Current therapeutic options and development strategies in cancer immunotherapy

A panel expert discussion

The increase in knowledge about the way the immune system functions is leading to exciting new therapeutic possibilities for cancer patients. A panel of experts met during the **ESMO Symposium on Immuno-Oncology** (15-16 November 2013, Geneva, Switzerland) to discuss:

- Critical components of immune therapy
- Disruption of homeostatic regulatory mechanisms
- Angiogenesis blockade
- Expansion of tumour-reactive T cells

Moderator:

George Coukos

Panel experts:

- Giuseppe Curigliano
- Jerome Galon
- Michael Kalos
- Cornelis Melief
- Laszlo Radvanyi

George Coukos: Thank you very much for your patience. This was, I think, a very intense couple of days; we had some wonderful quality science and clinical science presented. I was asked by the organiser to sum this all up. Of course by no means I am in a position to do so, but a better way I thought, was to do this is in a panel discussion.

Clearly we are in the era of heterotypic cell biology where the tumour microenvironment with its many components and subtypes; complex biology is now becoming very important in terms of gaining therapeutic benefit.

Coming back to the original scheme of at least how I see the world of immunotherapy combinations organised, is a focus on expanding tumour reactive cells, while at the same time addressing these very important mechanisms that we have been talking about of homeostatic regulation in the tumour microenvironment; these are stromal responses to chronic injury and inflammation and so they are from IDO to TGFß and so on. And also the role of angiogenesis in the tumour vasculature in organising the infiltration of T cells, There are many, many opportunities within reach and many in the context of clinical trials, and so the panel will hopefully help us address some of these.

Disruption of homeostatic regulatory mechanisms

What are the next targets for checkpoint blockade or immunomodulatory therapy? What are the opportune combinations strategies for immunomodulatory therapy? Where are we with biomarker development for patient selection?

George Coukos: We'll start with the most important or the most trendy, if you will, and promising area of disruption of homeostatic mechanisms and checkpoint blockade. The first question to the panel is what are the next targets for checkpoint blockade or immunomodulatory therapy? We heard a lot about PD1 pathway and CTLA4, what are in your mind the next low hanging fruit in this area?

Cornelis Melief: Well, many targets have been mentioned in the course of this; I mentioned the immunosuppressive cytokines like TGFß and IL -10, antibodies to these are already available and just need to become widely available to do clinical trials in combination and in those situations where the tissue and the serum levels tested show that this would be important. So I think the type of studies that Jerome Galon has been doing will become more and more important to tell us which immune checkpoint or other anti-immune inhibitory treatment the patients will need. Is there IDO expression? If you have it then you need IDO inhibitors, if you have IL-10 or TGF production, then you need to counteract that; but I'm not sure that all of the big pharma companies realise this yet. I mean, take anti-TGFß. It was developed to prevent lung fibrosis or other indications, not immunotherapy yet. But I think there is a great opportunity to use it for immunotherapy. So, in that sense I like the initiative of the Cancer Immunotherapy Network where, in the United States, increasingly this Network is being supported to make lawful immune reagents, therapeutic compounds available that are not easily obtained from industry. Once they have shown their effectiveness, and an example is IL-15 being produced by the NCI (National Cancer Institute) and NIH (National Institute of Health) if it will be shown like IL-7 and IL-15 are likely to work much better than IL-2 in vivo, if that can be shown in some good clinical trials, I think industry will definitely develop it further.

George Coukos: Are you aware of any comparative studies where people looked at the virus soluble factors that one has identified in the tumour microenvironment and compared them for their ability to suppress T cell binding or T cell function, for example, either ones you mentioned or potentially other ones? How would one prioritise what is the first and then the second and so forth?

Cornelis Melief: Well definitely I think STAT3 signalling, as a transcription factor, drives a lot of negative things so having a STAT3 inhibitor would be very effective; that would be actually my first choice because that would go to the root of things. But I am not aware that any company is developing a really, very strong STAT3 inhibitor.

