ESMO Immuno Oncology Congress 2017

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Summary

The ESMO Immuno Oncology Congress 2017 was dedicated exclusively to the rapidly evolving area of the development and use of immunotherapies against cancer. The main programme themes covered areas such as combining immune checkpoint inhibitors with chemotherapy or radiotherapy and beyond, patient selection and predictive biomarkers, management of side effects, resistance to therapy, imaging and nuclear medicine and design of immuno-oncology trials, what's new in immunotherapy for specific cancer types, technology developments, and future approaches like adoptive T cell therapy, personalised vaccines, cancer neoantigens, use of bacteria for immunotherapy. A brief overview of a sample of the abstracts presented at the ESMO Immuno Oncology Congress 2017 follows.

Introduction

The Immuno Oncology Congress 2017 organised by the European Society for Medical Oncology (ESMO) convened from 7 to 10 December 2017 in Geneva, Switzerland. This congress was dedicated exclusively to the rapidly evolving area of the development and use of immunotherapies against cancer. The main programme themes covered areas such as combining immune checkpoint inhibitors with chemotherapy or radiotherapy and beyond, patient selection and predictive biomarkers, management of side effects, resistance to therapy, imaging and nuclear medicine and design of immuno-oncology trials, what's new in immunotherapy for specific cancer types, technology developments, and future approaches like adoptive T cell therapy, personalised vaccines, cancer neoantigens, use of bacteria for immunotherapy.

Immunotherapy, which restores the patient's immune system and the ability to attack tumour cells, has altered the landscape of cancer treatment. The ESMO Immuno Oncology Congress provided a dedicated platform to inform healthcare professionals of the most recent and standard or care altering developments in the field.

The congress attracted 973 participants, which represented a 48.8% rise over the 2016 meeting. The majority (86.3%) of attendees were delegates, with 86 (8.8%) faculty members, 34 (3.5%) industry exhibitors, and 13 (1.3%) members of the press also taking part. While 8 of the 10 most represented countries were Western European, the second highest proportion (12.2%) of delegates travelled from the United States and the 10th highest percentage (2.2%) came from Iran. All global regions were represented, including Europe, North America, Asia, Central and South America, Africa, and Australia and the Pacific.

During registration, 703 delegates agreed to participate in a demographics survey. Although all ages were represented, the average delegate age was 43 years. Forty-two percent of delegates were female and the remainder were male. Most (44.5%) of the delegates were clinicians, followed by basic scientists (25.6%). The participants who benefited from a varied programme included medical oncologists (21.8%) and basic researchers (11.8%). Other primary career activities cited included immunologists, clinical researchers, radiation therapists, as well as other professionals.

The sessions provided examples of the application of immunotherapy to different tumour types, which were in tune with the interests of the congress participants. Approximately 51% of the survey respondents cited immunotherapy and tumour immunology as their primary topics of interest, with about 45% expressing an interest in tumour biology, clinical research, and anticancer agents. Translational research and biological therapy were an important focus for approximately 35% of delegates, and about 25% listed basic science, molecular pathology, and personalised cancer medicine as their main areas of interest. Other topics the attendees expressed interest in were tumour response evaluation, cancer in young adults, imaging, and numerous other topics.

These topics were also reflected in the 148 abstracts that were submitted, which was a 74.1% increase over the previous congress. India topped the list of the 5 countries submitting the most abstracts, with the US again occupying second place, followed the United Kingdom, Denmark, and Germany. Of the 97 accepted abstracts, 15.5% were selected for presentation in an oral session with the rest being presented as posters. The oral sessions covered new

therapeutic agents, as well as novel combinations of already approved drugs, patient selection and predictive biomarkers, including cancer neoantigens, and future approaches such as personalised vaccines. One robust area of discussion was why only a limited number of patients currently respond to checkpoint inhibitors.

A brief overview of a sample of the abstracts presented at the ESMO Immuno Oncology Congress 2017 follows.

Frontline Atezolizumab Added to Chemotherapy plus Bevacizumab Extends PFS In Non-Squamous NSCLC

Martin Reck, chief oncology physician, Department of Thoracic Oncology, Lung Clinic Grosshansdorf in Grosshansdorf, Germany presented interim findings from the phase III IMpower150 trial of first-line atezolizumab in combination with carboplatin and paclitaxel with and without bevacizumab. IMpower150 randomised 1202 patients with stage IV non-squamous non-small cell lung cancer (NSCLC) 1:1:1 to receive atezolizumab plus carboplatin and paclitaxel (arm A), atezolizumab with bevacizumab plus carboplatin and paclitaxel (arm B), or bevacizumab plus carboplatin and paclitaxel (arm C). Atezolizumab was administered at 1200 mg intravenously every 3 weeks and bevacizumab was given at 15 mg/kg. In each arm, carboplatin and paclitaxel were given on day 1 of each cycle for 4 to 6 cycles. Maintenance therapy was administered in arm A with atezolizumab alone, in arm B patients received bevacizumab plus atezolizumab, while patients in arm C received maintenance therapy consisting of sole bevacizumab. The median age of the patients was 63 years and 60% were previous smokers. The majority of patients were male and the ECOG performance status was 0 for 39% of patients in arm B and for 43% in arm C. Patients with known *EGFR* or *ALK* alterations were excluded from the study. The minimum follow-up for the analysis was 9.5 months. For the interim analysis, the study was only designed to compare arms B and C.

Treatment with combined atezolizumab plus bevacizumab, carboplatin, and paclitaxel delayed progression or death by 38% compared with bevacizumab and chemotherapy alone in patients with advanced non-squamous NSCLC. The objective response rate (ORR) in the atezolizumab arm B was 64% compared with 48% in the bevacizumab/chemotherapy arm C. Patients receiving the atezolizumab regimen demonstrated a median progression-free survival (PFS; primary endpoint) of 8.3 months compared to 6.8 months with bevacizumab and chemotherapy alone (HR 0.62; 95% confidence interval [CI] 0.52-0.74; $p < 0.0001$). The 12-month PFS rate was 37% in the arm B atezolizumab-containing regimen and 18% with arm C bevacizumab/chemotherapy.

