



Meeting Report

Highlights from the 2014 American Association for Cancer Research Annual Meeting

The 105th Annual Meeting of the American Association for Cancer Research (AACR) was held at the San Diego Convention Center from April 5 to 9, 2014. The conference theme – ‘Harnessing Breakthroughs – Targeting Cures’ – reflected the accelerating rate at which basic science is being translated to the clinic. Given the size of the meeting (18,500 registrants, 700 invited speakers, over 1000 oral presentations, and 6000 posters) this review is inevitably a snapshot. We have attempted to highlight major themes that spanned tumor types but are likely to be very important to future gynecologic research and treatment. These themes included the increasing excitement associated with immune-mediated therapy, improved understanding of tumor evolution and adaptation to molecularly targeted agents, and a growing appreciation of the importance of combined molecular therapies to enhance efficacy and pre-empt resistance. Within this context, the need for better cellular and animal model systems, and the use of innovative clinical trial designs featured strongly in the development of novel therapeutic strategies.

Immune-mediated therapy

The meeting featured plenaries and major symposia on immunotherapy, with a particular focus on immune checkpoint modulators. Long term durable responses in melanoma, renal and non-small cell lung cancer have been documented in a sizable fraction of patients treated with antagonist antibodies to the immune checkpoint molecules CTLA-4, PD-1 and PD-L1. Current research focus is rapidly shifting to strategies that increase the proportion of responders and targeting other solid and hemopoietic malignancies.

Dr. James Allison (MD Anderson Cancer Center, MDACC) was recognized by the G.H.A. Clowes Memorial Award Lecture for his pioneering studies on immune checkpoint inhibition in cancer. He described the evolution of our understanding of CTLA4 blockade, including the effects on T regulatory and effector cells, and approaches to increasing response rates with co-stimulatory molecules such as ICOS-L. Arlene Sharpe (Harvard Medical School, HMS) recounted fundamental studies that helped map the role of immune checkpoints in protection of normal tissue, such as the vasculature, the control of humoral activity, and the co-option of these processes in cancer. In addition to blocking the inhibitory effects of immune checkpoints, attention is focused at enhancing T cell responses through agonist antibodies that target co-stimulatory molecules such as GITR and OX40, described by Jedd Wolchok (Memorial Sloan Kettering Cancer Center, MSKCC). Steve Hodi (Dana-Farber Cancer Institute, DFCI) reported increased response rates by combining ipilimumab and nivolumab (PD-1) in melanoma,

and the potential inclusion of VEGF inhibition to relieve immunosuppressive effects. Antoni Ribas (University of California, Los Angeles, UCLA) described the use of BRAF inhibitors in mutant melanoma with ipilimumab; however, this combination resulted in excessive liver toxicity leading to early cessation of a phase 1 trial. Current focus is on combining nivolumab with BRAF inhibitors dabrafenib or vemurafenib. BRAF inhibition leads to T cell activation and may facilitate immune responses in addition to blocking growth of mutant melanoma cells. As studies expand to other tumor types, biomarkers for patient selection, informed by studies in melanoma and lung cancer, are likely to be instructive. The presence of tumor infiltrating lymphocytes, especially those in the epithelial fraction of the tumor, indicates immune responses that are poised for reactivation and increased likelihood of response. Accurate measurement of PD-L1 expression with improved antibodies should further facilitate patient selection. The recent success of checkpoint inhibitors is validating the field of tumor immunotherapy more generally. Stephen Rosenberg (National Cancer Institute, NCI) reported very long term responses, often in patients with bulky disease, commencing with systemic IL2 in melanoma, and more recently following *ex vivo* expansion and activation of tumor infiltrating lymphocytes (TILs), and the use of chimeric receptors to improve immune targeting. Dr. Rosenberg described innovative strategies to prime responses using a wide repertoire of neoantigens identified through next generation DNA sequencing.