Laszlo Radvanyi: I didn't have time to show the data but I think the nitric oxide pathway - iNOS and the nitric oxide pathway and nitrosylation both S- and tyrosine

nitrosylation - are really coming to prominence. I mean you have Vicente Bronte's data on nitrosylation which, incidentally preferentially causes the migration of myeloid cells into the tumours instead of T cells because the nitrosylation less affects the signalling of the receptor which is most highly expressed on myeloid cells than T cells because it blunts the signalling. We found in melanoma, I didn't have time to show the data, that there's an inverse correlation between nitrotyrosine and iNOS expression in melanoma and the infiltration of CD8 T cells. So I think it sets up a barrier, we notice in high areas of nitrosylation where there are no T cells and vice versa. There are a lot, a number of drugs out there, Pfizer has a drug aniline, there's doxycycline our good old antibiotic, which is known to be a nitric oxide iNOS inhibitor, so I think that's going to be an area to go after. And of course with TGFß, I think stay tuned for our TGFß dominant negative receptor transduced TIL (tumour infiltrating lymphocyte) trial), we're going to be starting to treat patients. So that's going to be a good 'proof of concept' to see whether blocking TGFß signalling will help.

Giuseppe Curigliano: And another potential target I believe is costimulatory growth factor receptor 1 that mediates microphage transition from M1 and M2; and there is a clinical trial, ongoing FDA registered, with paclitaxel plus this monoclonal antibody and it is very clear when you have M2 in the tumour microenvironment you promote growth and if you block this receptor you have a down regulation of proliferation. This is a very interesting target. Talking about TGFß inhibition for lung fibrosis, we are launching a trial in lymphangenic breast cancer because IntraImmune, the company developing this drug gave us the drug to do a trial in a special presentation of patients with breast cancer. It is lymphangitic breast cancer with a lot of tumour microenvironment, a lymphangitic spike to the chest wall and we will start this trial in a triple negative subpopulation because TGFß pathway is highly expressed in this subset. It is a small trial but they will give us the drug. We know there are money for translational research.

Michael Kalos: George, the first question that you posed is a big struggle for all of us. I mean, I don't know what drove ipilimumab to be the first molecule to be developed, other than that it happened to be the first antibody there and PD1 being the second one and then PDL1. I'm not sure that there was much more of a rational thought behind choosing those other than that they were the first to go, but finding some way to rationally define TIM3 and Lck, and determine in what way they may be different, what might CD137 give you that's different. I think that's our biggest challenge in terms of deciding where to go to next, because we all can enumerate all the targets that we can identify and say let's go after this guy next but thinking about how do you do it from a biological perspective rationally is our biggest challenge.

George Coukos: I think you're absolutely right and I think systems biology, systems immunology and ultimately systems pharmacology approaches are mandatory to start addressing some of these questions, I don't know a good way of doing that; for example, we recently looked at TILs in ovarian cancer and tetramer specific TILs which we know recognise antigen and we asked, what are the most common or prevalent inhibitory receptors? CTL4 and PD1 were invariably expressed on those, but Lck-3,

TIM-3 were not always there. Also that is, for example, one prioritisation that can be applied potentially in many other tumour types.

Jerome Galon: But the good news is that we have a lot of possibilities, already very powerful. There are a lot of co-inhibitory receptors, so-called checkpoint blockades, maybe they work through different mechanisms as well but certainly through immune related or autoimmune related mechanisms, so for sure other inhibitors, antibodies will be on top of the list. Activation of the T cells we heard about; the co-stimulatory molecules will be on top of the list as well. Also for the cytokines, same thing. We have the suppressive cytokines, TGFß was mentioned, and the positive ones also would be at the top of the list, IL-15, IL-21. Now the question is how to decide and how to prioritise. Because we have seen data showing the great impact of the combination so we need to combine or it will be optimal to combine and there's endless possibilities of combination but there will not be endless possibilities to test all of the combinations. It will not be possible to run phase III trials on any possible combination comparing everything with everything; so how to prioritise those is really the challenge. I'm not sure that we will be able to answer these questions with mouse models. Of course, we can dissect mechanisms to give some good ideas on how things are working but really to see which patients will benefit the most from different types of combination I think, unfortunately, we will have to test it in patients. One way to really select for patients is by biomarker and biomarker analysis and try to give the appropriate combination depending on the immune defect of the patient. I think that's really going to be the next wave of...

George Coukos: What you are proposing is the equivalent of a basket trial for the immunotherapy world where basically you have some biomarkers and depending on the expression, then you put the patient with the appropriate immunoregulatory drug plus a standard immunotherapy, say for example, PD1 or CTLA4 antibody. I'd like also any of the participants to give us any opinions.

