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104th American Association For Cancer Research (AACR) Annual Meeting; Breakthroughs In Science And Technology Changing Cancer Care

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After an unusually cold March, this year's cherry blossom season came right in time for the 18 000 or more scientists attending the 104th American Association for Cancer Research (AACR) Annual Meeting held in Washington, DC, April 6−10, 2013. This meeting is one of the biggest gatherings for cancer researchers worldwide. The organizing committee chaired by Jose Baselga from Harvard University and 16 cochairpersons, together with a 74-member Scientific Program Committee and over 60 subcommittees took on the herculean task to put together a program for the meeting. The meeting schedule included more than 100 educational, methods, and meet-the-expert sessions, more than 100 major and mini-symposia, and more than 5000 poster presentations spread throughout the four and a half days. The theme for the meeting, *Personalizing Cancer Care Through Discovery Science*, was chosen to acknowledge the accelerated pace of discoveries in basic, translational, and clinical cancer research. Next generation sequencing (NGS) as well as genome-wide association studies (GWAS) are important contributors to this new phase of personalized cancer care, therefore were major topics for several sessions at this meeting. In this short report, we will highlight a few of the talks given at the 104th AACR Annual Meeting, focusing on the NGS and GWAS subject areas.

Nowadays, NGS is making its way into the cancer clinic. Large-scale sequencing studies have demonstrated that it is possible to uncover many previously known and novel cancer-specific events, while strengthening our view that cancer is a highly heterogeneous disease.[1] Many success stories demonstrate the applicability of NGS technologies in cancer clinics and strengthen the view that, in time, NGS-based testing will become standard for every cancer patient. Of course, NGS is still in its infancy as a clinical tool, which was also emphasized by several presenters during the meeting.

NEXT GENERATION SEQUENCING IN THE CANCER CLINIC

At the meeting, one of the major symposia titled "Use of Next-Generation Sequencing: Implementation of Clinical Genomics" covered a variety of success stories, issues, and challenges. All the presenters outlined basic criteria for successful implementation of these sequencing technologies, current draw backs, and, most importantly, translating results into action for patient care. John Iafrate from Massachusetts General Hospital started his talk by summarizing the challenges and issues facing NGS in the clinic including universal testing of all tumors, clinical- or research-based sequencing, pool- or single gene-based assays, clinical validation, and specimen size and quantity. In addition, uniformity in bioinformatics, finance/billing, and proper patient consent are profound challenges according to Dr. Iafrate. He argued that in order to move NGS in to the clinic we need an effort to technically validate the technology. The issues he identified include which technologies to use, which reagents to use (home-brewed vs commercial kits), and what analytical sensitivity is required, and also whether we should look at hot-spots or whole-genes, whole exomes, or even whole genomes. He also raised the point that, for mutations, the FDA has, so far, only approved single assays, which are not applicable for NGS-based tests. Following this talk, John Carpten from the Translational Genomics Research Institute (TGen), discussed his experiences with using NGS in complementing clinical decision making. TGen's current strategy includes sequencing a low coverage long-insert whole genome at 5- to 8-fold coverage, together with a high coverage (100-fold) whole exome and transcriptome-sequencing (RNA-seq) from the patient's tumor together with a matched normal. Utilizing a highly optimized pipeline from library preparation, to sequencing, to data analysis helped them achieve a turn-around time of less than two weeks. While answering a question from the audience, Dr. Carpten indicated that, in 80−90% of the cases, they were able to make a clinical recommendation on the basis of the sequencing result. However, this is confounded by examples such as pancreatic cancers, where *KRAS* mutations are common and currently not actionable.

Arul Chinnaiyan from University of Michigan opened up by acknowledging that most tumors have a combination of private mutations and/or rare driver gene alterations, complicating the one-assay-fits-all approach. They currently use NGS for low coverage whole genome (5- to 15-fold), whole exome (70- to 100-fold), and RNA-seq from the same patient. They achieve a four-week turnaround and expect to reduce this to 2−3 weeks with faster sequencing technologies. He presented already published work describing their success stories in identifying recurrent translocations between *NAB2* and *STAT6* in solitary fibrous tumors[2] and recurrent *FGFR* gene fusions in many different solid tumors.[3]