Tumor evolution and resistance

The clinical success of targeted agents in common tumors with specific activating mutations, such as EGFR in non-small-cell lung carcinoma (NSCLC) and BRAF in melanoma, has been tempered by the almost invariant emergence of resistance. For tumor evolution to a resistance state to occur, three conditions must be satisfied – population diversity, selective pressure, and inheritance of a phenotypic trait. Next generation DNA sequencing studies highlighted a daunting level of intratumoral heterogeneity, facilitating rapid intrapatient evolution under the selective pressure of chemotherapy or targeted therapy. Charles Sawyers (AACR President, MSKCC) argued that the greater potency of newer targeted agents is leading to increasingly diverse mechanisms of resistance. He used the example of metastatic prostate cancer to demonstrate the range of different resistance mechanisms that are emerging to newer classes of anti-androgens such as Enzalutamide (MDV3100). Enzalutamide dramatically improves progression-free survival (HR: 0.186) and results in a marked delay in the median time to chemotherapy; however, a significant number of patients progress on

this therapy. Dr. Sawyers described how single nucleotide changes in the ligand binding domain of AR (F876L) or up-regulation of the glucocorticoid receptor and partial substitution for AR activity could result in resistance to Enzalutamide. He described how the Global Alliance for Genomics and Health (genomicsandhealth.org) aims to share information about resistance mechanisms to targeted agents. Jose Baselga (MSKCC) described the analysis of a breast tumor from a patient who had responded to an alpha-selective PI3K inhibitor but subsequently progressed. Genomic studies identified heterogeneous mechanisms for deactivating PTEN in multiple tumor sites collected at autopsy, providing resistance to the alpha-selective inhibitor. It is possible that PI3K-beta inhibitors may be effective in this setting and could be considered sequentially or in combination with alpha-selective PI3K inhibitors. Erica Jackson (Genentech) described adaptation of lung cancer cells to platinum through up-regulation of neuregulin. These findings mirror those described by Joan Brugge (HMS), involving expression of Bcl2 and multiple RTKs in breast cancer cells in response to the PI3K inhibitor lapatinib. Robert Schegel (Novartis Institute) described how treatment of GIST cells with imatinib leads to profound up-regulation of FGFR1/FGF expression after several days, emphasizing the need to perform longer duration *in vitro* screens to identify resistance mechanisms. James Brenton (Institute for Cancer Research) described extensive intrapatient heterogeneity in high-grade serous ovarian cancer (HGSOC) and the feasibility of cell-free circulating tumor DNA as a method to assess mutational diversity in a range of solid cancers. Peter Campbell (Wellcome Trust Sanger Institute) outlined comprehensive studies in breast cancer evolution revealed by next generation DNA sequencing and explained how the analysis of single nucleotide variants could be used to map mutational mechanisms operative during the phases of tumor expansion and clonal diversification.

Combination studies with molecularly targeted agents

Combination therapies seek to increase the therapeutic index by enhancing tumor kill at a similar or reduced level of toxicity associated with single agents. Pasi Janne (DFCI) described how, historically, the development of erlotinib in EGFR mutant NSCLC had followed a maximum tolerated dose model and left little 'head room' for the introduction of second agents, especially as these often had overlapping toxicity profiles with EGFR inhibitors. He described how intermittent and/or reduced dosing of erlotinib was allowing for co-administration of MEK inhibitors, and emphasized how combination strategies should focus on targeting resistance mechanisms to establish first-line agents, such as EGFR T790M mutations in NSCLC.

Gordon Mills (MDACC) outlined how the PI3 kinase pathway is activated in almost half of all HGSOCs and, with Ursula Matulonis (DFCI), described preclinical studies that identify impressive synergy between the PI3 kinase alpha inhibitor BKM120 and the PARP inhibitor olaparib in serous ovarian cancer. Dr. Matulonis described how these studies rapidly led to a phase I clinical trial, sponsored through the Stand Up to Cancer initiative, the outcome of which will be reported at ASCO in June 2014.

