The Present and Future of Precision Oncology

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The new era of precision medicine has begun. The recent advances in comprehensive genomic profiling and novel drug development have revolutionized the field of medicine. Cancer is known as a deadly disease that many patients have succumbed to. However, cancer presents an exceptionally promising opportunity that refines the principles and practices serving as the foundation for precision medicine. Innovation and breakthroughs that were made in the recent years in targeted therapy and immunotheray have turned the tide in the war against cancer, providing hope for cure in this devastating and relentless disease.

Precision cancer medicine has now become routine part of day-to-day oncology practice via both approved agents and novel clinical trials. For example, in my cancer clinic, all patients undergo next generation sequencing of tumor both from tumor tissue and blood cell-free DNA, based on which the best targeted therapies are carefully selected. As one of the national principal investigators of the NCI-MATCH (Molecular Analysis for Therapy Choice), a multi-arm clinical trial that provides the most cutting-edge precision medicine to more than 4,000 cancer clinics, and the upcoming SWOG (Southwest Oncology Group) DART (dual anti-CTLA4 and anti-PD-1 blockade in rare tumors), an immunotherapy basket trial for 130 rare tumors, I will discuss how we can facilitate precision medicine and new drug development in cancer treatment. I will also discuss how recent advances in cancer genomics and immune-oncology and innovative pathway-based clinical trial designs including umbrella and basket trials have all worked together to revolutionize new drug development in oncology.

1. Chae YK, Patel S, Kurzrock R, Giles FJ. SWOG DART (Dual Anti-CTLA4 and Anti-PD-1 Blockade in Rare Tumors). SWOG spring meeting 2015, San Francisco, USA;Early Therapeutics and Rare Tumors Committee Presentation Abstract #2.

WHAT SHALL WE TREAT: SYMPTOMS OR DISORDERS?
INSOMNIA AS AN EXAMPLE

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Everyone sleeps. Not one sleep is the same as the other, even in the same person. From the ancient era, many sleep related phenomena or symptoms were observed and documented. Nevertheless, those were just symptoms or signs of unknown or undefined normalcy or disorders.

A new medical discipline called sleep medicine was established a half-century ago. Many sleep related symptoms and sleep disorders were not well defined or classified until 1990 when the first edition of diagnostic manual of sleep disorders came out to the world. Despite this new field of medicine being developed and more sleep disorders being better-defined, we - both patients and clinicians - still focus too much on sleep symptoms rather than diagnosable disorders when targeting the treatment.

We often treat insomnia with medication in the sleep aid category. Sleep aids, however, rarely work. Why? It is because both the patient and the physician, too often, jump into the conclusion that insomnia (symptom) is insomnia (disorder) and as such decide to treat insomnia (symptom) using sleep aid. However, almost every sleep disorders category can manifest with insomnia symptom. What is one of the most common symptoms of the following major disorders: sleep apneas, restless legs syndrome, delayed sleep phase syndrome, or even narcolepsy? The answer is insomnia.

When hearing insomnia as a chief complaint, then we need more information starting with a good history taking starting to come with proper diagnoses for which the treatment will need to be tailored. Insomnia as a symptom can be resolved only when the underlying disorders are properly diagnosed and treated. Sleep aid won't treat insomnia.

Mitochondrial dysfunction and damage associated molecular patterns (DAMPs) in chronic inflammatory diseases

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Inflammation represents a comprehensive host response to external stimuli for the purpose of eliminating the offending agent, minimizing injury to host tissues and fostering repair of damaged tissues back to homeostatic levels. In normal physiologic context, inflammatory response culminates with the resolution of infection and tissue damage response. However, in a pathologic context, persistent or inappropriately regulated inflammation occurs that can lead to chronic inflammatory diseases.