On the other hand, Davis Solit of Memorial Sloan-Kettering Cancer Center and chair of the session, provided examples of "reverse-discovery" studies. They selected patients for sequencing on the basis of their remarkable clinical response during clinical trials that were otherwise unsuccessful. In his first example from a clinical trial in bladder cancer with the mTORC1 inhibitor everolimus, one patient demonstrated a remarkable full response with no relapse, while no other patient benefited from the treatment. Whole genome sequencing

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identified a mutation in *TSC1* that resulted in increased sensitivity to everolimus.[4]

In a larger bladder cancer cohort, they confirmed that *TSC1* mutations occur in 8% of the patients. His second example came from a patient receiving irinotecan (AZD7762) where whole genome sequencing revealed a mutation in the gene *RAD50* at a highly conserved position, together with loss of the wild-type allele. Screening components of the MRN complex (*MRE11A*, *RAD50*, *NBN*) in the Chk1-response-pathway among different tumor types revealed that, in 4 to 5% of the cases, these genes are mutated.

In summary, all presenters in this session agreed that NGS is a powerful tool for not only discovery but also clinical action. The common consensus was that fixed-tissue is an essential source for sample input because not all patients have their tumors resected but instead have biopsies. Therefore, establishing prerun QC matrices is critical in maintaining high quality input for sequencing-based assays. In terms of general applicability of NGS-based assays in the clinic, an optimal two-week turn-around time would be required; however, currently, less than four weeks is realistically achievable and acceptable. Also, a comprehensive committee called the "Tumor-board" is essential not only in interpreting the sequence data but also to make clinical recommendations for treatment. Finally, an issue raised not only by the presenters but also by the audience was "Who is going to pay for all this sequencing?". The common answer was that, for now, there is no standard to bill NGS-based tests to third-party health care subsidizers; however, there seems to be success in some limited cases.

CANCER TARGETS AND DISCOVERY

This session reported on efforts by the NCI's Cancer Target Discovery and Development network. The incentive is to consolidate the data generated by multiple centers, extract the most out of it using various analytical approaches, and make the data public. An investigator from the network, William Hahn from the Dana-Farber Cancer Institute, outlined their large-scale systemic assays. For gain-of-function events, they utilize open-reading-frame (ORF) libraries and siRNA libraries for loss-of-function events in hundreds of cell lines. He argued that the complexity enables higher power for finding significant correlations where small scale assays can be confounded by many factors including the choice of cell lines. One particularly interesting observation in a loss-of-function screen was the discovery that β-catenin driven cancers were dependent on the transcription factor YAP-1 and that YAP-1 and TBX5 were in a complex with β-catenin at target gene promoters.[5] He also described a model where the canonical β-catenin/TCF4 complex may be necessary for tumor initiation, while the β-catenin/YAP1 complex may be required for progression. Another investigator, Gordon Mills from the MD Anderson Cancer Center, described slightly different phenomena. He started by stating that cancer cells have hundreds of genomic aberrations contributing to a limited number of phenotypic outcomes that are cancer hallmarks. Of course, the question "Which aberrations are drivers, which are passengers?" is of the essence. He redefined drivers as any aberration that, if targeted, alters the phenotype of a tumor. Along these lines, he elucidated to three states of driver mutations. First, a hypermorph, a more active state, and second a hypomorph, a less active version of the wild-type protein. He also described a third state called neomorph, where the mutation causes the wild-type protein to gain a previously unknown and also unpredictable function. One classical example of a neomorph is the *IDH1*/*IDH2* mutations that result in an enzyme that generates 2-hydroxyglutarate, while the wild-type enzyme produces α-ketoglutarate. They performed large-scale mutant ORF screens in cell lines and assayed for proliferation and survival using cell viability assays against a variety of key signaling inhibitor compounds. Using this data set, they were able to discover the functional consequence of a recurrent *PIK3R1* R348* mutation. They overexpressed this particular mutation and a *PIK3R1* E160* mutation, for comparison, in BaF3 myoloid cells and assayed for survival against inhibitor compounds. While the E160* mutant showed resistance to AKT, PI3K, and mTOR inhibitors, the R348* mutant showed resistance only to mTOR but not AKT and PI3K inhibitors. In addition, the R348* mutant showed sensitivity to MEK inhibitors while the E160* mutant did not; however, this effect was not through RAS. Looking at the protein phosphorylation cascade showed that the R348* mutant increased phosphorylation of JNK and MEK but not p38 and JUN, while mutant RAS increased phosphorylation of p38 and MEK only. He also showed that the R348* mutant activates MKK7 while RAS activates MKK3, MKK4, and MKK6. In addition, the *PIK3R1* R348* mutant was localized to the nucleus while wild-type *PIK3R1* is not, further confirming that this recurrent variant is also a neomorph.

