YOUNG INVESTIGATOR WORKSHOP 2017

Immunosystems - Implications for Cancer Therapy

February 9 - 10, 2017
The University of Texas M. D. Anderson Cancer Center
Houston, TX

PROGRAM & ABSTRACTS

NORWEGIAN CANCER CONSORTIUM

investigatorworkshop.org

Program

Wednesday, February 8, 2017

7:00 pm	Bus departs Wyndham Hotel
7:30 pm	Reception – Residence of the Consul General of Norway, Morten Paulsen By invitation only – all participants, chairs and keynote speakers are invited 1024 Rocky Road, Houston, TX 77056
9:30 pm	Bus returns to Wyndham Hotel

Thursday, February 9, 2017
1MC 3rd Floor North, Main Ballroom

7:30 am	Bus departs Wyndham Hotel
7:45 am	Registration and poster set-up, light breakfast
8:30 am	Oliver Bogler – Welcome and Opening Remarks
8:40 am	Keynote Lecture – Patrick Hwu "The Interplay Between Tumor Biology and the Antitumor Immune Response"
9:40 am	Plenary Lecture – Åslaug Helland "Predictive Biomarkers for Response to Immunotherapy"
10:00 am	Plenary Lecture – Sattva Neelapu "Adoptive T-cell Therapy for B-cell Lymphomas"
10:20 am	Coffee Break
10:40 am	Plenary Lecture – Steinar Aamdal "Adjuvant immunotherapy for Malignant Melanoma"
11:00 am	Plenary Lecture – Naval Daver "Immune Checkpoint-based Approaches in Acute Leukemia and MDS"
11:20 am	Poster Session
12:30 pm	Lunch
1:30 pm	Workshops – breakout sessions by disease site; oral presentations by young investigators. See below for details by session.
5:00 pm	Bus returns to hotel
6:40 pm	Bus leaves hotel
7:00 pm	Social event – Gem Room at Houston Museum of Natural Science – registered participants, chairs and keynote speakers are invited to attend 5555 Hermann Park Drive, Houston, TX 77030
9:00 pm	Bus returns to hotel

Friday, February 10, 2017
1MC 3rd Floor North, Main Ballroom

7:45 am	Bus leaves hotel
8:00 am	Light breakfast
8:30 am	Keynote Lecture – James Allison "Immune Checkpoint Blockade in Cancer Therapy: New Insights, Opportunities, and Prospects for Cures"
9:30 am	Plenary Lecture – Don Gibbons "Exploring the Role for Immune Checkpoint Inhibitors in Differing Immunophenotypes of Early-stage and Metastatic Non-small Cell Lung Cancer"
9:50 am	Plenary Lecture – Arne Kolstad "Intra-nodal Immunotherapy for B-cell Lymphomas"
10:10 am	Coffee Break
10:30 am	Plenary Lecture – Cassian Yee – "Adoptive T-cell Therapy of Cancer: Personalized Medicine for Common Malignancies"

10:50 am Plenary Lecture – Yngvar Fløisand "*in vitro* Drug Sensitivity Screening for Acute Myeloid Leukemia: Identifying Novel Targets"
11:10 am Poster Session
12:30 pm Lunch
1:30 pm Workshops – breakout sessions by disease site; oral presentations by young investigators
5:00 pm Remove posters
5:15 pm Bus returns to hotel
6:10 pm Bus leaves hotel (bus available for both local and visiting young investigators)
7:00 pm Social event – George Ranch Historical Park – all registered participants, chairs, and keynote speakers are invited to attend
10215 FM 762 Rd, Richmond, TX 77469
10:00 pm Bus returns to hotel

Breakout Sessions

Melanoma **Chairs** **Cassian Yee, MD, Professor, Melanoma Medical Oncology, MD Anderson**
Steinar Aamdal, MD, PhD, Professor, Director – Clinical Research, Oslo University Hospital