Question from the audience: Listening carefully to Jerome, I was kind of thinking in the power of negative thinking. Looking at the tumours that are not infiltrated, and whether in those tumours do you see the signal for TGFß and related to STAT3 because those are the favourite targets by Dr Melief, so if it would happen that those tumours were reached in the negative signal, in the inhibitory signal type of thing that would make sense in terms of infiltration.

Jerome Galon: Looking at large cohorts of patients, at least with colon cancer, I was very astonished, I would say, by the fact we had a very hard time finding, let's say, bad immune genes. It was very striking initially. When I started all this, I was expecting to find the co-inhibitory receptors, the immunosuppressive cytokines, all the immune escape mechanism that had been very well described to find these associated with death of the patients, tumour recurrence, tumour progression but that's not what we found. We really found that the adaptive immune response associated with long-term survival; we had a hard time in finding immune suppressive mechanisms, so I think it's

more of a challenge to pinpoint the immune defects. In grouping patients with immune defects, we know for example, that we can clearly see a group of patients with increased VEGF expression, with sky high levels and this is not good, but it's not good only in the context in association with the cytotoxic T cells. Per se, the VEGF expression is not predicting the survival of the patients, so combination of markers will probably also be the way to go.

Cornelis Melief: Perhaps one of the most confounding factors is the lack of immunogenicity in many of the tumours; even viral tumours often are not infiltrated with the viral specific T cells. And why is that? Well, clearly these are self tissues not eliciting danger signals. So without any specific immunotherapy, either adoptive transfer expanding low numbers of specific T cells or appropriately vaccinating and then see what happens – if now T cells go into these tissues, which regulatory mechanisms will now pop up. And I think a signal of what could be expected is at the SITC (Society for Immunotherapy of Cancer) meeting last week it was presented that PDL-1 expression is no longer a predictor of clinical response; if you combine ipilimumab and PD1 blocking. This is probably because if you apply ipilimumab then it already drives more T cells and expands them in the tissue microenvironment, increasing the production of interferon gamma, driving the PDL1 expression. I expect the same thing from vaccination, but we need to monitor what happens if you try to push too much specific T cell response of tumours that, according to you, have no regulation to start with, now start. Because the self tissue defends itself against these attacks with upregulating protective molecules and the tumour is a self tissue.

George Coukos: So you have an adoptive resistance to immune pressure which may not be there at the beginning, at the steady state, is why Jerome cannot find it so evident.

Cornelis Melief: Right, I expect you start seeing it if you push the responses.

Laszlo Radvanyi: That adoptive resistance, I think that's the chicken and the egg all over again. I see that biomarkers are up for discussion and I think this is going to be a very tricky thing to work out - just what suppressive mechanisms intrinsically are driven by the tumour and what are driven by the immune response. Because we're seeing this in melanoma as well, that patients who do very well are paradoxically the ones that have a lot of CD8 cells, but actually have FOX3b cells. I couldn't show you the data, but if you look at those tumours, those patients' tumours grow TIL (tumour infiltrating lymphocytes) and the more CD8 and FOX3 cells they have when they go on to TIL therapy – those are the patients that live long and that suggests that there are feedback mechanisms, a strong localised immune responses. So I think in the future we need to tease apart, you know, I don't want to be simplifying the matter by categorising tumours that are cold, that are very intrinsically immunosuppressive through TGFß or other mechanisms STAT3, and other tumours that are more immunogenic - of course the mutanome comes in here as well - in terms of what's driving those T cell responses. And so we are going to have to tease all that out and

then find biomarkers and how they change on therapy and then be ready to change the way we modulate that. I just want to make another comment, something that Jerome said, you know we have to de-risk combinations as best we can with animal models, especially with targeted drugs, and that's the best we can do. I think we need to be more nimble and do smaller biomarker driven phase 1b trials and perhaps in a neoadjuvant setting, perhaps in smaller patient cohorts that are very biomarker driven before, after or during therapy and then you can do more combinations and figure out what is actually going on and what's driving the T cell response at the local level, what's not doing it, etcetera. So I think we have to change the way we do clinical trials too, to not just go ahead and pick something, as Michael said, and then rifle it into large phase 1's. For example, the PD1 trial that BMS did was a huge trial with hundreds of, why did you need to do that? You know, really, honestly.

George Coukos: So just to comment on the T-regs (T regulatory cells), we are seeing the same thing in ovarian cancer, that is: The more TILs you have, the more T-regs and I think this is what is at the base of the controversial predictive power of the regulatory T cells, which in some studies, are predictive of good outcome, in some studies they are predictable of bad outcome.

