Report from the Canadian Melanoma Conference

Advances in immune therapy

HIGHLIGHTS

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The 11th annual Canadian Melanoma Conference took place in Whistler, BC, from February 2 to 5, 2017. The meeting focused on recent research and treatment challenges in melanoma. Dr. Scott Ernst, Professor at the University of Western Ontario, chaired the meeting, which assembled international experts and participants from a wide range of clinical and research fields. Video from many of the conference presentations is now available on the Oncology Education website: www.oncologyeducation.com.

Below, we present summaries of just a few of the presentations, focusing on current challenges and questions around immunotherapy, targeted therapies, and their combination.

IMMUNE CHECKPOINT INHIBITORS: WHERE HAVE WE COME AND WHERE ARE WE GOING?

Jason Luke, MD, FACP, Medical Oncologist, University of Chicago Medicine, provided an overview of tumour immunology, described the mechanistic underpinnings of the anticancer immune response, discussed current use of immune checkpoint-blocking immunotherapy, and outlined a framework for identifying the highest-priority immune checkpoints and combination therapies for clinical investigation.

The path to killing a cancer would see a tumour grow and shed antigens that are picked up by dendritic cells, which would then send antitumour antibodies to destroy the tumour. As Dr. Luke stated: "While this process unfortunately doesn't occur naturally in our patients, it is key to the development of cancer immunotherapy." Two strategies are being tried to enable the immune system to fight cancer: blocking the mechanisms that prevent immune response, and enhancing the immune response. The first drug to be developed, ipilimumab, targeted the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) "off" mechanism, and more immune checkpoint inhibitors have been identified over the past 10 years, all of them potential targets for cancer immunotherapy.[1,2,3] "What really spurred the development of immunotherapies after ipilimumab was the long-term survival that was observed with CTLA-4 antibodies," said Dr. Luke. A retrospective analysis of more than 5,000 patients[4] found 22% progression-free survival (PFS) at 3 years, with patients tending to remain progression-free after that.

CLTA-4 studies have revealed a phenomenon of "pseudoprogression," where patients get worse before they get better. Dr. Luke highlighted that patients who have a delayed response phenomenon end up doing very similarly to those who have an early immune response. Studies also show that, while these drugs don't really cause side effects, the immune response can cause side effects that are autoimmune in character (hypophysitis, uveitis, pneumonitis, adrenal insufficiency, enterocolitis, arthralgia, pancreatitis, rash, hepatitis, hypothyroidism, dry mouth) and require a more nuanced, internal medicine-related approach to the evaluation of patients treated with immunotherapy.

BENEFITS OF COMBINING CTLA-4 AND ANTI-PD1 ANTIBODIES

In the current standard of care, agents are used to target programmed cell death protein 1 (PD1) and programmed death ligand 1 (PD-L1), which dampen the antitumour immune response. In at least half of all patients with cancer, the tumour actually displays the interferon gamma receptor that upregulates T cells and may lead to adaptive immune resistance. PD-L1 expression in the tumour microenvironment can inhibit antitumour T-cell activity, enabling apoptosis and tumour cell death. Over 40% of melanoma patients achieve 2- to 3-year survival with anti-PD1 antibodies. While PD1 acts on the effector of the immune system phase, CTLA-4 affects the priming phase. As such, these are mechanistically distinct and not overlapping approaches, and can therefore potentially be synergistic. However,

the combination produces a greater number of adverse events. "Patients can get extreme benefit, but they can also get extreme toxicity," noted Dr. Luke. He described recent attempts to refine this approach by using low-dose CTLA-4 antibodies (1 mg/kg dose of ipilimumab instead of 3 mg/kg) alongside a full dose of the PD1 inhibitor pembrolizumab. Early-phase trials showed a response rate (57%) similar to the ipilimumab plus nivolumab combination.[5] "Most people," said Dr. Luke, "now believe that lowering the dose of the CTLA-4 inhibitor ameliorates side effects to some degree. It will be interesting to see if these patients have the same degree of benefit as those on a higher dose. Other studies are looking at potentially reserving the addition of the CTLA-4 inhibitor for second-line therapy, and Dr. Luke's team is now running a clinical trial to see if they can start patients on CTLA-4 inhibitors only once they've shown clinical signs of progression, thereby reducing toxicities.