After two decades of disappointing results with cyclin dependent kinase (CDK) inhibitors, the interest in targeting these kinases has been invigorated by the recent exciting results with CDK4/6 inhibitors. Richard Finn (UCLA) presented the final results of the randomized phase II, open-label PALOMA-1 trial of the CDK4/6 inhibitor palbociclib and the aromatase inhibitor letrozole in postmenopausal women with locally advanced or metastatic HER2-/ER+ breast cancer patients. The combination nearly doubled progression-free survival *versus* treatment with letrozole alone, with manageable toxicity. A trend towards improved overall survival was seen in the palbociclib plus letrozole arm but those data need to mature before we know if they are statistically significant. A phase III trial, PALOMA-II, is underway. These findings suggest that other cancers, including gynecologic malignancies, may respond well to palbociclib alone or in combination. Interestingly, neither cyclin

D1 nor p16 status was a useful inclusion criterion in the PALOMA-I trial, indicating that preclinical studies in gynecologic cancers will be needed to identify target groups.

Cyclin E1 (*CCNE1*) participates in control of G1-S transition through interaction with CDK2 and is amplified in approximately 20% of HGSOCs. Amplification and expression appear to be an early event in the pathogenesis of HGSOC. The Cancer Genome Atlas (TCGA) had previously revealed that *CCNE1* amplification and *BRCA1* mutations are mutually exclusive. David Bowtell (Peter MacCallum Cancer Centre) described how mutual exclusivity could be explained by synthetic lethality of *BRCA1* mutation in *CCNE1* amplified tumor cells, and that this could be exploited using proteasome inhibitors such as bortezomib, which interfere with homologous recombination. However, the use of CDK2 inhibitors in patients is proving to be challenging as the majority of currently available agents inhibit other CDKs, including CDK9, resulting in significant dose-limiting toxicities.

Improved models to inform clinical trial design

There is a growing appreciation that the experimental models used to credential pathways and therapeutic targets are suboptimal. Recent publications have reviewed the serious problem of cancer cell lines misidentification [1] and have brought to light the sobering reality that the most commonly used ovarian cancer cell lines are poor models of the disease that they are supposed to reflect, especially human HGSOC [2, 3]. The development of authenticated cell lines and animal models with known clinical histories and well-documented histology is urgently needed. Dr. Andrea Bertotti (Institute for Cancer Research and Treatment) presented a comprehensive overview of patient-derived xenograft (PDX) model development, emphasizing the importance of reducing the interval of time between tumor harvest, culturing of the tissue in preservation media, and implantation into recipient mice, and how successful engraftment can be enhanced by Matrigel, antibiotics, and hormones for hormone-sensitive tumors. He articulated the need for rigorous quality control, including analysis of histology, STR fingerprinting, and genomic annotation to compare the fidelity of the PDX to the original patient sample. The limitations of PDX models are that they lack the ability to assess tumor-host interactions since these animals are immunocompromised. However, there is a growing interest in the development of 'humanized' PDX models where human hematopoietic stem cells are used to reconstitute the murine immune system. Michael Dyer (St Jude's) described the powerful integration of cell line and PDX models into the Pediatric Cancer Study, in which exome and whole genome have been obtained on hundreds of primary tumors. Modeled on human studies, he described phase I-III preclinical trials in mice that helped explain the lack of efficacy of olaparib in Ewing's sarcoma in a previous phase I human trial, and demonstrated impressive synergy of olaparib with temozolomide and irinotecan.

The limitations of PDX models can often be complemented by robust genetically engineered mouse models (GEMMs). Ronny Drapkin (DFCI) described the recent development of novel GEMMs that specifically target the fallopian tube secretory epithelial cells, either using the PAX8 promoter to drive Cre recombinase or the oviduct-specific glycoprotein (OVGP1) promoter to drive SV40 virus T antigen. In both cases, the mice develop precursor lesions in the fallopian tube epithelium that resemble human serous tubal intraepithelial carcinoma (STIC). These murine STIC lesions progress to advanced and widespread peritoneal disease with the morphologic and genomic features of human HGSOC. These models provide additional evidence that STICs are the likely precursors to high-grade serous carcinoma and provide very useful pre-clinical models to evaluate new drug combinations in immunologically intact animals.

