disease. And we found that ipilimumab did induce an increase in fecal calprotectin over time but it was not specific. And I have three examples of patients shown here. These are tables. On the x axis is time. In those little triangles are doses of ipilimumab. And the y axis is the amount of fecal calprotectin. This first patient did have an increase in fecal calprotectin but actually had no immune-mediated enterocolitis. The second patient did, indeed, have an increase in fecal calprotectin that did precede severe or moderate enterocolitis. So, that was what we had expected. But the third patient had a severe enterocolitis with no elevation in fecal calprotectin prior but did have an increase in fecal calprotectin after the enterocolitis had resolved. So, it was really non-specific and cannot really be used to monitor or to predict.
We also looked at humoral responses to enteric flora. These antibodies, which are to either microbial antigens or to pANCA at the time were being used in an exploratory fashion to try and differentiate Crohn’s disease and ulcerative colitis. I know that they are not completely validated and specific but we felt that they would try to at least give us directional support as to whether these were more of the CD or UC type of picture.

We found that ipilimumab induce an increase but it was non-specific and could not really be used to classify the patients. I will discuss the data in a second but I will point out that we also looked at similar humoral responses to tumor antigens, which are antigens that are only expressed in tumors. We found a very similar phenomenon, that ipilimumab would induce fluctuations in humoral response to these antigens. That probably has to do with the mechanism of action that ipilimumab not only activates CD8 T cells but also activates CD4 T cells and that probably helps in enhancing a plasma cell or humoral response.

So, for the data shown in the table here, each column represents a different antibody to a specific antigen. And we present these by the number of patients by worst grade enterocolitis. We had 115 patients treated in the first row. So, including any grade for patients who had enterocolitis and who didn’t. And you can see that out of those 115 only 10 to 25 percent actually had an increase in humoral responses to these antigens.

Interestingly, of those who had an increase, the majority actually never had any enterocolitis and you can see that in the second row. But 61 patients had no enterocolitis. And so you can see in the first column, out of the 18 patients who had a response to I2, 13 out of the 18 actually never even had enterocolitis. And finally, in the last row, of those patients who did have enterocolitis, there were 42, only a minority actually had a positive humoral response. And the frequency probably matches the general population as well. So, humoral responses could not be used to predict, nor could they really be used to classify the pathophysiology.
I will now turn to hepatitis. I know this is a liver conference. We have done more work on enterocolitis A) because it is potentially more severe and life-threatening; and B), the biomarkers on the assessment tends to be much easier. Patients can have endoscopy fairly routinely because there are fecal biomarkers. There is a lot of interest now in the microbiome. We can look at humoral responses. For hepatitis, we are limited to liver biopsies, but most of these patients who have end-stage cancer don’t want to undergo a liver biopsy. We are limited to serologies, to LFTs, which we do monitor but that doesn’t shed light, for the most part, on pathophysiology. We have been limited to try to explore the pathophysiology but, increasingly, I do think there is going to be a need to understand what is going on. Our biggest piece of information comes from a case series that Dr. Kleiner reviewed. He is the world’s expert in liver toxicity from ipilimumab because he has seen five patients who had severe immune-mediated hepatitis from ipilimumab. And what he observed is that these were really a non-specific inflammatory pattern. And the histology overlapped that with what you could see with acute viral hepatitis and drug reaction. And he concluded that this really required clinical pathologic correlation. Now, the majority of patients with ipi-induced hepatitis will resolve to high dose corticosteroids. Those who don’t may respond to mycophenolic acid.

Many times, these patients have metastatic melanoma to the liver and it can be hard to differentiate whether this is a mass effect or is really ipi-induced, or really an immune-mediated picture.

But other immuno-oncology agents that are being developed are likely to have a different type of hepatitis that may be not responsive to corticosteroids. Also, these immuno-oncology agents are going to be given together in doublets, they already are, and perhaps even triplets in higher order combinations.

And for me, at least, hepatitis is the most concerning of the immune toxicities we see because it is such a key organ. With enterocolitis if it is not responsive to corticosteroids, the surgeon can always go in and do a colectomy. But if we don’t have the appropriate algorithms for hepatitis, this is, obviously, a major problem in these end stage cancer patients.
We have also looked at dermatitis, which is less of a problem, although fatal events have been observed. And this is a case series from the NCI of eight patients who had immune-mediated dermatitis.

I should mention that in those five cases, we had excluded viral etiology. We had excluded other concomitant drugs. In this case series, we excluded other concomitant drugs that may have caused the dermatitis. But the histology and the clinical pattern really represented a typical drug reaction. There was predominately a T cell infiltrate. Interestingly, some of these patients had eosinophilia in their blood. And it was distinct from autoimmunity and GVHD.
As I mentioned, there are other checkpoint receptors besides anti-CTLA-4 that are being developed. There are other co-stimulatory agonists that are being developed that target receptors such as CD137. So, you will be hearing more about these, guaranteed, over the next several years. This does lead to an interesting academic point in that we are intervening by targeting single molecules in the immune system. And for the most part, entry of these patients into clinical trials requires no history of an autoimmune disease. So, from an academic standpoint, this really represents and experiment in patients where we are manipulating single immune pathways and, potentially, by combining multiple pathways. And I think that this may help shed light on autoimmunity, maybe.

This also raises another related question. Does the safety profile of ipilimumab --- mostly enterocolitis, skin, and liver --- shed light on the role of CTLA-4 in preventing autoimmunity in those organs? It is just a question.
This is my last slide. Immuno-oncology is an emerging treatment modality. It has already demonstrated survival in at least two tumors. For ipilimumab, the enterocolitis picture, and the hepatitis and the skin appears to stem from classic autoimmune conditions, but more study is needed about the mechanism of action of these toxicities. As was mentioned by Mark, we do need to be able to predict who is going to be at risk. And that probably represents the other hand of understanding the pathophysiology. And once we understand what is really happening, we might be able to identify who is at risk. Thank you very much.