These studies demonstrate the potential for large-scale screens to help better understand disease states and to design novel treatment regimens for such cases. In concordance with the overall theme of the meeting, large-scale sequencing and/or screening efforts will be invaluable in helping patients in the clinic as well as the tumor boards make well informed recommendations.

GENOME WIDE ASSOCIATION STUDIES: THE NEXT STEPS

As a methods workshop, the Saturday session titled "GWAS: The Next Steps" balanced education for interested scientists and reporting recent findings in the field. Christopher Amos' presentation titled "Post-Genome-Wide Association Studies" set the stage by reminding the audience that while there are many single nucleotide polymorphisms (SNPs), about one in 400 bases, only less than 0.01% are thought to be associated with diseases. He went on to report results from a recent study on the influence of common genetic variations in lung cancer describing *CHRNA5*, *CHRNA3*, and *CHRNB4* as susceptibility genes for smokers but not for never-smokers, while *TERT*, *CLPTML1*, *BAT4*, and *RAD52* are susceptibility genes across all cancer subtypes. Brooke L. Fridley from the University of Kansas Medical Center discussed analytical approaches for the identification of new association loci and emphasized the importance of incorporating statistics, bioinformatics, and multiple data types. Alvaro N. A. Monteiro from Moffitt Cancer Center emphasized the importance of data integration in "Functional Analysis of Predisposition Loci", drawing examples from a recently published[6] large collaborative study of which he was part. A majority of disease-associated variants are found outside of coding regions, presumably in distal regulatory elements. eQTL analysis, associating gene expression with genomic variants, is therefore one of the most important steps in data integration.

In the final presentation of the Saturday session, Rosalind A. Eeles from the Institute of Cancer Research, UK discussed the

"Clinical Evaluation of GWAS Findings". In her view, the goals of GWAS studies are to "refine risk assessment, target screening, develop biomarkers, find associations with disease behavior, and apply to targeted treatment". She suggested that complementing screening with genotyping for risk alleles may improve patient management. Dr. Eeles supported these suggestions with some results from her recent paper:[7] approximately 30% of the familial risk for prostate cancer (PC) is now explained by 23 newly discovered and 44 previously known susceptibility loci. Men in the top 1% of the risk distribution have a 4.7-fold increased risk of PC compared to the risk average. Thus, incorporating genotyping information can substantially affect a patient's risk estimate. On the basis of familial risk alone, an unaffected man at age 50 with a father diagnosed with PC at age 60 has a 20% chance of PC during his lifetime, but if genotyping information were to put his risk into the top 1% of the distribution, his lifetime risk for PC is estimated to be over 60%. Such substantial modifications of the risk assessments can ultimately translate into improved screening strategies. Mathematical modeling showed that by removing patients assessed to be in the lower 1% of the risk distribution, almost 16% of screenings can be avoided while missing only 3% of the cases. On the opposite end of the risk spectrum, with an almost two-thirds lifetime risk in the top 1%, it may instead be advisable to forego PSA screening and go straight to biopsies.

GENETIC EPIDEMIOLOGY IN THE POST-GWAS ERA

Dr. Eeles was also the opening speaker of the mini-symposium titled "Genetic Epidemiology in the Post-GWAS Era" on Tuesday afternoon. In this session, she extended her previous talk to discuss the design of the iCOGS custom Illumina genotyping array probing 211 155 variants and also gave a brief overview about the massive organizational effort by COGS (Collaborative Ovarian, prostate and breast Gene-environment Study), a consortium of consortia including, among others, PRACTICAL, the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome, which contributed over 50% of the samples of the more than 100 000 samples in COGS.