Question from the audience: You have mentioned something about, but I would like you to be more specific about what you think are the main limitations for an appropriate biomarker development.

Cornelis Melief: We need clinical specimens that will allow us to ask the right questions and get the right answers. As Lazlo pointed out, we need biopsy material, pre and post. Also to find ways to change the paradigm that says you can't get this material - I realise that many times it's hard to get. But we have to design trials that give us that information, in my mind.

Question from the audience: Would then, paraffin embedded tissue sections be enough for that or do you need cryomaterial.

Cornelis Melief: Yes, there are platforms that you can use FFPE that will give you answers.

Giuseppe Curigliano: Two large trials have been launched, one in metastatic breast cancer and another in non-small cell lung cancer. The trial in breast cancer is the AURORA trial; more than 3000 patients will be biopsied at first relapse and a wide genome analysis will be performed on all these tissue samples, including genes relating to the immune system. This is a very important effort of the Breast International Group and the North American Breast Cancer Group with an investment of 25 million dollars from the Breast Cancer Research Foundation and one of the topics that will be addressed is the immune response in these patients. So this is an important topic to be addressed, biopsying and rebiopsying in order to better understand driver pathways in metastatic breast cancer, at least.

George Coukos: So going back into the immune modulatory checkpoint blockage, clearly there are some patients who respond very well and some patients don't respond; if you have a patient who has PDL1 expression in the tumour and has pre-existing T cells in the tumour and does not respond to PD1 therapy, what is your knee jerk reaction, in terms of where should one look to decipher the complexity of the system?

Giuseppe Curigliano: A good question. So what I know in my field is that if you have high PD1 expression usually you also do not respond to trastuzumab in the metastatic setting. So in my opinion, you should overcome this resistance, by blocking another checkpoint, by continuing the monoclonal antibody targeting the pathway that determines the oncogene addiction in that specific tumour. So let's say combination strategy, in an ideal trial is in a neo-adjuvant setting; so we proposed a trial to Bristol-Meyer that high-intensity modulated ultrasound (HIFU) ablation of primary tumours with one shot of ipilimumab in order to explore the mechanism of over response by the immune system and specifically on the primary breast tumour. This trial will never start, there is no interest finally. And the second trial is the PANACEA trial that is for patients that are progressing on trastuzumab first-line treatment and at progression we randomise to trastuzumab alone plus chemotherapy versus trastuzumab plus anti PD-1 monoclonal antibody. It is very interesting. How to regain response to a monoclonal antibody by modulating the checkpoint of immune modulation. So I think this is being investigated. What we need to do to better understand the mechanism of action of all the drugs currently used, about cisplatin or low dose of cyclophosfamide in metronomic chemotherapy, let's say. Also, imatinib has immune-modulatory effect. There was a paper, very interesting, published on trabectedin; it seems it works by stopping the microphage conversion from M1 to M2. So explore new ways of giving all the drugs with immunomodulatory therapy because I believe we can achieve a lot of results. First of all moving again in the neoadjuvant setting because I believe this is a very important point, we have the window of opportunity trial, you know the biopsy you expose to one agent or two agents another biopsy and at surgery you will collect information. And breast cancer is a good model for this.

George Coukos: But ultimately the patient, let's say in the PD1 trial who does not respond and has all the biomarkers that should predict a good response, one should think that T cells are ultimately anergic, one cannot push them enough. So Lazlo, you have done a lot of TIL work, so what is your interpretation of T cell anergy in the tumour microenvironment. What else do we need to do to juice them up?

Laszlo Radvanyi: Well, first of all, I'm not a believer in exhaustion, I think that's an unfortunate term. I think what we are seeing now is that PD1 positive, BTLA positive and all these other Lck3, TIM3 activation markers, these are markers of highly activated T cells and because of the way the immune system evolved to roll back runaway immune responses, it's a natural response of the immune system to roll it back and so in terms of anergy, there's been some studies to show, you know, there are mechanisms that can induce anergy such as DGK alpha signalling but we haven't

seen that in our TIL; this is one of the benefits of adoptive T cell therapy, I think, you know we're discussing all these ways of manipulating the immune response with immune modulators and drugs and other strategies and looking at biopsies, but in a way, adoptive T cell therapy with tumour infiltrating lymphocytes takes away all that mystery, takes away all those unknowns. You pull the T cells out of that immunosuppressive environment, you wake them up, you expand them with interleukin 2 - we've seen that seems to wake up a lot of the cells - and then you can re-infuse these cells back into the patients at very very high numbers and they can then do better what they are supposed to be doing. I think adoptive T cell therapy with TILs has shown that these cells are really not anergic, you can wake them up, if they were anergic or inactivated you can reactivate them. I think Carl June's data as well shows that.