Recent scientific advances have integrated the role of innate immune response to be an important arm of inflammatory process. Accordingly, dysregulation of innate immunity has been increasingly recognized as a driving force of chronic inflammatory diseases. Mitochondria have recently emerged as organelles which govern fundamental cellular functions including cell differentiation, cell death, metabolism and cellular signaling that are important in innate immunity and inflammation-mediated diseases. As a natural consequence, mitochondrial dysfunction has been highlighted in a myriad of chronic inflammatory diseases. Moreover, the similarities between mitochondrial and bacterial constituents highlight the intrinsic links in the innate immune mechanisms that control chronic inflammation in diseases where mitochondrial damage associated molecular patterns (DAMPs) have been involved.

Here in this presentation, the role of mitochondria in innate immune responses is discussed and how it pertains to the mitochondrial dysfunction or DAMPs seen in chronic inflammatory diseases is reviewed.

ASSESSMENT OF A CELLULAR IMMUNE RESPONSE FROM PERIPHERAL BLOOD OF NON-HUMAN PRIMATE AFTER PORCINE ISLET XENOTRANSPLANTATION

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Introduction: Outstanding results from nonhuman primate study put islet xenotransplantation with immunosuppression closer to the clinical application. To establish successful immune suppressive protocols, immune monitoring by which the fate of graft could be predictable would be critical. However, there are few reports showing predictive immune parameters associated with the fate of the graft in pig to nonhuman primate islet xenotransplantation model. Implementation of an appropriate monitoring method to detect the development of detrimental porcine antigen-specific cellular immune responses is also necessary. In addition, clarifying the causes of islet death in the chronic phase after islet transplantation is important.

Methods: Porcine islets were transplanted to diabetic nonhuman primate under immunosuppression. During observation period, the number and ratio of T cell subsets were analyzed by flow cytometry from peripheral blood of seven-teen transplanted monkeys to find out graft-fate predictive immune parameters. ELISpot assays were conducted on serial stocks of peripheral blood mononuclear cell (PBMC) samples previously isolated from four NHP recipients transplanted with porcine islets to validate the essay's utility to monitor the porcine antigen-specific cellular immune responses. Furthermore, RNA sequencing with peripheral blood and bioinformatics analysis in two monkeys was undertaken to find out or predict potential cause(s) of graft failure after 100 days after transplantation.

Results: After the depletion of $CD3^+$ T cells with rATG with immunosuppression, mean recovery time of $CD3^+$ T cells was 38.2 ± 47.7 days. $CD4^+$ T cells were the dominant populations in $CD3^+$ T cells before the anti-thymocyte globulin treatment. However, $CD8^+CD28^-CD95^+$ effector memory T cell's rapid expansion reversed the ratio of $CD4^+$ versus $CD8^+$ T cells. T lymphocyte subtype analysis with graft survival day revealed that $CD4^+/CD8^+$ T cell ratio was significantly associated with early graft failure. The optimal conditions for the ELISpot assay were defined as 2.5×10^5 responder cells incubated with 5.0×10^5 stimulator cells in 96-well, flat-bottom plates without further co-stimulation. Using donor splenocytes as stimulators, a serial interferon-gamma (IFN-γ) ELISpot assay with PBMCs from the monkeys with prolonged porcine islet grafts (>180 days) demonstrated that the number of donor antigen (not islet-specific)-specific IFN-γ-producing cells significantly increased upon overt graft rejection. By using novel bioinformatics tool, I found that highly relevant activated 'immunologic' pathways were indeed manifest in graft failed animal compared with control one in chronic phase. In line with this notion, I further confirmed that the porcine islets were heavily infiltrated with $CD3^+$ T cells by immunohistochemistry on biopsied liver samples.

Conclusions: $CD4^+/CD8^+$ T cell ratio could be used as a surrogate marker to predict early graft failure in porcine islet xenotransplantation in NHPs with immunosuppression. The use of recipient PBMCs in a porcine antigen-specific IFN-γ ELISpot assay may be a reliable method for monitoring T-cell-mediated rejection in pig-to-NHP islet xenotransplantation. Finally, a new bioinformatics analysis combined with peripheral RNA sequencing could unveil insidious immune rejection in the chronic phase after pig-to-NHP islet xenotransplantation.