Thursday, February 9

1:30 pm Welcome and opening remarks by session chairs
1:45 pm Lawrence Kwong "Novel Rational Combined BRAF Inhibition and Cytokine Therapy for Melanoma"
2:05 pm Weiyi Peng "Defining Immune Resistance Associated with Tumor Intrinsic Glycolytic Activity in Melanoma and Lung Cancer"
2:25 pm Jennifer Mcquade "High Body Mass Index (BMI) Is Associated with Improved Clinical Outcomes in Metastatic Melanoma Patients Treated with Anti-PD1: Differences by Gender"
2:45 pm Raya Leibowitz-Amit "Regulation of Immune Checkpoint Genes Revealed by a Melanoma Tumor Cancer Genome Atlas (TCGA) Analysis – Potential Implications for Improving Immunotherapy"
3:05 pm Break
3:25 pm Alexandre Reuben "Genomic and Immune Heterogeneity Contribute to Differential Responses to Targeted Therapy and Immune Checkpoint Blockade in Melanoma"
3:45 pm Changlin Zhang "KMT2A Promotes Melanoma Growth by Targeting the iNOS/COX-2/hTERT and NF-kB/CBP Signaling Pathways"
4:05 pm Saikiran Sedembi "A novel Non-genotoxic p53 Activator Rallies NK Cells Via Stress-induced Ligand Expression on Melanoma Cells"
4:25 pm Gustavo Schvartsman "Incidence and Outcomes of Central Nervous System Metastasis in Metastatic Melanoma Patients Treated with Anti-PD1 Therapy"
4:55 pm Go to bus to return to hotel

Friday, February 10

1:30 pm Xianghou Xia "INFα-2b Inhibitory Effects on CD4+CD25 +FOXP3+ Regulatory T-cells in the Tumor Microenvironment of C57BL/6 J Mice with Melanoma Xenografts"
1:50 pm Anna Przybyla "Defining the Activation State of Melanoma Antigen-specific CD8 Cells as Naïve/Resting Memory or Effector Cells"
2:10 pm Yao Wang "Downregulation of C-FLIP Enhance PD1 Blockade in Efficacy in B16 Melanoma"

2:30 pm Noha Abdelwahab Hassan “Use of Immune Checkpoint Inhibitors in the Treatment of Patients with Melanoma and Preexisting Autoimmune Diseases: A Systematic Review of Case Reports”
2:50 pm Chan-Keng Yang “Pembrolizumab Expanded Access Program (EAP) in Taiwan for Patients with Progressive Advanced Melanoma after Prior Ipilimumab Treatment”
3:10 pm Break
3:25 pm Daniel Araujo “Prognostic Relevance of Baseline Neutrophils and Nautrophil-to-Lymphocyte Ration in Metastatic Melanoma Receiving Anti-PD1 Therapy”
3:45 pm Bettina Weigelin “Cytotoxic T-cell Cooperation is Required to Overcome Melanoma Resistance to Immunotherapy”
4:05 pm Sang Kim “Successful Treatment of Arthritis Induced by Checkpoint Inhibitors with Tocilizumab While Preserving the Anti-tumor Immunity”
4:25 pm Wrap-up discussion
5:00 pm Take down posters
5:15 pm Go to bus to return to hotel

<u>Lung</u> **Chairs** **Don Gibbons, MD, PhD, Assoc. Professor - Thoracic/Head and Neck Medical Oncology, MD Anderson**
Åslaug Helland, MD, PhD, Senior Consultant, Oslo University Hospital

Thursday, February 9

1:30 pm Welcome and opening remarks by session chairs
1:45 pm Orazio Fortunato “Circulating miRNAs in Lung Cancer are Associated to Pro-tumorigenic and Immunosuppressive Microenvironment”
2:05 pm Carlo Genova “Immune Cell Sub-populations as Potential Predictors of Response to Nivolumab in Non-small Cell Lung Cancer”
2:25 pm Takashi Sato “Intratracheal Delivery of Immunostimulatory Oligonucleotides Using Biodegradable Polyketal Nanoparticles: Effect on Murine Lung Cancer”
2:45 pm Yo-Liang Lai “Radiation Reduces B Cell Representation in TC-1 Mouse Lung Adenocarcinoma Cells”
3:05 pm Break
3:25 pm Jie Zhang “Cancer Associated Fibroblast Conferred Resistance to Anticancer Drugs in Lung Cancer”
3:45 pm Federica Biello “Nivolumab in Advanced Non-small Cell Lung Cancer: A Comparison among Different Radiological Criteria for Assessing Response”
4:05 pm Miho Kono “Metachronour Second Malignancy after Treatment of Limited-stage Small Cell Lung Cancer: Incidence and Survival”
4:25 pm Seyed Javad Maghaddam “Reprogramming Lung Tumor Microenvironment by Targeting Cytokine Network as a Preventive and Therapeutic Strategy for K-ras Mutant Lung Cancer”
4:55 pm Go to bus to return to hotel