Cornelis Melief: I have a question here because in the CAR (chimeric antigen receptor) therapy you have a very effective CD/ CD137 signalling domain inserted. I haven't seen this used large scale in adoptive T cell transfer but it arguably would work exactly the same way perhaps you don't need it, but you can replace it with CD137 monoclonal antibody.

Laszlo Radvanyi: Yeah, we were actually funded to do a clinical trial by the Melanoma Research Alliance with Jeff Weber's group at Moffett four years ago, a one million dollar grant, in which we proposed to do a anti-4-1BB and unfortunately we were ready to get the antibody and everything looked ready to go, and then, bad timing, BMS had those toxicities when they dose escalated and they yanked the funding out, despite the fact that we were arguing until we went blue in the face that the doses that we wanted to use in the dose escalation phase I in combination with TIL were way lower than that they were using. So this risk, overly risk aversive attitude set us back four years. And there are newer technologies right now where I think we're at a realm where we routinely gene modify T cells and gene modify TIL's as well very efficiently, very practically with very high efficiency that's going to get better and better with GMP quality processes so one could put signalling domains. You know we're actually working on developing a novel signalling domain that can allow you to fire anti-4-1BB in a B cell or whatever you want, I won't go into the details but that's the kind of new things that one could think of.

Angiogenesis blockade

Is tumour vessel normalization important for immune therapy? What are the opportune targets for immunomodulatory antiangiogenesis therapy? What are the appropriate combinations?

George Coukos: We will move on to the next target. Does anyone in this room believe that angiogenesis blockade has a role to play in immune therapy? So, is normalisation of the vessels important? We just heard that radiation might be doing some of that...

Giuseppe Curigliano: Are you sure that anti-angiogenic agents normalise, first of all. This is also a matter of discussion. We have a flop for anti-angiogenics, at least in breast cancer. Finally, there is just improvement in progression-free survival but no effect on overall survival. I just published a trial on sunitinib on triple-negative breast cancer; more than 200 patients - sunitinib alone versus chemotherapy of the choice of the investigator and the final result is that sunitinib is detrimental in these patients. They were first-line treatment and almost all died. So I believe that bevacizumab or older anti-angiogenic agents can remodulate the stroma but personally I don't believe they are so important to enhance an immune response..

George Coukos: So, Jerome, you mentioned that you found VEGF as a negative predictive factor for the TIL signature.

Jerome Galon: But without any significant impact on the survival by itself. It was within the context of TIL infiltration and also we quantified the level of vessels in these patients and there is no relationship between the VEGF expression and the level of vessels. And these 'so called' anti-angiogenic therapies, if they work it may not be through normalisation. We know that anti-VEGF or VEGF is acting directly on immune cells blocking the maturation of dendritic cells, its acting directly on T-regs so we know that they are direct actions of these agents on the immune system, so there might be opportunities to combine these 'so called' anti-angiogenic therapies with other immune modulators, but I believe that if they work it will be more through immune regulation, maturation of dendritic cells, these types of things, rather than normalisation of the blood.

Cornelis Melief: So I would say that most of the angiogenesis being produced is due to factors produced by the myeloid cells, so if you suppress the myeloid cells by other means, such as appropriately timed chemotherapy, or CSF1 receptor therapy it will be interesting to see if then the angiogenesis inhibitors still add on, on top of that. That would be an interesting question to research.

George Coukos: It certainly is a very complex topic.

Michael Kalos: I just think that anything we can do to disrupt the equilibrium that the tumour has established is a potential opportunity to make a difference and the beautiful picture recently put out in the immunotherapy publication with different stages, all of them are on the cover of that reference, you had the publication right there, I think all of those are intervention points that we need to fully explore and we shouldn't let out preconceived notion about normalisation of vasculature affect our opportunity to go in there. We have very exciting products that we can test in each of these cases.