Advances in Diabetes Treatment

Jongoh Kim

The goal of diabetes treatment is to prevent diabetic complications using the most suitable combination of medications. Though the risk of diabetic complications is clearly associated with elevated glucose levels or hemoglobin A1c, the benefit of intensive glycemic control guided by hemoglobin A1c has not been encouraging, particularly in terms of reduction in macrovascular risk. Recently, there came out new classes of diabetes medications. Studies suggest that some of GLP1 (glucagon like peptide 1) receptor agonists and SGLT2 (sodium glucose co-transporter 2) inhibitors have cardioprotective and renoprotective effects independent of glucose lowering effects. It is important to consider potential benefits of different diabetes medications beyond glucose control in optimizing diabetes treatment.

Defining and understanding adaptive resistance in cancer immunotherapy

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Despite the unprecedented tumor regression and long-term survival benefit observed with anti-Program Death (PD) (anti-PD-1 or anti-B7-homolog 1 (B7-H1)) therapy in patients with advanced cancers, a large portion of patients do not benefit from such treatment and a fraction of responders relapse after a period of response.

Current experimental and clinical efforts to overcome resistance and improve efficacy of anti-PD therapy require a clear definition and understanding of the type of resistance involved, whereas, in our opinion, current efforts using random combinations with available treatment regimens should be abandoned in near future.

We categorized three types of resistance, namely target-missing, primary, and acquired resistance, and discussed their definitions, potential mechanisms, and solutions to overcome these types of resistance. These discussions are based on the four classifications of tumor immunity in the microenvironment (TIME) that we have proposed and requires reliable, accurate tissue sampling and appropriate interpretation of results. These categories and classifications can serve as a starting point to dissect complicated interactions between tumor and immune system, as well as the heterogeneity of the tumor microenvironment in patients with advanced cancer.

We believe that fundamental understanding of cellular and molecular mechanisms underlying these types of true resistance is the key for targeting the right targets in combination with or beyond anti-PD therapy in the future.

Thyroid Update

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Thyroid disorder is very common yet there are a lot of controversies remain with respect to various issues. These include but are not limited to goal of TSH values, proper iodine intake, normal TSH values, thyroid nodule management etc. Patients get inundated with medical information from medical blog and google search, often times leading to mismanagement of his /her own medical condition.

Learning objectives include

- Be able to decide when the patient needs treatment for subclinical hypothyroidism
- Thyroid disorder in pregnancy
- Be able to suspect testing issues rather than true disorder (such as biotin interference)
- What is new in thyroid nodule management/thyroid cancer management

CLONAL EVOLUTION AND HISTOLOGIC TRANSFORMATION OF LUNG ADENOCARCINOMAS INTO SMALL CELL CARCINOMAS DURING TARGETED TREATMENT

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Histologic transformation of EGFR-mutant lung adenocarcinoma (LADC) into small cell lung cancer (SCLC) has been described as one of the major resistant mechanisms for EGFR tyrosine kinase inhibitors (TKIs). These cases typically present an aggressive clinical course after the transformation leading to a rapid deterioration of patient's condition. However, the molecular pathogenesis has been elusive. We investigated 21 patients with advanced *EGFR*-mutant LADCs, which were transformed into EGFR TKI-resistant SCLCs. Among them, whole-genome sequencing was performed for nine tumors acquired at different time points from four patients to reconstruct their clonal evolutionary history and to detect genetic predictors for small-cell transformation. Our findings were validated by immunohistochemistry in 210 lung cancer tissues.