OTHER TARGETS

Chen et al[6] drew out the immunity cycle, adding on potential targets at different phases. At each level, there are stimulatory immune factors that could potentially be agonized to drive the immune system, along with inhibitory factors that could be blocked to enable release of a greater degree of immunity. Dr. Luke emphasized that understanding cancer immunity involves much more than checkpoint immune therapy — it requires thinking holistically about where the lesion in the immune response is slowing down immunity and preventing rejection of the tumour. His team at the University of Chicago is currently investigating agents targeting both stimulatory and inhibitory checkpoints. "We're also looking at combinations with other classes of agent: metabolic, cytokine, oncolytic viruses, targeted therapies, chemotherapies and radiation."

He described some of the most interesting potential combinations addressing ligand interactions and the innate immune system to amplify the benefit of immunotherapy. When the immune system doesn't succeed in clearing a tumour, T cells become exhausted or dysfunctional, and gain characteristics that can be targeted through PD1 inhibitors and other antibodies. An anti-lymphocyte-activating gene (LAG3) antibody + PD1 inhibitor combination is showing some promise in mouse models, and a phase 1-2 trial has already accrued more than 100 patients. A T-cell immunoglobulin mucin family member 3 (TIM3) + PD1 inhibitor combination is being looked at in mouse lung cancer models, where it is showing increased survival. Multiple studies of agents targeting inhibitory checkpoints are now underway.[7] Agonistic checkpoints are also being targeted, with tumour necrosis factor receptor superfamily member 4 (TNFRSF4/OX40) and TNFRSM9/CD137 agonism appearing to augment tumour-specific T-cell memory response. Ongoing trials of urelumab combined with nivolumab in melanoma show a 70% disease control rate and a 40% overall response rate, with no increase in toxicity. "We will need randomized trials to show that this is better than PD-L1 alone," Dr. Luke stressed. Many companies are looking at the possibility of blocking 2 receptors at once: an agonistic checkpoint and an inhibitory checkpoint.

SMALL-MOLECULE APPROACHES

There may be a role for small-molecule inhibition of checkpoints with agents such as BRAF and MEK inhibitors, however there were concerns that MEK inhibition could be

immunosuppressive, as the receptor sits downstream of T-cell activation. "The idea was that if you gave a MEK inhibitor, you wouldn't be able to activate any T cells," explained Dr. Luke, adding, "That turns out to be not exactly correct." Recent data show that MEK has an impact over time, and trials of targeted immunotherapy triplets, with BRAF + MEK + PD1/PD-L1 are showing interesting short-term effects.[8,9] Adverse events were over 50%, though most were manageable, and a number of phase 3 trials of multiple triplet combinations are now launching. Dr. Luke pointed out that these trials involve continuous-dose BRAF + MEK on top of immunotherapy, and considered that it might be better to pulse these drugs, as there is some indication that continuous administration can be deleterious. "Dr. Ribas and Dr. Flaherty both showed that BRAF inhibition can lead to immune infiltration," he noted, "so treating until resistance may drive immunosuppression. The T cells come in, but then they leave. The question is: can we get them to keep coming, potentially with a pulse approach, as opposed to continuous dosing?"

A small molecule inhibitor, epacadostat, acts on indole-2,3-dioxygenase (IDO), which converts tryptophan to kynurenine, an immunosuppressant. IDO is downstream of gamma interferon, and Dr. Luke's team found that blocking IDO and PD-L1 was synergistic in mouse models. Phase 1-2 results of the combination of the IDO inhibitor epacadostat + pembrolizumab in patients with melanoma have now been reported at multiple meetings.[10] Dr. Luke noted that in the first 25 unselected patients, the response rate was 55%, and there was no added toxicity from epacadostat. An international randomized phase 3 trial comparing epacadostat + pembrolizumab vs pembrolizumab + placebo and has already accrued 600 patients.

Other interesting molecules include IPI-549, a selective inhibitor of phosphoinositide-3-kinase-gamma (PI3K-gamma),[11] which can increase the number of effective CD8 cells and raise immune activity, improving the effectiveness of an ipilimumab + nivolumab combination. "The idea is that, by blocking the macrophages, we may be changing the inflammation status of the tumour to allow those T cells to be more active," explained Dr. Luke. A phase 1 trial is already in progress. Purinergic (adenosine) immune regulation is also attracting attention. The release of adenosine throughout the body happens in the context of the fight or flight response, and also occurs in tumours. Adenosine is directly immunosuppressive, and will selectively suppress the activity of CD8 cells, so that blocking access would effectively increase the number of CD8 cells. A number of adenosine receptor inhibitors are now entering clinical trials.