Giuseppe Curigliano: The only preclinical and clinical data that support efficacy of antiangiogenic therapy is combination with metronomic chemotherapy. There is a lot of data on this with low dose cyclophosphamide, the study researchers believed that it was an anti-angiogenic effect of low dose cyclophosphamide but now we know that it

was a downregulation of T-regs. So let's think of trials perhaps with bevacizumab and low dose chemotherapy in order to understand if there can be a combination that can be effective against the tumour. This is a good observation since there is a huge amount of data from Canadians and from the United States also.

George Coukos: There are also data on the effects of angiogenesis therapy on vaccine modulation and so forth; I think it is clearly a very interesting area of investigation.

Expansion of tumor-reactive T cells

Are vaccines still useful? What is the ideal patient population for vaccine therapy? What are critical ingredients for developing effective cancer vaccines? What are the opportune combinations strategies for vaccine therapy? Is chemotherapy deleterious or helpful? Are there effective approaches to in situ vaccination? What are the opportune combinations strategies for in situ vaccine therapy? Will adoptive T cell therapy be effective in solid tumors? What are the opportunities in custom design of engineered T cells? What are the opportune combinations strategies for adoptive T cell therapy?

George Coukos: So are vaccines still useful, what is the ideal population of patients to vaccinate? So maybe we can start from there.

Cornelis Melief: If I may start, I think the ideal population is those with premalignant disease followed by the adjuvant setting, followed by the neoadjuvant setting followed by those with overt bulky disease where you need to do other things, debulking plus combination therapy to have an effect. But I am convinced in most patients with failed cancer therapies, that vaccination alone will not be effective and will require additional combination therapy such as what I discussed but in addition with antiangiogenesis and checkpoint blockers and other. So combination therapy clearly is what is needed if you use vaccines for patients with more advanced disease, and as we saw in the patient with premalignant disease, it might work by itself, but also there, there was a correlation with those with high levels of T-regs, the T cells expanded insufficiently in response to the vaccine and there was no clinical benefit. So there was a clear cut correlation there, so even in half of the patients with premalignant disease, you need to do something else.

Giuseppe Curigliano: I completely agree with you, there is a trial with trastuzumab in DCIS, in ductal carcinoma in situ, over-expressing cerbB2 and we proposed to GSK block, and we use the same vaccine that we used in the adjuvant setting for DCIS for premalignant breast cancer. I really believe the post neoadjuvant setting could be an area where you can explore any immune approach because these are patients with very poor prognosis specifically triple negative cerbB2 positive and so in that setting you can obtain at least a proof of concept that an immunostrategy can impact on a

clinical endpoint. Because we need to realise is to have an outcome on the clinical endpoint, either disease-free survival or overall survival. It's important to develop markers, it's important to stratify patients but we need signal of activity that you can have a difference between the treatment, observational and treatment arm.

Michael Kalos: When I came to Penn five years ago, I gave a talk on how we have to change how we think about vaccines targeting these self antigens, I have been told 'Michael, a lot of people have spent a lot of years working on these antigens, so temper your message.' But I have to say we are never going to succeed until, I mean, Ton Schumacher's talk was so wonderful. He told us what the relevant, what the potent T cells are. I firmly believe that we are never going to be dramatically successful in vaccines unless we start thinking much more carefully about the target antigens. Cornelis, your beautiful data with HPV - it's a non-self antigen and low and behold I have seen strategies that have worked in different settings. And I was part of that group that did all the work the vaccine work but we have to look at ourselves and say.

George Coukos: Sorry to interrupt. I'll give it to you on self-antigens but when you have new epitopes, again mutated peptides are new epitopes, or viral antigens are new epitopes, I think there is a good chance there.

Michael Kalos: I agree.

Giuseppe Curigliano: The failure of vaccine, finally, is related to the fact that a large amount of data comes from metastatic disease, when you have mechanisms of tolerance, you cannot test efficacy of a vaccine in this setting. Absolutely not. How many trials do you have in the adjuvant setting or in the premalignant disease? That is why we need to change our way of designing trials with vaccine. Maybe you can observe something interesting, that's why I propose this one.