Friday, February 10

1:30 pm Jin-Lin Huang “Expression of Sydecan-1 in Pulmonary Lymphoepithelioma-like Carcinoma is Correlated with Early Stage and Good Prognosis”
1:50 pm Jhajaira Araujo “Repeated Observation of Immune Gene Sets Enrichment in Women with Non-small Cell Lung Cancer”
2:10 pm Bertha Rodriguez “PD-L1 Checkpoint Blockade in Combination with MEK Inhibition Reduces Lung Tumor Growth”
2:30 pm Nobuaki Kobayashi “Development of a Novel Anti-cancer Immune Therapy Using the Synthetic Oligonucleotide Containing Poly-G Motif”

2:50 pm Break
3:10 pm Limo Chen “CD38 as a Novel Immune Checkpoint and a Mechanism of Resistance to the Blockade of the PD-1”
3:30 pm Parag Parekh “Theranostic Probes for Targeted Immunotherapy”
3:50 pm Triparna Sen “Combining Immune Checkpoint Targeting and DNA Damage Repair (DDR) Targeted Therapy in Small Cell Lung Cancer (SCLC)”
4:10 pm Wrap-up discussion
5:00 pm Take down posters
5:15 pm Go to bus to return to hotel

Lymphoma and Other Hematologic Malignancies

Chairs **Sattva Neelapu, MD, Assoc. Professor, Lymphoma/Myeloma, MD Anderson**
Arne Kolstad, MD, PhD, Senior Consultant, Oslo University Hospital
Naval Daver, MD, Asst. Professor, Leukemia, MD Anderson
Yngvar Fløisand, MD, PhD, Senior Consultant, Oslo University Hospital

Thursday, February 9

1:30 pm Welcome and opening remarks by session chairs
1:45 pm Zijun Xu-Monette “The Potential Role of CD37 Tetraspanin in Adaptive Immunity and Sensitivity to PD-1 Blockade in Diffuse Large B-cell Lymphoma”
2:05 pm Liang Wang “PD-L1 is Up-regulated by EBV-driven LMP1 through NF-κB Pathway and Correlates with Poor Prognosis in Natural Killer/T-cell Lymphoma”
2:25 pm Jinsheng Weng “TCL1-specific T-cell Receptor Gene Transfer Redirects T Lymphocytes to Display Effective Antilymphoma Reactivity”
2:45 pm Giacoma de Tullio “αβ-Double Negative T-cells (αβ-DNTs): Role on Tumor Surveillance and Relevance as Prognostic Factor of Clinical Outcome in Lymphoma and Solid Tumors”
3:05 pm Break
3:25 pm Roza Nurieva “Absence of Grail Promotes CD8+ T-cell Anti-tumor Activity”
3:45 pm Fuliang Chu “Dickkopf-3 (DKK3) Regulates Follicular Cytotoxic T-cells Function in Follicular Lymphoma”
4:05 pm Alberto Mussetti “Role of Cell Source and Graft Composition in Haploidentical Transplantation Using Post-transplant Cyclophosphamide”
4:25 pm Ricardo Weinlich “Necroptosis as a Prognostic Marker and Possible Target in Cancer”
4:55 pm Go to bus to return to hotel

Friday, February 10

1:30 pm Else Marit Inderberg “Tapping CD4 T-cells for Cancer Immunotherapy”
1:50 pm Nadia Mensali “A Universal Killer T-cell for Adoptive Cell Therapy of Cancer”
2:10 pm Beibei Zhang “HSP90 Inhibitors Specifically Target FLT3-ITD-driven AML and Bypass TKI Resistance”
2:30 pm Mateusz Rytelewski “Deciphering the Role of Metabolic Reprogramming in the Immuno-modulation of the Leukemic Microenvironment”
2:50 pm Break
3:05 pm Rohit Mathur “Exploring Mechanisms Associated with Loss from Immune Surveillance During Early Progression from Smoldering Multiple Myeloma to Symptomatic Multiple Myeloma”
3:25 pm Benedicte Sjo Tislevoll “Early Changes in Intracellular Signalling Networks of Acute Myeloid Leukaemia in Response to Chemotherapy”

3:45 pm	Hila Shaim “NK cell Dysfunction in CLL Is Associated with Poor Prognosis and Is Mediated through SHP-1”
4:05 pm	Carmen Swanepoel “Investigating the Suitability of Standardized Euroflow Flow Cytometry Panels for the Characterisation and Diagnosis of Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia (CLL/SLL) at Tygerberg Academic Hospital (TAH), South Africa”
4:25 pm	Wrap-up discussion
5:00 pm	Take down posters
5:15 pm	Go to bus to return to hotel