INNATE IMMUNITY AND ONCOLYTIC VIRUSES

Toll-like receptor (TLR) agonists are infection-sensing molecules that can detect the presence of foreign molecules and may have a role in treatment-resistant noninflamed tumours, alongside PD1 antibodies. A number of these agonists are currently used in dermatology and will be coming forward as selective partners for cancer immunotherapy to drive inflammation in the tumour microenvironment. The cyclic GMP-AMP synthase (cGAS) protein appears to be involved in immune system recognition of tumours, signaling through the stimulator of interferon genes (STING) pathway to activate the interferon response. Early mouse studies show that 3 doses of a cyclic dinucleotide molecule that directly agonizes STING can eradicate the tumours, and they do not come back, even in rechallenge. A phase 1 trial of the intratumoural STING agonist, MIW815, has been completed, and the next step will be to add a PD1 inhibitor.[12]

VIRAL THERAPY

Oncolytic viruses lead to a number of downstream responses, including the direct activation of STING and the interferon response, to preferentially kill cancer cells. A number of oncolytic virustherapies are being developed, and talimogene laherparepvec (T-VEC), derived from herpes simplex virus, type 1 (HSV-1), is approved in the US for advanced melanoma, though not yet in Canada. T-VEC is made up directly in a syringe and injected to deliver the virus right to the tumour. T-VEC replicates selectively in the tumour cells, prompting the release of dendritic and CD4 helper cells, the downstream activation of CD8 T-cells, and local lysis of the tumour, with the possibility of a more global response. This has already been taken forward into clinical trials. The first report of phase 1b T-VEC + ipilimumab looked quite promising: whereas ipilimumab would otherwise have about a 10% response rate, the addition of T-VEC shows a 25% complete response rate.[13] Dr. Luke cautioned that studies involve patients with earlier-stage cutaneous melanoma, though in one trial that included patients with visceral lesions[14], these were also found to shrink. Dr. Luke described this approach as "conceptually promising."

RATIONALLY GUIDING IMMUNOTHERAPY DRUG DEVELOPMENT

Gajewsi[15] presented a model contrasting T-cell inflamed vs noninflamed tumour microenvironments. In the first, an immune response can be promoted, while in the second, immunotherapy doesn't really work, and patients derive almost no benefit from PD1 blockers. To date, most responders correspond to T-cell inflamed phenotypes, identifiable on immunohistochemistry. Dr. Luke proposed using gene expression profiling as a diagnostic test to more holistically look at the tumour microenvironment and genes associated with gamma interferon to see whether they are upregulated or not. He suggests moving away from unselected clinical trials, to reflect new understanding that the biology is actually different between these different kinds of cancer. In tumours that are T-cell inflamed, blocking the immune-inhibitory pathways, possibly by combining PD1 and IDO inhibitors, may become a new standard of care. In noninflamed tumours, something needs to be done to drive inflammation in the tumour before giving PD1. Dr. Luke considered that the best way to do that might be to inject an oncolytic virus, which would cause the interferon-gamma response.

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SEQUENCING TARGETED AND IMMUNE THERAPIES

Keith Flaherty, MD, Director of the Henri & Belinda Termeer Center for Targeted Therapies in Boston, addressed the difficult clinical issues oncologists face in terms of sequencing in targeted therapy, immunotherapy

FROM FAITH TO ENLIGHTENMENT

Dr. Flaherty describeds a need for scientific principles to guide the sequencing of treatments for melanoma, and for tools to use in clinical practice to triage patients effectively. He points to data from a number of trials that enable comparison of shorter-term outcomes and long-term benefit. It may take time, but nearly 20% of patients eventually achieve complete or near-complete responses. "In melanoma, we're learning that even crude instruments like CAT (computed axial tomography) and PET (positron emission tomography) scans can delineate patients who are going to do well in the long term, even if they might have some evidence of residual disease," said Dr. Flaherty.