After a minimum follow-up of 9.5 months, there was a 22.5% reduction in the risk of death with the atezolizumab combination compared with bevacizumab and chemotherapy alone; median overall survival (OS) was 14.4 (95% CI 12.8, 17.1) versus 19.2 months (95% CI 16.8, 26.1) in favour of the atezolizumab arm B (hazard ratio [HR] 0.775; 95% CI 0.619, 0.970; $p = 0.0262$). Further OS analysis is planned for the first half of 2018.

Patients were also tested for a tumour T-effector gene expression signature and the data were analysed according to programmed cell death-ligand 1 (PD-L1) expression. PD-L1 expression on immune and tumour cells did not appear to impact efficacy, as patients testing negative for the marker showed an improvement in PFS with atezolizumab (HR 0.77; 95% CI 0.61, 0.99). However, there was a 50% reduction in the risk of progression or death with atezolizumab in patients testing positive for PD-L1 on immune and tumour cells (IHC1/2/3; HR 0.50; 95% CI 0.39, 0.64).

In the T-effector signature wild-type population, the addition of atezolizumab reduced the risk of progression or death by 49%. The median PFS was 11.3 months with the atezolizumab combination versus 6.8 months with bevacizumab plus chemotherapy alone (HR 0.51; 95%

CI 0.38, 0.68; p < 0.0001). The ORR in this group was 69% with atezolizumab compared with 54% in arm C patients on bevacizumab/chemotherapy. The 12-month PFS rate was 46% with the atezolizumab combination compared to 18% with bevacizumab/chemotherapy.

Each of the agents showed similar toxicity profiles as observed in previous trials. Serious treatment-related adverse events were reported in 25.4% of patients treated with the atezolizumab regimen compared with 19.3% of patients treated with bevacizumab/chemotherapy.

Professor Reck also presented preliminary findings for the comparison of arm A comprised of atezolizumab plus chemotherapy without bevacizumab versus the control arm C of bevacizumab plus chemotherapy. In this analysis, no PFS benefit was seen when atezolizumab/chemotherapy was administered without bevacizumab, HR 0.936; 95% CI 0.787, 1.112. The ORR was also similar between the two groups (49% versus 48%). The OS data for this comparison were immature.

Atezolizumab is currently approved as a treatment for patients with metastatic NSCLC following progression on a platinum-containing regimen. For lung cancer, bevacizumab is approved for patients with non-squamous NSCLC in combination with carboplatin and paclitaxel. Several studies are assessing atezolizumab as a treatment for patients with lung cancer as part of various combinations or as monotherapy. The PD-L1 inhibitor is being looked at with nab-paclitaxel and in combinations with pemetrexed and other chemotherapy agents. The combination of atezolizumab and bevacizumab is being assessed in several solid tumours, with promising findings reported in renal cell carcinoma. NCT02366143. Reck *et al.* Abstract LBA1_PR

Practice point and future research opportunities

The rationale supporting the combinations explored in the trial include that bevacizumab may enhance the ability of atezolizumab, an inhibitor of PD-L1, to restore anti-cancer immunity by inhibiting vascular endothelial growth factor (VEGF)-related immunosuppression, while the chemotherapy of carboplatin plus paclitaxel may induce immune responses.

This trial demonstrated a significant and clinically relevant improvement in PFS favouring the addition of atezolizumab to bevacizumab and chemotherapy. The findings show that there is a way to improve the efficacy of platinum-based chemotherapy in patients with advanced non-squamous NSCLC. There were no new safety signals or toxicity issues with this combination so it appears to be a feasible approach for this group of patients.

IMpower150 is the first phase III randomised trial to formally evaluate the combination of immunotherapy and chemotherapy versus chemotherapy frontline. Importantly, the combination of chemotherapy and immunotherapy was beneficial regardless of PD-L1 expression or a T-effector gene signature. It was also beneficial in patients with alterations in EGFR and ALK, who usually do not do well with immunotherapy. This trial shows that by combining chemotherapy and immunotherapy, the need for patient selection according to a particular biomarker is deleted. This strategy has the potential to benefit large numbers of patients with advanced NSCLC without the practical difficulties of biomarker testing. These exciting results pave the way for a new standard of care in advanced non-squamous NSCLC.

Pegylated Human IL-10 (AM0010) in Combinationwith Anti-PD-1 Blockade Demonstrates Efficacy in Patients with Mrcc

Aung Naing of the department of Oncology, MD Anderson Cancer Centre in Houston, USA, described the novel AM0010 as PEGylated human IL-10**,** which is anti-inflammatory and stimulates the cytotoxicity and proliferation of CD8+ T cells at higher concentrations. Previously, 4 of 16 heavily pre-treated patients with poor- or intermediate-risk metastatic renal cell carcinoma (mRCC) achieved partial response (PR) with sole AM0010 treatment. Since both IL-10 receptors and PD-1 are expressed on activated and exhausted CD8 T cells, the investigators evaluated the combination of AM0010 plus an anti-PD-1 antibody in patients with RCC in this phase Ib trial.

The study recruited 38 patients with mRCC from 20 February, 2015 to 18 November, 2016; of these, all patients received AM0010 at 10 or 20 ug/kg daily subcutaneously and 29 patients also were treated with intravenous (i.v.) nivolumab at 3mg/kg every 2 weeks and 9 patients received i.v. pembrolizumab at 2mg/kg every 3 weeks. Three patients had favourable risk, 30 had intermediate or poor-risk by International Metastatic Renal Cell Carcinoma Database Consortium (IMDC), and 5 patients did not have available data. The patients had received a median of 1 prior therapy (range 1 to 3), including at least one VEGFR tyrosine kinase inhibitor. Tumour responses were assessed by Immune-Related Response Criteria (irRC). Serum cytokines, blood derived T cells, clonal identity of peripheral T cells, and tumour DNA sequence and mRNA profiling were assayed.

One patient that had received prior AM0010 was included only in the safety analysis, which showed that AM0010 plus nivolumab or pembrolizumab was well tolerated. Treatment-related adverse events (TRAEs) were reversible and transient. Grade 3/4 TRAE included anaemia in 10 patients, thrombocytopenia in 7 and hypertriglyceridemia in 6 patients on AM0010 plus nivolumab or pembrolizumab. Two patients had a reversible cytokine release syndrome with splenomegaly and increased immune mediated red blood cell phagocytosis that was most likely precipitated by T-cell activation, as both patients achieved PRs. No grade 3/4 anaemia or thrombocytopenia occurred in patients treated with the 10 ug/kg AM0010 dose plus either nivolumab or pembrolizumab.