Keynote Speakers

Patrick Hwu, MD

- Head, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center
- Department Chair, Department of Melanoma Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center
- Department Chair, Department of Sarcoma Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center
- Co-Director, Center for Cancer Immunology Research, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center
- The Sheikh Mohammed Bin Zayed Al Nahyan Distinguished University Chair

Dr. Patrick Hwu is a leading tumor immunologist focused on the areas of vaccines, adoptive T-cell therapies, and immune resistance. His research and clinical efforts have led to insights and advances in the understanding of the interactions between tumors and the immune system, and the development of cellular therapies. He is the principal investigator on several NIH R01 translational immunotherapy grants, and other peer-reviewed grants. Several novel, ongoing clinical trials have resulted based on his group's work, which include a trial of T-cells modified with chemokine receptor genes to enhance their migration to the tumor. Most recently, his preclinical studies have focused on combinations of immune checkpoint blockade and T-cell therapy, as well as rational combinations of targeted therapies and immunotherapies. Both of these concepts are being moved into the clinic to improve treatment outcomes for our patients.

James Allison, PhD, Chair, Immunology

As an immunologist, Dr. James Allison's fundamental discoveries include the definition of the structure of the T cell antigen receptor, demonstration that the T cell molecule CD28 provides costimulatory signals necessary for full T cells activation, and that the molecule CTLA-4 is an inhibitory checkpoint which inhibits activated T cells. He proposed that immune checkpoint blockade might be a powerful strategy for therapy of many cancer types, and conducted preclinical experiments showing its potential. He was involved in the development of ipilimumab, which was approved by the FDA for treatment of metastatic melanoma in 2011. His development of the concept of immune checkpoint blockade has transformed cancer therapy and saved thousands of lives.

Dr. Allison is a member of the National Academy of Sciences and the National Academy of Medicine. He has received numerous awards, including the Lifetime Achievement Award from the American Association of Immunologists, the Lloyd J. Old Award and Pezcoller Foundation-AACR International Award from the American Association for Cancer Research, the Novartis Award for Clinical Immunology, the Medal of Honor in Basic Research from the American Cancer Society, the Harvey prize in Human Health from the Israeli Insitute of Technology, the Economist Magazine Innovation Prize for Biomedicine, the Breakthrough Prize in Biosciences, the Szent-Gyorgyi Prize for Progress in Cancer Research, and Lasker-Debakey Clinical Medical Research Award, and the Wolf Prize in Medicine.

Melanoma

Novel rational combined BRAF inhibition and cytokine therapy for melanoma

Gabriele Romano and **Lawrence Kwong**

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Despite enormous recent advances in the treatment of melanoma including BRAF inhibitors and immune checkpoint antibodies, 50-60% of treated patients relapse within 16 months. This relapse is due to the presence of minimal residual disease (MRD) that eventually overcomes therapy and regrows. Moreover, the combination of the two therapeutic approaches has so far induced severe gastrointestinal toxicities in two clinical trials. Thus, there is an urgent need to identify additional safe and durable combination therapies that can target the MRD and prevent tumor relapse. Here, we describe a novel synergistic combination of BRAF inhibition and a specific cytokine therapy that we have preliminarily validated in mouse studies. We have recently generated a novel sophisticated mouse model in which tumor regression and relapse can be precisely controlled by chemically turning on and off the oncogenic BRAF signal. We have now conducted an in silico, longitudinal RNA-sequencing analysis of both BRAF inhibitor (BRAFi)-treated mouse and human patients, and demonstrated that an initial strong CD8/CD4-postive immune infiltration eventually decreased over time, returning to baseline when MRD or relapse was reached. This suggested that the residual disease was immune suppressive or evasive. Indeed, using severely immunocompromised NSG mice as hosts resulted in larger MRD and faster tumor relapse after BRAF inhibition, confirming the essential role of the immune response in controlling MRD. A subsequent systems biology network analysis identified specific cytokines that were predicted to regulate this immune response, and which also decreased over time; hence, we hypothesized that sustaining the expression of these cytokines would also sustain the CD8/CD4 response to target the MRD. Our preliminary results showed that our top candidate cytokine, when delivered intratumorally as a recombinant protein, is synergistic with BRAFi: the majority of combination-treated mice completely rejected the tumor (5/7) and all mice indefinitely delayed tumor relapse (7/7), whereas only one singly-BRAFi-treated control tumor avoided relapse (1/5). We are now testing this combination in larger numbers, as well as testing different delivery routes. Our next step includes a mechanistic evaluation of the both the action of the cytokine as well as of its induction. Overall, our long-term goal is to preclinically optimize the delivery of a specific cytokine-BRAFi combination therapy, with an eye towards eventual clinical applicability.