A meta-analysis of phase 2 and 3 BRAF and BRAF + MEK randomized trials[1] emphasizes that achieving short- to intermediate-term benefits is the top priority only for patients with the highest disease burden. In patients with moderate and low disease burden, there is a need for greater confidence in how to navigate them through short- to intermediate-term time frames in order to achieve long-term benefit. Looking at progression-free survival (PFS) data with followup as long as 5 years, Dr. Flaherty pointed out that long-term survivors include not only patients who have benefitted from multiple lines of therapy, but also patients who achieved progression-free status on frontline BRAF/MEK combination therapy.[2] He stresses the need to find out more at a clinical and molecular level about these patients, to make it possible to "prospectively think about triaging patients who have that phenotype or genotype to receive this exact therapy," knowing "with high confidence, they would be alive, well and progression-free 5 years down the line."

Studying population subsets from multiple targeted therapy trials with long-term followup makes it possible to discriminate between patient outcomes, using features of the disease available in clinical practice. Data now support anecdotal awareness that patients with low disease burden, normal lactate dehydrogenase (LDH) and involvement of 2 or fewer organ sites stand to have exceptional outcomes. This suggests that patients are potentially getting a benefit from multiple lines of therapy and that it is possible to identify co-occurring genetic alterations in this subpopulation of patients.[2] "Our initial goal with this type of data was to look at common co-occurring alterations with preclinical evidence suggesting a basis for therapeutic resistance." They now have extensive data from 200 patients and are starting to see that patients with CDKN2A alterations have significantly poorer outcomes. "There is probably going to be a basis in the future for going forward beyond just BRAF, NRAS and NF1 testing, in order to look at the next level of tumour suppressor gene loss and how that might inform our thinking in terms of the patient's ability to benefit, not just in the short-term or intermediate timeframe, but long-term."

There are data available to suggest that fairly basic immune profiling enables some separation of better and worse candidates for PD1 antibody therapy.[3] "The presence of CD8, and then a marker on top of that, seems to provide reasonably clean separation in terms of non-response to therapy," said Dr. Flaherty. "As more evidence accumulates, it suggests that we may be able to go, not just to the level of mutation burden analysis or counting the mutations, but really deeper into the field of trying to predict mutative neoantigens, of trying to understand who are those patients who have a T-cell clone that sets them up to be one drug away from response to therapy. It's an enticing concept, but of course not quite ready to reduce to practice just yet."

IMMUNE CONSEQUENCES OF TARGETED THERAPY

Dr. Flaherty described his team's interest in the concept of the immune consequences of targeted therapy, and the intersection between the 2 therapies. They were looking at serial tumour biopsies, focusing on signal transduction events in tumour cells, and the pathologists first had to examine the lymphocytes that were clouding their view of tumour cells in the initial analyses of samples — robust T-cell infiltrates present after 1 to 2 weeks of BRAF inhibitor-based monotherapy, or BRAF/MEK combination therapy. Those lymphocytes have markers of activation, as well as markers of exhaustion. In some cases, there was coordinated upregulation of PD-L1 expression on tumour cells at the same time. This work stimulated discussion regarding the possibility of encouraging some of the mechanisms of immune escape to turn off with oncogene targeted therapy.

They investigated the possibility (proposed by Dr. Georgina Long's group in Sydney) of a functional component[4], exploring the concept in an immune-competent model, the BRAF/PTEN model, with early results suggesting that BRAF inhibitor therapy has its antitumour effect in part because of this immune effect[5]: depleting CD8 T cells ameliorates the antitumour effect of BRAF inhibitor therapy. Dr. Flaherty then discussed the first evidence from a molecularly targeted therapy, a MEK inhibitor following immunotherapy, which raised the possibility that there might be a leftover effect of immunotherapy that could be potentiated by using molecularly targeted therapy to counter other measures of immune evasion. The NRAS mutant population in this study had better PFS if they had received prior immune checkpoint antibody therapy before MEK inhibition. He pointed out that these trials were conducted before PD1 antibodies became first-line therapy, and that it is now more difficult to study the possible potentiating effect of upfront immunotherapy.

CROSS-RESISTANCE?

Dr. Flaherty described a best-case scenario in which 5-year surviving patients of molecularly targeted immunotherapy were a nonoverlapping subpopulation of patients. In this case, the challenge would simply be to figure out how to identify this group prospectively. However, evidence to date appears to indicate that immune markers present in this group may also be present in patients who develop cross-resistance.[6] He raises the possibility "that we have a whole subpopulation of patients who are not being reached by the available treatments; even if we put them together, we wouldn't solve that problem."