As of 11 August 2017, 34 patients were evaluable for efficacy. Of these, 14 (41%) patients achieved PR and 13 (38%) patients showed stable disease (SD). Eight patients with SD also showed tumour reduction of more than 30% following irRC (in progress). Neither median progression-free survival nor median overall survival has been reached with a median follow-up of 13.5 months (range 0.5 to 29.83 months) for the nivolumab arm.

mRNA analysis done to distinguish patients showing a response from those experiencing progressive disease revealed that responding patients had a higher degree of CD8+ T cell invigoration. Based upon the safety analysis, the investigators determined the recommended phase II (RP2) dose as 10 ug/kg. NCT02009449. Naing *et al.* Abstract 3O

Practice point and future research opportunities

The combination of AM0010 with nivolumab or pembrolizumab was safe in patients with mRCC. The observed efficacy is very promising, which warrants the ongoing further study of AM0010 at the RP2 dose of 10 ug/kg plus nivolumab or pembrolizumab.

Promising Activity Seen with E7046, a PGE2 Receptor EP-4 Inhibitor that Targets Immunosuppressive Myeloid Cells in the Tumour Microenvironment

Lead author David S. Hong of the Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Centre in Houston, USA explained that E7046 is a selective small molecule antagonist of the prostaglandin E_2 receptor-type-4 that prevents the differentiation of monocytic myeloid lineage cells into a pro-tumorigenic phenotype in the tumour microenvironment (TME). Dr Hong and collegues evaluated E7046 monotherapy in a first-in-human phase I study in patients with diverse cancer types.

The study enrolled 30 adult patients aged ≥18 years, with a median age of 58 (range 24 to 78) years. The patients had selected advanced cancers, with the majority (40%) of patients having colorectal cancer (CRC), 20% with pancreatic cancer, and 13% of patients with squamous cell carcinoma of the head and neck (SCCHN). All patients had high levels of myeloid infiltrate and had received 2 to 7 prior lines of therapy. In the dose-escalation phase cohorts, 6 patients per cohort received 125, 250, 500, and 750 mg doses of oral E7046 administered once per day in a 21-day cycle. The primary objectives included safety/tolerability, and determination of the maximum tolerated dose (MTD) and/or recommended phase II dose (RP2D). Secondary objectives included pharmacokinetics (PK) and initial anti-tumour activity and exploratory objectives included pharmacodynamic (PD) assessments on immune cells in the tumour infiltrate and in peripheral blood, as well as, the metabolic response by ^{18}FDG-PET.

No dose limiting toxicities (DLTs) were observed in 30 patients on E7046; the MTD was not reached. The most frequently reported drug-related adverse events (AEs) were diarrhoea by 20% of patients, and 13% of patients each reported decreased appetite, fatigue, and nausea. Grades 3/4 drug AEs occurred in 4 patients and included diarrhoea, anaphylactic reaction, hypersensitivity, hyperuricemia, rash, generalised rash. Drug-related serious AEs of rash, allergic reaction, and hyperuricemia occurred in 4 patients, and one patient each had fever and grade 2 acute renal failure. Three patients discontinued treatment due to AEs that included bowel obstruction, allergic reaction, and abdominal pain. No drug-related deaths occurred.

PK assessment showed that E7046 exposure was dose proportional up to 500 mg with no incremental increase in exposure at 750 mg. E7046 was extensively metabolised, with an elimination half-life of approximately 12 hours. The accumulation of the drug on multiple dosing was approximately 2-fold.

Treatment is ongoing in 2 patients. The preliminary efficacy analysis showed no objective responses; however, 4 patients demonstrated durable stable disease (SD) or clinically SD lasting more than 4 months, and 4 patients showed metabolic responses according to ^{18}FDG-PET. NCT02540291. Hong *et al.* Abstract 4O

Practice point and future research opportunities

Novel E7046 monotherapy was tolerated in heavily pre-treated patients with diverse myeloid-rich tumours. The maximum tolerated dose was not reached and both a metabolic response and stable disease were demonstrated, which warrant further investigation of E7046.

Omaveloxolone Combined with a Checkpoint Inhibitor Shows Clinical Benefit in Patients with Unresectable or Metastatic Melanoma

Findings presented by Sapna Patel, Assistant Professor, Department of Melanoma Medical Oncology, University of Texas MD Anderson Cancer Center in Houston, US indicated that omaveloxolone could inhibit myeloid derived suppressor cells (MDSCs), thereby enhancing the activity of checkpoint inhibitors, such as nivolumab or ipilimumab in metastatic melanoma. Omaveloxolone reduces the production of reactive oxygen and nitrogen species by MDSCs resulting in restored immune surveillance.

The investigators evaluated the safety and efficacy of omaveloxolone in combination with either ipilimumab or nivolumab an open label, multicentre, phase Ib/II trial. Thirty-nine patients were enrolled with unresectable or metastatic melanoma. The patients underwent a screening biopsy and had more than 5% of tumour cells that were positive for inducible nitric oxide synthase (iNOS). Twelve patients were treated with omaveloxolone at doses up to 150 mg plus ipilimumab and the remaining 27 patients received omaveloxolone plus nivolumab, for a median duration of 13 weeks. Both checkpoint inhibitors were administered per standard protocol. Of the 30 patients having evaluable tumour restaging, 7 (23%) patients were naive to checkpoint inhibitors and 23 (77%) had received prior treatment with a checkpoint inhibitor. Serial biopsies were collected at weeks 2 and 13.

The median time to response was 19 weeks following treatment. The overall response rate (ORR) was 27%, which included 2 complete response (CR) and 6 partial responses (PR). The ORR was 57% in checkpoint inhibitor-naive patients, including one CR. In patients who had been refractory to prior checkpoint inhibitors, the ORR was 17% with omaveloxolone plus nivolumab, including one CR. Omaveloxolone was associated with decreases in tumour iNOS, and with decreased expression of both PD-L1 and IDO-1.

The maximum tolerated dose for omaveloxolone was not established since no dose-limiting toxicities occurred. Omaveloxolone was well tolerated in combination with ipilimumab or nivolumab. No serious adverse events (AEs) related to omaveloxolone have been reported to date. The most commonly reported treatment-related AEs included fatigue, nausea, pruritus, transaminase increases, and decreased appetite.