Laszlo Radvanyi: I wouldn't throw out self antigens, overexpressed self antigens just yet. There's a trial, you know, with the HER2 positive and HER1 plus that clearly showed some clinical benefit in terms of relapse-free survival in breast cancer patients. So the issue here is: Can we find vaccines, even if they are against over-expressed self antigens that can then cause antigen epitope spreading? I think that we know, even from advanced cancer trials, that patients that respond are not responding because of the antigen in the vaccine has actually eliminated those cells expressing the antigens as a method of self selection, but in turn they elicited other responses. I think maybe that is the area where basic immunology and tumour immunology can come in and try to tease out what is really regulating that antigen spreading or epitope spreading that is occurring in some patients but not in others in response to that overexpressed self-antigen vaccine. That's going to be an interesting area, maybe checkpoint blockade, of some immunoregulatory pathways, some immunostimulatory pathways are different - patients' immune systems are hot-wired differently. I think there are opportunities in years of research to try to understand what is going on at a more basic level in that regard.

George Coukos: Cornelis already answered the chemotherapy question so let's move on – in the case of vaccination, in situ vaccination, so the effects of chemotherapy, the immunogenic cell death by radiation are these opportunities for combinations today with other modalities?

Cornelis Melief: My feeling about the type of immunogenic cell death induced by anthracyclines and oxaliplatin for example is that we can do so much better in vaccinating with proper vaccine concepts that we don't need actually immunogenic cell death because from what I have seen it is a weak effect, much weaker than potent vaccines. I am not saying it could not help if you do not vaccinate but there are ways to vaccinate against these mutant antigens or viral antigens with proper combination therapy.

George Coukos: Ok, so tumour reactive T cells, the last is adoptive T cell therapy we have heard wonderful results on leukaemia from Carl June. The big question is, will this work in solid tumours?

Michael Kalos: Yes - and they will work dramatically. I think an activated T cell takes no hostages. I mean - PD1, PDL1 blockade that's the tip of the iceberg of what can happen because even in that setting, that's a really compromised setting where the tumour has the balance. There's early data from Rosenberg's data on the TIL - and the TIL - there're not as potent as some of these engineered cells - very dramatic responses in melanoma, there's data in sarcoma that shows dramatic responses. We have early data at Penn with RNA engineered cells that I think Carl talked about. I think this is going to be a real breakthrough if it isn't already, in solid tumours. Maybe I'm being optimistic here, but there's going to be some dramatic success there.

George Coukos: So you think that the properly activated T cell, with the right receptor or the affinity receptor will drive through any tumour and eradicate it?

Michael Kalos: Most tumours, I would say, maybe not pancreas. We saw yesterday a case report of a patient who died, I think probably because of bleeding in the brain because T cells went to the brain where there was no level antigen expression. We do autopsies in patients and we unfortunately had the opportunity to do one of those at Penn and these engineered T cells, these T cell receptor engineered cells they're everywhere, they do not know about boundaries, I think, in part the boundaries are our interpretation of negative data.

Cornelis Melief: What that means is that target selection becomes all the more important.

Michael Kalos: Critical, absolutely,

Cornelis Melief: Because vaccines against CEA are nontoxic but adoptive transfer with high affinity CEA T cells destroys the large intestine.

Michael Kalos: So the big challenge in regard to CARS is how you define the appropriate threshold for receptors because a lot of the CARS are derived from antibodies, which require 20,000 molecules on the surface whereas the CAR may require 50. And for TCRs the enhancement process for self antigens gives you entirely unpredicted degenerate specificities, so that is what I see as the big consideration.

Giuseppe Curigliano: I have one comment on this, just one. You know there is an FDA approved procedure that is Provenge for the treatment of prostate cancer and I am reminded of the total of the cost of this type of treatment so, which was very high; and can we afford the cost of these procedures in a world of economic crisis?

Michael Kalos: I can speak to that a little bit – I can't speak to the cost of the CAR therapy because I know nothing about that cost, but Provenge was a great first entrée into cell therapy. Nobody, none of us cell therapists want to diss it but, for good reasons, the European authorities balked at it. Complete ongoing response with a single infusion of cells is possible now, and I know leukaemia is special, but we have patients out three years with a single infusion of T cells. How much does that have to cost to make that worthwhile? Economically, right? So I think that once these, when we show adoptive cell therapy to be a disruptive technology that works as robustly as I propose to you it does, the cost will fall into place without much debate.

Cornelis Melief: But the cost to be implemented in hopefully routine blood transfusion settings, because it's an advanced personalised blood product, as I see it.

Michael Kalos: You're asking, if it can...

Cornelis Melief: It can.

Michael Kalos: Carl made the point that when bone marrow transplantation was started the same argument was made, absolutely it can, yes.

George Coukos: Well, this was a wonderful meeting, thank you very much for your patience, attendance and participation.