CONCOMITANT TARGETED/IMMUNE THERAPY

"Is there really a basis for being interested in putting the treatments together?" Dr. Flaherty asked. Early outcomes in the multiple ongoing studies combining BRAF/MEK and PD1 or PD-L1 antibodies[7] look much like outcomes of BRAF/MEK therapy on its own: nearly all patients have some degree of tumour regression, and a significant fraction have complete or near-complete responses. The added benefit appears to come with time. The combinations may not be improving outcomes in patients who wouldn't benefit from either PD1 antibody-based therapy or BRAF/MEK targeted therapy, but "it is possible that those patients who would get some short- and intermediate-term benefit can have that more reliably turned into longer-term benefit." Dr. Flaherty stressed that more time and more patients in trials are needed to truly understand if this is a real effect. MEK inhibitors have caused some concern,[8] however combination studies suggest that MEK inhibition, via direct effects on T cells and the tumour microenvironment, may help to unlock the full antitumour potential of PD-L1 inhibition.[9] It may be consolidating long-term benefit within the responding patient population, and outcomes observed both in the BRAF mutant and wild-type settings.

Dr. Flaherty concluded by pointing out that, in the treatment of BRAF mutant melanoma, there is compelling evidence that both targeted therapy and immune therapy are effective in the first line, and that they are less efficacious in patients with high disease burden. As evidence continues to build support for the idea that targeted therapies have an immunologic effect, the challenge will be to find out how to identify patients who stand to benefit from both targeted and immune therapies, and how to time their administration.

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CTLA-4 AND PD1 INHIBITOR COMBINATIONS: EFFICACY AND TOXICITY CONSIDERATIONS

Michael Postow, MD, Melanoma and Immunotherapeutics Service, Memorial Sloan Kettering Cancer Center, presented the comparative efficacy and toxicity of these agents, used alone and in combination. He cautioned that many of the combinations discussed are not yet approved for use in Canada.

In patients with BRAF wild-type melanoma considered for immunotherapy, first-line treatment would usually involve a PD1 agent, either alone or in combination with a CTLA-4 inhibitor. Dr. Postow presented available evidence on the additional efficacy brought by combining these agents, as well as considerations of side effects.

COMPARATIVE EFFICACY

In the Checkmate 067 study[1] comparing nivolumab (Nivo) + ipilimumab (Ipi) vs Nivo alone vs Ipi alone, 18-month results showed higher response rates for the combination (ORR=57.6%, 95% CI: 52.0, 63.2 for combo vs ORR=43.7%, 95% CI: 38.1, 49.3 for Nivo; ORR=19.0%, 95% CI: 14.9, 23.8 for Ipi). Duration of response was lowest for Ipi (14.4 months, 95% CI: 8.3, NR) vs 22.3 months for Nivo (95% CI: 20.7, NR) and was not reached for Nivo + Ipi (95% CI: 20.5, NR). Dr. Postow expressed eagerness to see overall survival (OS) results at AACR in April 2017. He stresses the difficulty in identifying populations who will benefit from the combination. While the phase 1 study of Ipi + Nivo in pretreated patients showed long-term OS of 68% at 3 years, it is more difficult to see differences in OS in phase 3 studies, because patients cross over. In the Checkmate 069 phase 2 study[2] where crossover from ipilimumab monotherapy to nivolumab monotherapy was allowed, 2-year survival of 54% was seen in the Ipi group vs 64% in Ipi + Nivo, and more grade 3–4 side effects when agents were combined (Nivo 19.8%, Ipi 27%, and Nivo + Ipi 56.5%).[1]