The phase II portion of this trial will investigate omaveloxolone plus nivolumab in patients refractory to prior anti-PD-1/PD-L1 therapies. NCT02259231. Patel *et al.* Abstract 5O_PR

Practice point and future research opportunities

Omaveloxolone has a novel mechanism of action of blocking MDSCs, which are known to suppress the immune response. This study tested a new combination therapy in patients with melanoma and demonstrated encouraging response rates with omaveloxolone plus ipilimumab or nivolumab in patients who were either checkpoint inhibitor naive or resistant. The combination was well tolerated and may address some of the immune escape mechanisms that limit the activity of current checkpoint blockade therapies.

More data are needed before a final call can be made on whether there is a place for this combination in the current treatment portfolio. The next step should be a randomised trial to investigate whether omaveloxolone provides additional benefit when combined with the checkpoint blockade backbone, for example, comparing the efficacy of PD-1 blockade alone versus PD-1 blockade plus omaveloxolone.

A First-In-Human Phase I Clinical Trial of the IVAC MUTANOME, which Targets Individual Mutant Neoantigens, for the Treatment of Melanoma

Mathias Miller, Biopharmaceutical New Technologies Corporation, Mainz, Germany, described the IVAC® MUTANOME as a highly potent personalised neo-antigen-encoding RNA vaccine approach that harnesses the individual patient's mutation profile. He further explained that the genome of cancer cells is inherently instable and promotes multiple genomic alterations and epigenetic changes, leading to a unique molecular profile in each tumour. Recently, a series of independent reports stated that neo-antigen specific T-cell responses are seminal for the clinical efficacy of immune checkpoint inhibitors; however, less than 1% of mutations appear to raise a spontaneously occurring T-cell response, which results in only patients with a high burden of mutations profiting from currently approved therapies.

The IVAC® MUTANOME was designed to overcome this restriction by identifying the individual mutation repertoire by next-generation-sequencing. From this repertoire, 10 potentially immunogenic mutated sequences per patient are selected and incorporated into a poly-epitopic RNA vaccine, the IVAC MUTANOME®, that is tailored to activate and expand the individual patient's T cells in response to the unique mutation signature.

Dr Miller and colleagues conducted this phase I first-in-human trial that began in 2013 in patients with stage III and IV melanoma to study the feasibility, safety, tolerability, immunogenicity and the potential anti-tumour activity of the IVAC® MUTANOME approach. By November 2016, 13 patients with melanoma had evaluable data, which revealed each patient had a strong poly-neo-epitopic immune response against the vaccine antigens. In the overall population, 60% of the 125 selected neo-epitopes elicited a T-cell response.

Analysis of the safety profile following IVAC MUTANOME® treatment showed that the therapy was well tolerated and no severe adverse drug reactions were reported. The investigators observed promising indications of clinical activity. NCT02035956. Miller *et al.* Abstract 6O

Practice point and future research opportunities

This study showed that vaccination with IVAC® MUTANOME was safe in patients with melanoma and patients showed a high response to the antigens in the vaccine. The investigators observed encouraging clinical activity that warrants further clinical development.

Deciphering the Intra-Tumoural T Cell Receptor Repertoire in Patients with NSCLC

Kroopa Joshi of the Cancer Immunology Unit, UCL Cancer Institute, London, UK pointed out that his group had previously demonstrated the importance of the clonality of cancer mutations in predicting overall survival in non-small cell lung cancer (NSCLC) and the response to checkpoint blockade. In the TRACERx study, genomic analysis demonstrated that intratumoural heterogeneity associated with an increased risk of recurrence or death.

The investigators reasoned that the level of mutational burden and genomic heterogeneity could be reflected in the adaptive anti-tumoural immune response in these patients, leading to an evaluation of the T cell receptor (TCR) status in these patients. They compared TCR sequencing data from multi-region tumour specimens with normal lung samples in patients participating in the TRACERx study having either genetically heterogeneous (high ITH) or homogenous (low ITH) NSCLC tumours.

This analysis revealed that the TCR repertoire was distinct in tumour specimens and differed from the TCR in normal lung samples. The majority of CDR3 sequences were unique to either compartment, which suggested to the investigators a repertoire of T cells that was spatially confined to the tumour microenvironment, possibly driven by the presence of tumour antigen. They also observed a lower degree of overlap in the TCR repertoire between matched normal tissue and tumour tissue compared to the TCR repertoire across multi-region tumour specimens.

TCR repertoire heterogeneity was found to reflect neoantigen heterogeneity; there was a higher degree of TCR repertoire overlap, as assessed by the Jaccard index of the 100 most abundant TCRs, in patients with low ITH tumours compared to high ITH tumours. A correlation between TCR clonality and neoantigen load was also found in patients with low ITH tumours. The authors postulated that TCR clones present across multiple regions of the tumour may expand in response to common neoantigens found in cancer cells, and efforts are currently underway to determine the antigen specificity of these TCRs. Joshi *et al.* Abstract 7O

Practice point and future research opportunities

Taken together, these findings demonstrate a heterogeneous spatial distribution of tumour infiltrating lymphocytes among patients with NSCLC. The observations described are indicative of a dynamic intra-tumoural T cell response that may be accounted for by differences in the genetic heterogeneity in the mutational and predicted neoantigen burden observed in NSCLC.

TGF-B Signalling Attenuates Tumour Response to PD-L1 Checkpoint Blockade by Contributing to Retention of T Cells in the Peritumoural Stroma

Sanjeev Mariathasan, Senior Scientist, Oncology Biomarker Department at Genentech in South San Francisco, US and colleagues investigated the biology behind primary immune escape and responsiveness to anti–programmed death inhibitor ligand 1 (PD-L1) using tumour samples of patients with bladder cancer who were taking part in the phase II IMvigor210 trial of the anti–PD-L1, atezolizumab. IMvigor 210 met its primary endpoint of objective response in all patients and also in subgroups of patients having varying PD-L1 expression on tumor-infiltrating immune cells detected by immunohistochemistry (IHC) using SP142. Atezolizumab was approved in the US for the treatment of metastatic urothelial cancer based on findings from IMvigor210.