CLOSING REMARKS

In the subsequent talk, Sara Lindstrom from Harvard reported on her work on refining the location for causal variants for breast cancer by analyzing 12 selected regions, which extended to cover a total of 5.5 Mbases, previously associated with breast cancer. On the basis of data from a cohort of 2288 women diagnosed with breast cancer and 2323 controls from different ethnicities they attempted to identify causal SNPs with a Bayesian assuming one causal SNP per region and identical priors. The strongest associated SNPs were, on average, 80 kb apart from the GWAS signal, but the analysis currently did not provide unequivocal evidence for a single causal variant.

Yian Ann Chen from Moffit reported on her work implicating variants in long noncoding RNAs (lncRNA) in the etiology of epithelial ovarian cancer (EOC). She analyzed variants associated with EOC in three previous GWAS studies and showed that 1737 variants fell onto 63 unique lncRNAs, and 651 were included in the analysis after LD reduction. Four lncRNAs (WT1-AS, Hoxa11as, AK082072, and H19) are associated with EOC Risk. Follow up validation studies using 9854 EOC cases and 17633 controls from the COGS initiative, among the top four lncRNAs, only one SNP was available. Association of this variant in WT1-AS, a putative regulator of the tumor suppressor WT1, was replicated. Integration of expression data from the Cancer Genome Atlas further showed that WTA1-AS expression was significantly higher in carriers of the variant allele.

Alan Fu, a graduate student at Yale and winner of the 2013 AACR Scholar-in-Training award, described his functional study of the germline polymorphism rs2682818, which was associated with an increased risk for B- and T-cell non-Hodgkin Lymphoma (NHL) in a study involving 455 cases and 527 controls. He showed that this variant located in the stem-loop of the pre-miR-618 is associated with a reduced expression of the mature miRNA, probably through attenuating pre-miRNA processing. His RIP-Chip-based analysis identified targets of miR-618, which were enriched for genes implicated in NHL including TP53 and BRCA1. The impact of polymorphisms on the action of miRNA was also the subject of the presentation by Brid Ryan and Curtis Harris from the National Cancer Institute. However, their analysis focused on variants affecting the recognition site for miRNA rather than the pre-miRNA itself. One specific variant affecting the dopamine receptor was reported for the first time to be associated with a reduced risk for lung cancer. She elucidated that the explanation for this association, that it may affect the nicotine reward pathway and stimulate smoking, is most likely incorrect, because the association remained valid after adjusting for smoking status even in never-smokers. Dr. Ryan concluded that her findings, therefore, suggest a novel role of the dopamine receptor signaling pathway in the etiology of lung cancer.

In the final presentation of this session, Xia Pu from MD Anderson gave an example of using association studies to identify markers for adverse treatment effects by identifying SNPs associated with risk for pneumonitis and esophagitis, two main modes of clinically significant toxicities after radiation therapy, in a cohort of 393 Caucasian nonsmall-cell lung cancer patients. More than a hundred associations were found at a p-value less than 10^{-4}, with the strongest associations located on chromosomes 10q (OR = 3.2) and 4p (OR = 0.17). In addition, CART analysis identified interactions of four SNPs each for the two investigated toxicities.

The 104th AACR Annual Meeting showcased the remarkable work conducted by thousands of scientists worldwide toward understanding the disease, diagnosing it early, and discovering novel therapeutic options. In terms of the subject areas covered in this short report, both NGS and GWAS are proving invaluable in further complementing our knowledge. GWAS studies can lead the way to understand the group of patients that are at higher risk for developing cancer, so that we can implement effective prevention policies. Of course, when and if prevention fails, we need tools like NGS to enable the prescription of a personalized treatment regimen for the patient. However, in terms of general applicability of NGS, there are major hurdles to overcome. From a regulatory view, a new standard may be needed to justify the utility of such technologies in the clinic. From a clinical view, we need comprehensive understanding of the outcomes of mutations in genes and an array of targeted-compounds that can achieve clinical success. Also, from a financial view, we need acknowledgment of NGS as a clinical tool by third-party health care subsidizers. Finally, it is reassuring that, even at this early stage, NGS-based tests are providing clinical benefits to a

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number of cancer patients, who otherwise have failed all the possible treatment options available as of today.

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Notes

Disclaimer. The views and opinions expressed here are those of the presenters at the 104th AACR annual meeting and/or the authors and do not reflect the views of the National Cancer Institute or its employees.

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