TOXICITIES

Dr. Postow pointed out that many of the toxicities with the combination resolved relatively early, with median resolution at about 5 weeks.[3] Looking at the number of doses of Nivo patients received, he finds that half of patients had 4 doses or less. This means that half of the 51% who had 2-year PFS received less than 4 doses of combination therapy and did well without further treatment.[2] "That needs to be taken into account in our toxicity assessments," stressed Dr. Postow, "and we need to look beyond just rates of grade 3–4 toxicity." He suggests that it might be better to have a short term of toxicity that lasts just a month or 2, rather than lower-grade toxicities that last a longer time. He also proposes considering how much immunotherapy is required to produce an effect: "We have seen some amazingly quick and thorough responses to a single dose of immune therapy." His team is proposing a phase 2 study to test adaptively dosed combination immunotherapy. All patients would receive 2 doses of Ipi (3 mg/kg) + Nivo (1 mg/kg). At a CAT scan at 6 weeks, patients who showed clinical benefit (no new lesions, and/or shrinkage of existing lesions) would then receive maintenance Nivo, while patients with stable or progressive disease would receive another 2 doses of Ipi + Nivo.

Long et al's study presented at ASCO 2016[4] showed that a lower dose (1 mg/kg) of Ipi, in combination with pembrolizumab (Keynote 029) brought an overall response rate (ORR) of 57% and reduced the rate of grade 3–4 toxicities. An ongoing phase 3b/4 study of 340 patients will compare Ipi 3mg/kg + Nivo 1 mg/kg against Ipi 1 mg/kg + Nivo 3 mg/kg, with a primary endpoint being the rate of grade 3–5 adverse events.

Dr. Postow also suggested looking more deeply into toxicities, pointing out that in the phase 2 study, many toxicities were detected by lab values, not symptoms, and that longer-term effects associated with these changes in lab values are not well understood.

He presented experience of adverse effects in clinical practice at his institution showing that, of 64 patients on the combination, 72% required 1 or more doses of steroids; 22% required infliximab for steroid-refractory diarrhea; and 3% required mycophenolate for steroid-refractory transaminitis.[5]

Translating this evidence into patient advice, Dr. Postow considered that patients can be informed that combination therapy brings a 3/4 chance of their disease being stabilized or improving, along with a 3/4 chance they will need steroids.

FUTURE DIRECTIONS

Combination immunotherapy is more effective than single agents in the short run, but OS data are still needed. Side effects are higher, but need to be considered in context of shorter treatment duration. Less Ipi reduces toxicity, but more data are needed to be sure it doesn't compromise efficacy in the long run. Finally, there is the significant challenge of comparing this combination to other combinations without randomized controlled trials.

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PREDICTIVE/PROGNOSTIC IMMUNOLOGICAL FACTORS CONFIRMED AND POTENTIAL

Antoni Ribas, MD, PhD, Professor of Medicine at UCLA, Director of the Tumour Immunology Program at the Jonsson Comprehensive Cancer Center, and of the Parker Institute for Cancer Immunotherapy, described recent advances in understanding the biological processes that lead to response to immunotherapy.

T CELLS AND TUMOURS

PD-1 blockade induces response by inhibiting adaptive immune resistance. Dr. Ribas displayed slides of tumours that were PD-L1 negative (more cancer cells) and positive (fewer or no cancer cells) to show that CD8 cells are found only when it is positive. The negative tumour has no T cells in the middle, and the CD8s are seen only outside. Wherever the CD8s are, PD-L1 is expressed. When PD-L1 therapy is effective, T cells are attacking the tumour because they have the right receptor; it will not work if there is no T-cell receptor, and therefore no antigen. Activity depends on a T cell that has the ability to recognize a mutation in the tumour, that tries to attack the tumour and makes interferon gamma. However, the tumour protects itself by expressing PD-L1, which tells the T cells not to attack. When a PD-L1 antibody is provided, the T cells infiltrate and attack the cancer.[1-3] This is what is called adaptive immune resistance, where the tumour tries to turn off the immune system, not throughout the body, but specifically the T cells that attacked the tumour.[4]

Biopsies may display CD8, PD1 and PD-L1, or an absence of CD8 and PD1, but still have PD-L1. Dr. Ribas highlights that if there are T cells that trigger PD-L1 expression, the patient is prepared to respond to immunotherapy. If not, even if there is PD1 expression, response cannot occur. "What kills the cancer is the T cells, not the antibody," he stresses. The majority of patients in a study of PD1 resistance[5] had at least half the pre-existing number of T cells ready to attack; the others have very few T cells and these may be patients that experience pseudo-progression, which Dr. Ribas now believes to be indicative of delayed progression. "We've biopsied some," he says, "and found that in patients who had very few T cells at the beginning, it took longer for that T cell to build up, and they were unlikely to respond." Transcription analysis reveals how these tumours differ.