Dr. Mariathasan discussed the findings of the exploratory analyses in the biomarker component of the IMvigor210 trial, which was done in 300 patients with evaluable pre-treatment tumours. The investigators performed CD8 IHC analysis to define immune deserts where no T cells could be detected near the tumour, plus excluded and inflamed subtypes, and also did whole-transcriptome RNA sequencing to identify pathways associated with response. They also performed targeted mutational profiling (FoundationOne) to estimate the tumour mutation burden (TMB) and whole-exome sequencing to predict putative neoantigens. The inhibition of tumour growth was evaluated in EMT6-grafted BALB/c mice following treatment with anti– transforming growth factor β (TGF-β) and/or anti–PD-L1 antibodies.

Using these methods, the investigators elucidated the drivers of efficacy and of primary resistance to the anti-PD-L1 checkpoint inhibition. The anti-PD-L1 response was associated with CD8+ T-effector gene expression and, to an even greater extent, TMB. However, tumours having high expression of the cytokine TGF-β tended to be unresponsive to immunotherapy; a signature of TGF-β signalling in fibroblasts was identified that associated with resistence to atezolizumab, which was observed particularly in patients with CD8+ T cells that were excluded from the tumour parenchyma and were found instead in the fibroblast- and collagen-rich peritumoural stroma.

The mouse model recapitulated this immune excluded phenotype and showed that therapeutic administration of a TGF-β blocking antibody together with anti–PD-L1 reduced TGF-β signalling in stromal cells, facilitated T cell penetration into the centre of the tumour, and provoked vigorous anti-tumour immunity together with tumour regression.

The authors concluded that 3 types of tumour microenvironment contributed to immunotherapy resistance: An immune desert, which occurs in approximately 25% of bladder cancer, T cell excluded tumours where T cells appear in the stromal microenvironment rather than the tumour and occurs in 50% of bladder cancer, and T cell inflamed tumours where T cells have penetrated into the tumour, which is seen in 25% of bladder cancer. Furthermore, the authors theorised that T cell excluded tumours may be augmented by a factor secreted by the tumour that builds a collagen-rich barrier around the tumours. High expression of TGF-β and TGF-β-induced stromal genes in excluded tumours were associated with non-

responders, suggesting that TGF gene products may function to prevent T cell tumour penetration. NCT02108652. Mariathasan *et al.* Abstract 8O_PR

Practice point and future research opportunities

This study provided valuable evidence of the determinants of response to cancer immunotherapy, which is crucial to improve the therapeutic benefit to more patients. The investigators found that T-cell immunity and TMB associated with response to atezolizumab in metastatic urothelial cancer, whereas TGF-β signalling in the stroma was a negative indicator of response, especially in immune-excluded tumours. These data contribute to the explanation of why only in a subset of patients respond to PD-L1 inhibitors. TGF-β is a soluble protein that is known to suppress immune responses through a variety of mechanisms and this analysis of gene expression in clinical trial biopsies indicated that the gene signature of active TGF-β is in action in a fraction of bladder carcinoma patients who do not respond to PD-L1 blockade, and also seems to correlate with the inability of T killer lymphocytes to penetrate the tumour.

These findings are important to lung, pancreatic and colorectal cancer where the T cell excluded phenotype is also common. These findings open the door for improving therapeutic responses to PD-1/PD-L1 blockade by simultaneously targeting the TGF-β pathway. In bladder cancer, a clinical trial that selects patients with a T cell excluded tumour phenotype, based on a TGF-β signature, for treatment with an anti-TGF-β agent plus PD-1 or PD-L1 blockade may be warranted, which could then be studied in other cancers with the same phenotype.

AM0010 (Pegylated Human IL-10) Combined with an Anti-PD-1 Agent Shows Clinical Benefit in Advanced NSCLC

Deborah Wong, Haematology/Oncology, of the UCLA - School of Medicine in Los Angeles, USA reported results from a trial of AM0010 in patients with non-small cell lung cancer (NSCLC). AM0010 is PEGylated IL-10, which has been reported to stimulate the cytotoxicity, survival, and proliferation of intratumoural, antigen activated CD8+ T cells both in pre-clinical cancer models and in patients. Since AM0010 activates antigen stimulated CD8 T cells, which may be inhibited by PD-1, there is a strong rationale behind combining AM0010 with PD-1 inhibitor, which may remove this block.

The study enrolled 34 patients with NSCLC who had received a median of two prior therapies. AM0010 at 10 to 20 ug/kg subcutaneously was administered each day to all patients; 5 patients also received intravenous (i.v.) pembrolizumab at 2 mg/kg every 3 weeks and 29 patients received nivolumab i.v. at 3 mg/kg every 2 weeks. Tumour responses were assessed by immune-related response criteria (irRC). Immune responses were measured by analysis of serum cytokines (Luminex), activation of blood derived CD8 T cells (FACS), and peripheral T cell clonality (TCR sequencing). Tumour tissue was analysed for tumour mutational burden by whole exome sequencing (WES) and mRNA expression of immunotherapy markers was assessed using Nanostring.

After a median follow-up of 14.9 (range 5.6 to 30.3) 27 patients were evaluable for response. AM0010 in combination with either nivolumab or pembrolizumab provided a partial response (PR) in 10 (36.4%) patients and 12 (44.4%) patients achieved stable disease (SD). The median overall survival (OS) was 19.7 months, and the 1-year survival rate was 71%.

Twenty patients had undergone PD-L1 expression analysis. Patients having tumours showing high levels of PD-L1 expression (defined as >50%) achieved an objective response rate (ORR) of 80%, consisting of 4 PR; additionally, one (20%) patient had SD and also showed 47% reduction of tumour burden. Patients with non-PD-L1 expressing tumours (<1%) achieved an ORR of 25%, which consisted of 3 PR, and another 7 (64%) of patients showed SD. Patients having intermediate PD-1 expression (1 to 49%) achieved an ORR of 50% consisting of 2 PR with one (25%) patient with SD.