Centrality of TP53 mutation in HGSOC

Essentially all HGSOCs have either mis-sense or non-sense mutations in *TP53* and the studies on fallopian tube precursor lesions indicate

that *TP53* mutations are the earliest genetic defects in HGSOC. A major question in the field is whether mutations in p53 are simple loss-of-function effects, dominant-negative, or gain-of-function events. Carol Prives (Columbia University) presented data showing that mutant p53 drives expression of genes involved in the biosynthesis of sterols, specifically the mevalonate (MVA) pathway. The mutant protein accomplishes this by binding to the promoters of mevalonate genes and activating transcription in a manner that is partially dependent on Sterol Regulatory Element-Binding Proteins (SREBPs). She went on to show that wildtype p53 normally suppresses the mevalonate pathway, whereas loss of p53 results in robust MVA pathway expression and mutant p53 results in a further boost of MVA pathway activation. Guillermina Lozano (MDACC) described how mutant p53 is inherently unstable but that tumor specific alterations stabilize the protein and thus confer upon it gain of function activities. Thus, it appears that a majority of *TP53* mutations exhibit gain-of-function activities that may be amenable to therapeutic intervention. Interestingly, a number of p53 reactivating compounds are in development that aims to re-direct the mutant protein towards a more wildtype conformation. Whether all p53 mutants behave similarly or exhibit unique properties in the setting of HGSOC remains to be determined.

Metabolism

The identification of germline mutations in mitochondrial proteins SDH, FH and IDH1 has been associated with paragangliomas and pheochromocytomas and has helped to decipher the role of cellular metabolism in cancer initiation and growth. Sandra Orsulic (UCLA) described elegant studies identifying that the dysregulation of SDHB occurs in a subset of ovarian cancers. Functional studies showed that gene knock-down was associated with increased growth, epithelial-mesenchymal transition (EMT), accumulation of succinate and a hypermethylated epigenome. Comprehensive metabolic analyses revealed that SDHB knockdown leads to reprogrammed central carbon metabolism and decreased mitochondrial reserve capacity. Importantly, these analyses show that this metabolic dysfunction can be therapeutically targeted.

Cervical cancer – HPV vaccine – presidential report

The President's Cancer Panel members, appointed by the President of the United States and reporting directly to him, have identified a critically low HPV vaccination rate in the USA. In 2012, only 33.4% of girls and 6.8% of boys ages 13–17 completed the three doses of the HPV vaccine, compared with Australia (71.2%), the United Kingdom (60%) and the provinces in Canada (50–85%) [4]. The panel emphasized that increasing vaccine uptake in the USA in girls to 80% would prevent 53,000 additional cases of HPV-mediated cancers. In addition, a rise in HPV positive oropharyngeal cancers, which is more prevalent among men, indicates the importance of vaccinating boys. The panel recommended measures to increase vaccine uptake including 1) reducing missed clinical opportunities to recommend vaccine administration, 2) increasing parent and caregiver education about HPV-related disease and vaccination and 3) increasing access to the vaccine by providing sites for vaccination that are not limited to a clinical setting, such as pharmacies. The panel also recommended involvement of the Centers for Disease Control and Prevention (CDC) to further validate the impact of vaccination; encouragement of healthcare payers to adequately reimburse payment for vaccination; research into more convenient dosing schedules; and development of next generation vaccines with improved

coverage and that are easier to store and administer. Cause of the strikingly low vaccination rate in the USA, identified through structured surveys, includes the parental perception that the vaccine was not needed (19%), failed recommendation by the physician (14%), safety concerns (13%), lack of awareness about the vaccine (13%) and the misperception that the vaccine was not needed because the daughter was not yet sexually active (10%). These results strongly indicate not only the need for parent/caregiver education but also the need for education of pediatricians on the safety, efficacy and need for HPV vaccination. Finally, given the importance of the panel's report and its implications for public health, delivered at one of the nation's largest annual cancer meetings, it is sobering that the presentation was attended by approximately only 40 people.

Concluding remarks

There is little doubt that the rapid development of genomic technologies over the past decade has changed the way we approach basic and translational cancer research. The real-time characterization of patients' tumors is a reality in many centers and is now driving clinical trial design and impacting patient care. These advances were prominently highlighted during the 2014 AACR Annual Meeting and are a testament to the importance of team work across the spectrum from bench to bedside. It has been said that 'the biological and medical sciences are in a golden age.' [5]. With continued and sustained support from government agencies, foundations, and philanthropies, research will continue to change the face of cancer and its impact on our patients.

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