THE INTERFERON GAMMA RECEPTOR PATHWAY

The IPRES, or innate anti-PD-1 resistance signature, is not a perfect predictor of response, indicating that there may be something at work beyond just the interaction between the T cells and the tumour. It may be in the makeup of the tumour itself, and the interferon (IFN) gamma receptor pathway would seem to be of particular importance. Dr. Ribas referred to 2 recent papers from his lab looking at innate and acquired resistance on PD1, and on alteration in the interferon receptor pathway that prevents cells from signalling. That happens within the tumour[6] and emphasizes the importance of knowing the exact steps involved in the circuit between interferon gamma production and tumour expression of PD-L1. Interferon gamma has its own receptor, IFNGR, with 2 chains, and it enters through JAK 1 and 2, and the phosphorylates STAT transcription factors, before going into the nucleus and binding to promoters, among them interferon response factor 1 (IRF1). IRF1 bonds to PD-L1, leading to the turning on of PD-L1. If interferon does not, through this circuit, lead to expression of PD-L1, there will be no response.

If there is a mutation somewhere that prevents adaptive expression of PD-L1, Dr. Ribas considers it will be useless to give a PD1 blocking antibody because PD1 is not what is limiting response. Dr. Daniel Shin, a clinical fellow in Dr. Ribas' lab, screened 58 human melanoma cell lines established from patients and looked for the baseline PD-L1 expression. Five were found to have PD-L1 expression over the background level and would be considered negative. But when given interferon gamma, they went from low to high levels and most responded. In the great majority of cases, the circuit was working. When levels were low and stayed low on interferon gamma, they found that JAK1 had a mutation in both alleles that led to truncation of the protein that prevented the pathway between interferon to PD-L1 from working.[7] "If we add back the wild type (WT) gene," says Dr. Ribas, there is an increase in PD-L1 upon exposure to interferon alpha, beta, and gamma."

They sequenced 23 patients treated with PD1, with and without response, and found overlap between the 2 groups in terms of mutational load. They then looked at other mutations in the interferon gamma receptor pathway responsible for the cell line not responding. Mutations that crippled the protein and were deleterious in both alleles were found in only 1 patient. "It was a JAK1 loss of function homozygous mutation in a patient who was not a responder and had the highest mutational load," says Dr. Ribas, "which tells us that it doesn't matter how many mutations that tumour had, the T cells that tried to attack it couldn't lead to PD-L1 expression. And then there's no point in giving a PD1 blocking antibody."

As Dr. Ribas states, these observations also improve understanding of what happens with patients who respond but then progress. "The good news is that the majority of patients who respond to this therapy respond long-term.... but a subset lose their response over time. My best estimate is one third of patients." In the phase 1 trial of pembrolizumab, 204 of 650 participants had objective responses. At around 2 years, 30% of them had lost the response. The same pattern occurred in the phase 3 trial of pembrolizumab and ipilimumab.[8-9] Ribas' group then compared patients who lost response in phase 1 to 3 trials of pembrolizumab. Only a few had suitable baseline and relapse biopsies, but comparing sequencing before and after showed that tumours at baseline and progression (even 2 years later) were over 90% similar: the same tumour experiences relapse, not a different cell or tumour. They focused on new mutations that were homozygous and found only one likely to be important, and that was in JAK1 and JAK2,[10] where they

crippled the protein. "What turns out to be the most important," says Dr. Ribas, "is why we use interferon alpha in the adjuvant setting: it gives growth a rest in cells. Interferon interferes with the growth of barely infected cells, that's why it was called interferon in the first place." A wild type cell exposed to interferon goes into growth arrest after 2 or 3 days of exposure.

"Knowing all of this now," says Dr. Ribas, "we're starting to understand why tumours can respond or not respond. At baseline, the tumour wants to signal through this pathway to turn off the T cell that's attacking it. But as we block PD1 continuously, if there are residual cells left, they realize that all the other effects of interferon are no good any more; it doesn't want to signal any more, so it blocks this and then it can lead to acquired resistance because now this wants to get rid of the beneficial anti-tumour effects interferons can have."

"Immunotherapy is no longer magic," Dr. Ribas concludes, "but is turning into a real science, and the concepts described help to understand the biological processes that lead to response or no response."

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