AM0010 plus anti-PD-1 therapy was well tolerated. All treatment–related adverse events (TRAEs) were reversible and transient. Grade 3/4 TRAEs included thrombocytopenia in 8 patients, anaemia in 7, fatigue in 6, rash in 4, pyrexia in 2, hypertriglyceridemia 3 patients, and one patient had pneumonitis. NCT02009449. Wong *et al.* Abstract 9PD

Practice point and future research opportunities

AM0010 in combination with either pembrolizumab or nivolumab was well tolerated in this study of patients with advanced NSCLC. Efficacy was improved regardless of PD-L1 status and the observed CD8 T cell activation is promising. Efficacy was substantially improved by the combination treatment in patients with high PD-L1 expression to 80%, which compares favourably with previously published studies of pembrolizumab monotherapy showing typical response rates of 44%. These findings encourage the continued study of AM0010 in combination with an anti-PD-1 therapy.

Preoperative SIRT Promotes the Recruitment of Tils into the Tumour Microenvironment in HCC

Lead author Ligia Craciun of the Pathology department, Institut Jules Bordet, Université Libre de Bruxelles in Brussels, Belgium and a team from Belgium investigated the tumour microenvironment following the intra-arterial therapies, transarterial chemoembolization (TACE) and selective internal radiation therapy (SIRT) with ^{90}Yttrium in patients with hepatocellular carcinoma (HCC). Since high levels of tumour-infiltrating lymphocytes (TILs) are associated with a better prognosis in HCC, the researchers investigated the extent to which TACE and SIRT modified the tumour microenvironment by evaluating TILs in patients who underwent partial hepatectomy for HCC. For this analysis, sections for digital image analysis (DIA) were prepared from paraffin blocks and immunohistochemistry for CD3, CD4, CD8, CD20 and Granzyme B was performed. TILs were quantified as the percentage of positive cells per analysed area after necrotic zones were excluded.

TILs from 3 patient cohorts were compared. All patients underwent hepatectomy for HCC; this included 12 patients who underwent preoperative SIRT, 16 with preoperative TACE, and 32 patients underwent hepatectomy but received neither TACE nor SIRT. Patients in the 3 cohorts had similar tumour characteristics, and the median times between SIRT or TACE and partial hepatectomy were 16 compared to 11 weeks, respectively.

This analysis demonstrated that only preoperative SIRT significantly increased CD3+ TILs, including both CD4+ and CD8+ subpopulations. Preoperative TACE did not significantly affect CD3+ TILs, or the CD4+ or CD8+ subpopulations, compared to the surgery only cohort. Significantly increased expression of Granzyme B was also demonstrated in only SIRT patients. Additionally, CD20+ B lymphocyte infiltrates were similar across the 3 groups. Craciun *et al.* Abstract 10PD.

Practice point and future research opportunities

These findings indicate that SIRT, but not TACE or surgery alone, alters the tumour microenvironment by increasing TIL levels and intratumoural cytotoxic activity in resected HCC. These results suggest that local attraction/activation of effector T cells may contribute to the anti-tumour effect of SIRT and support the clinical development of therapeutic approaches that combine SIRT and immunotherapy.

IMA950 Multipeptide Vaccine Adjuvanted with Poly-ICLC in Combination with Standard Therapy Shows Promise in Patients with Newly Diagnosed HLA-A2 Glioblastoma

Valérie Dutoit, of the Oncology department, Geneva University Hospital in Geneva, Switzerland, and colleagues carried out a phase I/II trial to evaluate the safety and immunogenicity of IMA950, a novel multipeptide therapeutic vaccine comprising 9 glioma-associated CD8 peptides and two tumour-associated CD4 peptides that was adjuvanted with Poly-ICLC. The trial enrolled 16 patients with newly diagnosed HLA-A2-positive (HLA-$A2^+$) glioblastoma who were treated with the standard of care, which included surgery and 6-week chemo-radiation therapy plus 6 to 12 adjuvant cycles of temozolomide. One week following the end of chemo-radiation therapy, IMA950 was injected.

The investigators initiated treatment in the first 6 patients by injecting peptides intradermally (i.d.) and Poly-ICLC intramuscularly (i.m.) to evaluate the regimen. After low levels of vaccine-induced CD8 T cell responses were detected, the vaccination protocol was modified to combine the peptides and adjuvant prior to injection, which was given i.m. to 6 patients and subcutaneously (s.c.) to 7 patients, in order to determine the optimal injection route. The primary endpoints of the study were safety and immunogenicity, and secondary endpoints included overall survival (OS) and progression-free survival (PFS) at 6 and 9 months.

After modification of the vaccination protocol, multipeptide responses were detected in 46% of patients compared to 17% of patients with the initial protocol was used. The median OS was 21.2 months.

Analysis of the vaccine-induced T cell responses, revealed that 63% of patients responded to one CD8 peptide and 37% of patients responded to two or more CD8 peptides. The vaccine-induced CD4 T cell responses that were detected in the majority of patients were of Th1 phenotype. The IMA950/Poly-ICLC vaccine was well tolerated, with local inflammation at the injection site reported as the most common adverse event. A few patients experienced cerebral oedema, which was manageable with steroids. The authors are continuing to evaluate the clinical results and determine their association with immunological data to determine the most efficacious route of vaccination for use in further trials. NCT01920191. Dutoit *et al.* Abstract 11PD

Practice point and future research opportunities

This study established a regimen for administering the IMA950/Poly-ICLC vaccine, which was found to be safe and immunogenic in patients with newly diagnosed HLA-$A2^+$ glioblastoma. The study also revealed encouraging preliminary median OS results. The continued efforts of the investigators are warranted and further findings are anticipated.

Blocking the CD47-Sirpα Axis Alone or Combined with Autophagy Depletion may be a Novel Therapeutic Approach in Glioblastoma

X. Zhang of the Department of Microbiological and Biochemical Pharmacy, School of Pharmacy, Fudan University, Shanghai, China reported results of an assessment of the anti-tumour benefit of the CD47-targeted fusion protein, SIRPα-Fc, when used alone to block the CD47-SIRPα axis or in combination with autophagy depletion in glioblastoma.

Autophagy was evaluated by confocal microscopy, transmission electron microscopy, and western blot and the synergistic anti-tumour effect of simultaneous blockade of the CD47-SIRPα axis and autophagy were studied using glioblastoma xenograft tumours. The investigators established syngeneic immunocompetent glioblastoma models to elucidate the role of adaptive immune response in SIRPα-Fc-treated glioblastoma.