We identified that EGFR TKI-resistant LADCs and SCLCs shared common clonal origin and underwent branched evolutionary trajectories. The clonal divergence of SCLC ancestors from the LADC cells occurred even before the first EGFR TKI treatments, and the complete inactivation of both *RB1* and *TP53* were observed from the early LADC stages in our sequenced cases. We extended the findings by immunohistochemistry in the early LADC tissues of 75 patients treated with EGFR TKIs; inactivation of both Rb and p53 was strikingly more frequent in small cell-transformed patients, compared with non-transformed patients (82% vs. 3%; odds ratio=131; 95% CI=19.9−859). Among patients registered in a predefined cohort (n=65), an *EGFR*-mutant LADC harboring completely inactivated Rb and p53 had 43-times greater risk of small-cell transformation (relative risk=42.8; 95% CI=5.88−311). Branch-specific mutational signature analysis revealed that APOBEC-induced hypermutation was frequent in the branches toward small-cell transformation. EGFR TKI-resistant SCLCs are branched out early from the LADC clones harboring completely inactivated *RB1* and *TP53*. The evaluation of *RB1* and *TP53* status in LADCs receiving EGFR TKI treatment is informative in predicting small-cell transformation.

Immunotherapy in skin cancers; moving beyond melanoma

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Cutaneous SCC (cSCC) is one of the most common malignancies and its incidence has rapidly increased in the US in recent years. While surgical excision is curative in most, a subset of patients develops recurrent unresectable and/or metastatic disease. While advanced cSCC can cause significant functional morbidity and disfigurement, there are no effective therapeutic approaches that provide meaningful clinical benefit, indicating a major unmet need. Chronic UV-induced DNA damage and alteration of p53 tumor suppressor gene are early events in carcinogenesis but subsequent molecular mechanisms for transformation of precancerous conditions to invasive cSCC are elusive. It is believed that alterations of host immunity and development of various immune evasion pathways such as upregulation of immune checkpoints may play a critical role in the progression of invasive cSCC. Preliminary data of phase I study and our institutional experience at MGH with anti-PD-1 therapy in advanced cSCC indicate effective anti-tumor activity, but also suggest there are non-redundant adaptive immune evasion mechanisms beyond the PD-1:PD-L1 axis present in non-responders. The identification of predictive biomarkers and alternative immune evasion mechanisms which are targetable are needed to develop combinatorial immune therapeutic strategies in advanced cSCC.

Merkel cell cancer (MCC) is a relatively rare cancer. The advanced MCC is poor with 2-year survival rate is around 25%. Interestingly, MCC has 2 distinctive etiologic factors, UV exposure and Merkel Cell Polyomavirus (MCPyV). Traditionally, advanced stage MCC is treated with platinum-based cytotoxic chemotherapy with limited efficacy. Recently, immune checkpoint inhibitor has shown promising efficacy in both UV-induced and MCPyV-related MCC with overall response rate of 33%. Several immunotherapy clinical trials are ongoing to optimize the clinical benefit and MGH is planning to launch the high-risk/advanced MCC clinic/research team.

Fecal transplant from young host to aged host improves mortality and enhances innate immune response with *Clostridium difficile* infection in a mouse model

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BACKGROUND: *Clostridium difficile* infection (CDI) is not only the most common pathogen to cause health care–associated infections, but also a serious issue for a rapidly aging population, with 15% of population in the US 65 and older. CDI disproportionately affects the older population, resulting in 3-10 times higher incidence in people 65 and older and making them sicker with CDI, resulting in higher deaths and worse outcomes even when controlling for other confounding factors. Here, using an aged mouse model, we show worse outcome with CDI. In addition, we evaluate the changes in immune response and intestinal microbiota with aging and the effects of exchanging intestinal microbiota with young mice.

METHODS: Aged (18 months) and young (8 weeks) mice were infected with *C. difficile* using an established model of infection. For our infection experiments, aged and young mice were infected and followed for clinical outcome with blood and tissue samples collected to measure immune response. Stool was collected to analyze the microbiome. For our intestinal microbiota exchange experiments, dirty cages were exchanged between aged and young mice to equalize the intestinal microbiota before infection. They were then infected with *C. difficile* and data collected regarding clinical outcome, immune response, and microbiome.