The results of these assays showed that SIRPα-Fc demonstrated potent anti-glioblastoma efficacy by increasing macrophage phagocytosis and cytotoxicity of glioblastoma cells. Autophagy and autophagic flux were markedly stimulated by SIRPα-Fc in the glioblastoma cells through inactivation of Akt/mTOR. Blocking this autophagy using pharmacological inhibitors or siRNA resulted in enhanced SIRPα-Fc-induced macrophage phagocytosis and cytotoxicity against glioblastoma cells. Simultaneously blocking the CD47-SIRPα axis with SIRPα-Fc and blocking autophagy in tumour-bearing mice increased macrophage infiltration and tumour cell apoptosis, which elicited enhanced anti-glioblastoma efficacy and prolonged survival in these tumour-bearing mice compared with SIRPα-Fc treatment alone. The anti-glioblastoma efficacy of SIRPα-Fc was observed to also include adaptive immune responses that mainly included $CD8^+$ T cells. Zhang *et al.* Abstract 12PD

Practice point and future research opportunities

Findings from this study indicate that SIRPα-Fc could elicit potent anti-glioblastoma efficacy, which could be enhanced when combined with autophagy inhibition, suggesting a potential therapeutic approach for glioblastoma through disrupting CD47-SIRPα axis alone or combined with autophagy.

Functional T Cell Responses Detected in Patients with Melanoma Resistant to Checkpoint Inhibitors

Rikke Andersen, Centre for Cancer Immune Therapy, Department of Haematology and Department of Oncology, Herlev and Gentofte Hospital, Herlev, Denmark and colleagues evaluated the immune system of melanoma patients progressing on, or following treatment with a PD-1 or anti-CTLA-4 checkpoint inhibitor to determine whether they generated functional tumour-specific immune responses. The investigators isolated tumour-infiltrating lymphocytes (TILs) from metastatic melanoma lesions and expanded the TILs. Tumor-specific immune responses were assessed by co-culture assays of TILs and autologous tumour cells. TILs from 19 metastases were obtained from 19 patients who progressed on or following treatment and were assessed for T cell recognition of autologous tumor cells; all 19 of these patients had received anti-PD-1, and 16 had received anti-CTLA-4 therapy.

These assays showed that functional anti-tumour immune responses could be detected in 15 (79%) patients. Fourteen (74%) patients each had $CD8^+$ and $CD4^+$ TILs that were able to recognise autologous tumours. A large percentage of CD8+ TILs recognised tumour cells, mean 25% (range 1.1% to 84%), which was similar to or higher than the percent reported in cohorts of unselected patient populations with metastatic melanoma in previous studies.

The investigators also conducted a phase I/II clinical trial, wherein TILs were administered in concert with lymphodepleting chemotherapy, pegIFNa2b, and IL-2 to 12 patients with checkpoint inhibitor-resistant melanoma. Two partial responses were observed, of which one is ongoing, out of 12 patients with anti-CTLA-4/anti-PD-1-resistant melanoma who were treated with TILs. The team is conducting additional histology analyses to identify the location of the immune infiltrates. NCT02379195. Anderson *et al.* Abstract 13PD

Practice point and future research opportunities

This study demonstrated that the tumour microenvironment of patients who failed previous treatment with checkpoint inhibitors is heavily infiltrated by tumour-reactive T cells. Furthermore, the phase I/II showed that these patients are able to respond to infusion of unselected autologous TILs. These results represent a novel immune re-activation strategy that warrants further testing in anti-PD-1-/anti-CTLA-4-resistant melanoma.

Anti-CD137 Stimulation Benefits Expansion of Tumour Tils From Sarcoma For ACT Immunotherapy

Morton Nielsen, Centre for Cancer Immune Therapy, Department of Haematology and Department of Oncology, Herlev and Gentofte Hospital, Herlev, Denmark, explained that tumour specific tumour-infiltrating lymphocytes **(**TILs) can be expanded in vitro that have the ability to induce complete and durable tumour regression in some patients with melanoma following adoptive cell transfer **(**ACT).

With colleagues, Dr. Nielsen investigated the feasibility of expanding TILs from sarcomas and performed functional in vitro analyses on them. They used tumour samples obtained from 28 patients having 8 different sarcoma subtypes. TILs were isolated and expanded in growth medium containing IL-2. The effect of adding an agonistic CD137 antibody (Urelumab, BMS) and/or an anti-CD3 antibody (OKT3) to the medium was also explored. Phenotypic and functional analyses were performed by flow cytometry and IFNγ-Elispot, while cytotoxicity analyses were performed using Xcelligence.

Expansion to a minimum of 40 million TILs was possible from 25 of the tumour samples and the mean expansion time was 32 days (range 14 to 61 days). The majority cell type (87.7%; range 36.4 to 99.1%) was CD3+; of these, 66.7 % (range 16.3 to 99.1%) were CD4+, and 21.8% (range 0.1 to 50.6%) were CD8+. This yield was increased with the addition of anti-CD137 and/or OKT3. Adding anti-CD137 directed the phenotype significantly towards an increased percentage of CD8+ TILs and in some cases NK cells and γδ cells.

Reactivity against autologous tumour cells was demonstrated by TILs from 11 of 22 tested tumour samples obtained from 7 of the 8 different sarcoma subtypes, according to IFNγ-Elispot analysis. The investigators verified these findings in an intracellular cytokine release assay using flow cytometry and in cytotoxicity assays. In some cases the fraction of reactive cells was more than 20%. This reactivity against autologous tumour cells could be increased in all 4 tested samples by stimulating the TILs with anti-CD137. In some cases, the observed reactivity was as high as previously only seen in melanoma samples. Nielson *et al.* Abstract 14PD

Practice point and future research opportunities

In this study, TILs were expanded from 90% of tested sarcoma tumour samples that included a population of CD4+ and CD8+ cells, with CD4+ being predominant. Half of the TIL cultures showed in vitro tumour reactivity. Adding anti-CD137 influenced the phenotype, and functional capacity of the expanded TIL. The planed initiation of clinical testing of TIL based ACT in sarcoma patients is warranted.

LAG-3 is Expressed on Activated Tils and Predicts Resistance to Inhibitors of the PD-1 Axis in Advanced NSCLC

Ila Datar, Department of Pathology, Yale University School of Medicine, New Haven, CT, USA, and colleagues measured levels of PD-1, LAG-3, and TIM-3 in non-small cell lung cancer (NSCLC) tumour tissue to assess their functional role in inhibition of the programmed cell death protein-1 (PD-1) axis. The research team performed mass cytometry (CyTOF) on immune cell suspensions extracted from 20 primary human NSCLC tumour samples to map the distribution of PD-1, LAG-3 and TIM-3 and also analysed RNA levels of these markers in TCGA NSCLC datasets. They determined the association of these proteins with CD4/CD8 mRNA and the mutational burden. Using multiplex quantitative immunofluorescence (QIF), they measured the levels of CD3, PD-1, LAG-3 and TIM-3 in 66 pre-treatment tumour samples obtained from NSCLC patients receiving PD-1 axis blockers in 2 independent cohorts: the Yale cohort with 42 patients comprised the Training set, and the Validation set included 24 patients from combined Cleveland Clinic/University of Navarra patients.

These analyses revealed that PD-1 was mostly expressed on T- and natural killer (NK) T cells in primary NSCLC; PD-1 LAG-3 expression was higher in $CD8^+$, $CD4^+CD25^+FOXP3^+$ and NK T cell subsets, but was low or absent in antigen-presenting cells (APCs). TIM-3 was found to be broadly expressed in adaptive and innate immune cells, and showed the highest levels in APCs.

Expression of all 3 markers in T-cells was associated with lymphocyte activation (CD69/HLA-DR), effector function (Granzyme-B) and proliferation (Ki-67).

LAG-3-expressing T-cells associated to a greater degree with early activation and effector function than TILs expressing PD-1 or TIM-3. PD-1 and LAG-3 transcripts evaluated using TCGA strongly correlated with CD8 mRNA, while TIM-3 was associated with CD4 mRNA. Limited association was observed between the markers and tumour mutational burden.

Overall survival was shorter in pre-treatment specimens having elevated LAG-3 from both cohorts of patients treated with PD-1 blockers. This association was not observed with elevated PD-1 or TIM-3 protein levels. Datar *et al.* Abstract 15PD

Practice point and future research opportunities

This study demonstrated that PD-1, LAG-3 and TIM-3 have variable expression and are associated with T-cell activation and effector function in NSCLC. Elevated T-cell LAG-3 in baseline tumour samples may be predictive of primary resistance to inhibitors of the PD-1-axis.

INO-5150 Immunotherapy Shows Promise in Biochemically Relapsed Prostate Cancer

IIdiko Csiki, Clinical Development, Inovio Pharmaceuticals Inc. in Plymouth Meeting, USA, presented findings from an interim data analysis showing that treatment with INO-5150 immunotherapy generated antigen-specific CD8+ killer T cell responses, as measured in peripheral blood from patients with recurrent prostate cancer. INO-5150 is an active immunotherapy that targets both prostate specific antigen (PSA) and prostate specific membrane antigen (PSMA), which are present in the majority of prostate cancer cells. INO-5150 was designed to activate the patient's immune responses and to specifically target prostate tumours expressing PSA and PSMA.

Dr Csiki and colleagues conducted this phase I open-label, multicentre study of the safety, immunogenicity, and efficacy of INO-5150 in patients with recurrent prostate cancer. All patients were post-definitive therapy and showed rising PSA counts after surgery and/or radiotherapy, together with a PSA doubling time (PSADT) greater than 3 months. The patients also had more than 150 ng/dL testosterone levels, were not receiving concomitant androgen deprivation therapy (ADT), and had no evidence of metastases. The trial had 4 treatment arms in which INO-5150 was administered as monotherapy or with INO-9012, a DNA-based IL-12 immune activator. Arm A comprised 16 patients who were treated with 2 mg of INO-5150, arm B patients (n=15) received 8.5 mg INO-5150, arm C patients (n=15) received 2 mg INO-5150 plus 1 mg INO-9012, and in arm D 16 patients received 8.5 mg INO-5150 plus 1 mg INO-9012. INO-5150 was delivered intramuscular in 4 doses followed by electroporation on day 0, weeks 3, 12 and 24.

The immunology results after a 72-week follow-up of 58 evaluable patients demonstrated that INO-5150 treatment stimulated IFN-γ reactivity, as measured by ELISPOT, in 35 (60%) of patients across all 4 combined treatment arms. Antibody titres against PSA and PSMA were observed in 6 (10%) and 5 (8%) patients, respectively. In the overall population, 19 (38%) patients had CD38 and perforin-positive CD8 T cell responses; the highest number of these responses was observed in arm A, where 8 (57%) patients showed CD38 and perforin-positive CD8 T cell responses and also showed high PD-1 expression of 68.6% at week 27.

An evaluation of myeloid derived stem cells (MDSC) showed that the frequency of MDSCs was decreased in patients having a more than 70% PSA decline, as compared to patients with radiographically confirmed disease progression. Moreover, patients with specific CD8+ T cell responses experienced dampening in the rise of PSA and significant increases in PSADT; the dampening of the percent of PSA rise was median 28.0 versus 53.4 ($p = 0.02$) compared with non-reactive patients. The median day 0 PSADT improvement was from 8.2 to 19.0 months at the time of the last observation ($p = 0.002$) across all cohorts combined; again, the highest numerical improvement of 26.6 months was observed in patients receiving INO-5150 as monotherapy in arm A. At median follow-up of 436 days (range 85 to 582 days) 56 of 61 (90%) patients were free from metastasis.

Eleven grade 3 serious adverse events (SAEs) were reported in 6 patients and 82% of patients had grades 1 to 3 AE's that were mostly related to injection site reactions. The investigators are performing additional analyses and prolonged follow-up to further determine

the correlation of immunologic efficacy and clinical benefit. NCT02514213. Csiki *et al.* Abstract 17PD

Practice point and future research opportunities

INO-5150 +/- INO-9012 was safe and well tolerated in patients with recurrent prostate cancer. Immunotherapy with INO-5150 induced cellular immune responses and had an effect on both PSA and PSA doubling time. This study provided encouraging immunologic and clinical data demonstrating that this immunotherapy agent can generate antigen-specific CD8+ killer T cell responses in the blood. Further evaluation of this product in prostate cancer patients should be undertaken.

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Affiliations and Disclosure

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Disclosure

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