RESULTS: In the infection experiments, higher mortality was observed in aged mice with CDI, as well as weaker neutrophilic mobilization both in peripheral blood and intestinal tissue and depressed pro-inflammatory cytokine production in early infection. Later in the infection, inflammation was equal to or higher than in young mice. Fecal microbiota analysis revealed significant differences. At phylum level there was a significantly lower number of Bacteroidetes in aged mice. At genus level three signature genera – Bacteroides (Bacteroidetes), Alistipes (Bacteroidetes), and rc4-4 (Firmicutes) – were found to be much lower in aged mice. Intestinal microbiota exchange by cage switching increased innate immune response in early infection and improved survival in aged mice. Microbiome analysis revealed that the Bacteroidetes phylum in general and the three signature genera in particular – Bacteroides, Alistipes, and rc4-4 – were significantly augmented in aged mice with cage switching. Microbial diversity did not increase in aged mice with cage switching.

CONCLUSIONS: Our model recreated worse outcome seen in elderly patients with CDI and demonstrated lower innate immune response during early infection. The results show that there are key components of intestinal microbiota – in the Bacteroidetes and Firmicutes phyla – that affect the outcome in CDI. The change in innate immune response in the aged mice suggest that this effect of microbiota on CDI outcome is mediated through immune response. These findings provide new insight into the mechanism of CDI in the aged host.

Single cell RNA sequencing analysis of *Hhip*$^{+/-}$ age associated emphysema model reveals cell type specific changes related to inflammatory pathways

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Introduction: HHIP (Hedgehog Interacting Protein) is a well-replicated genome-wide association study gene associated with COPD. Recently we have described Hhip haploinsufficient mice (*Hhip*$^{+/-}$) as an animal model of COPD that recapitulates age and cigarette smoke related development of emphysema with characteristic peri-brochial lymphoid aggregates. However, the cell autonomous and non-cell autonomous role of HHIP for maintaining tissue integrity and immune regulation remains elusive. To gain a systematic understanding of the molecular events in diverse cell types underlying the pathogenesis of COPD, we performed unbiased single-cell RNA sequencing (sc-RNAseq) in *Hhip*$^{+/-}$ and age matched wild type (WT) lungss.

Method: Whole lungs from 4 month and 11 month *Hhip*$^{+/-}$and age matched WT mice (n= 2 each), representing the early and late phases of disease, were enzymatically digested. Sc-RNAseq was performed using Chromium system and sequenced with Illumina HiSeq 2500 and analyzed with Seurat R package.

Results: Transcriptomes of 20,653 cells were sequenced, with 18,450 cells passing quality control. Cells were classified into 25 distinct clusters, ranging from resident immune cell subtypes to structural cell types. 4 month *Hhip*$^{+/-}$mice showed increased number of CD8+ T cells and NKT cells compared to age matched WTs, whereas 11 month *Hhip*$^{+/-}$mice demonstrated increased B cells and CD8+ T cells, consistent with increased lymphocyte aggregates on histology. Comparison between 11-month to 4-month WT lung cells revealed an alveolar type 2 cell-specific response to reactive oxygen species, suggesting age-related gene expression changes in structural cells. *Hhip* expression was mainly found in a subset of fibroblasts, where *Hhip* haploinsufficiency resulted in increased TGFB and cytokine related pathways. Even before histological changes lung destruction and inflammatory phenotypes manifested in *Hhip*$^{+/-}$ mice, pervasive changes of molecular states were found in immune cells that have minimal expression of *Hhip*. Macrophages and NKT cells demonstrated elevated expression of integrin pathway-related genes at 4 months in *Hhip*$^{+/-}$lungs, which difference persisted in NKT cells at 11 month of age; CD8+ T cells had upregulated leukocyte adhesion molecules. Differential gene expression signature expanded to type 2 alveolar cells suggesting extrinsic response to cytokine signaling.

Discussion: With the application of single-cell transcriptomic profiling in a murine model targeting COPD GWAS gene, HHIP, we established a comprehensive atlas of cell types in murine lung related with emphysema progression and identified cell type-specific molecular pathways altered in alveolar destruction and inflammation processes. These findings may provide insight into the specific cellular and molecular processes involved in age-related COPD.