Defining immune resistance associated with tumor intrinsic glycolytic activity in melanoma and lung cancer

Weiyi Peng, Tina Cascone, Jodi McKenzie, Rina Mbofung, Leila Williams, Simone Punt and Patrick Hwu

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Recent studies have demonstrated that T cell-mediated immunotherapies are promising treatments for a variety of cancers, including melanoma and lung cancer patients. The objective response rates of combined PD-1 and CTLA-4 pathway inhibitors in melanomas and non-small cell lung cancers (NSCLCs) are approximately 50% and 20%, respectively. However, the vast

majority of patients fail to respond to therapy or will ultimately relapse after exhibiting an initial response. We and others have demonstrated that tumor oncogenic pathways exert immunosuppressive effects, which limit the efficacy of immunotherapies, by inhibiting tumor infiltration of T cells and reducing tumor susceptibility to T cell-killing. Although oncogenic activation of the β-catenin and PI3K pathways has been reported to be associated with immune resistance, these pathways do not account for all of the tumors with an immune resistance phenotype. Therefore, identification of additional tumor intrinsic pathways that contribute to the establishment of an immunosuppressive microenvironment will lead to combinatorial strategies to improve the efficacy of immunotherapy. Here, we utilized two global genetic approaches to identify novel tumor-associated immune-suppressive pathways. In one pre-clinical model of melanoma, we used a new high-throughput shRNA screening platform to interrogate the mechanisms immune resistance in tumors Briefly, patient-derived melanoma cells were transduced with a DECIPHER pooled lentiviral shRNA library. After puromycin selection, shRNA-transduced tumor cells were exposed to their autologous tumor-infiltrating T cells (TILs). Analysis of the intensity of gene-specific shRNA sequences in tumor cells with or without treatment of autologous TILs identified genes that contribute to resistance of tumor cells to T cell-mediated killing. The shRNAs that increase the tumor susceptibility to T cell-mediated killing should demonstrate the loss or decreased intensity of the individual shRNAs in viable tumor cells. In addition to the high-throughput shRNA screening platform, we also interrogated the samples of melanoma and NSCLCs from the TCGA datasets, were stratified based on the expression levels of T cell signature genes into T cell-inflamed and non-T cell inflamed groups We performed network-based analyses using the Ingenuity Pathway Analysis to identify pathways that are differentially expressed between the two groups. Interestingly, Results from both shRNA library screening platform and TCGA analyses demonstrated that aerobic glycolysis, the first recognized cancer metabolism reprogramming phenotype, is associated with immune resistance phenotypes. We further tested whether enhanced aerobic glycolysis contributes to the immunosuppressive tumor microenvironment. In melanomas, we observed increased mRNA expression of ALDOA, a gene encoding for a critical enzyme in the glycolysis pathway, in tumors with poor T cell infiltration. Overexpressing ALDOA in Mel 2400, a BRAF-mutated melanoma cell line, reduced susceptibility of tumor cells to autologous TIL-mediated killing. Pretreatment of tumor-reactive T cells with lactic acid, a terminal product of glycolysis, impaired the effector function of TILs. Taken together, our results suggest that tumor metabolic reprogramming favoring aerobic glycolysis promotes resistance to T cell-mediated immunotherapy in melanoma and NSCLC patients. Further studies will be performed to determine the predictive significance of glycolysis-related genes in melanoma and NSCLC patients receiving immunotherapy and the therapeutic potential of combining immunotherapy and glycolysis inhibitors.

High body mass index (BMI) is associated with improved clinical outcomes in metastatic melanoma patients treated with anti-PD1: differences by gender

Jennifer Mcquade and Michael Davies

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Obesity is increasingly recognized as a prognostic factor across several malignancies. We previously reported a link between obesity and improved outcomes in BRAF-mutant metastatic melanoma (MM) patients treated with dabrafenib and trametinib that was more pronounced among men. In a multi-national cohort of 331 MM patients treated with anti-PD1, we examined WHO BMI categories at immunotherapy treatment initiation in relation to survival outcomes.

Underweight patients (<2%) were excluded due to possible cancer-related cachexia. Kaplan-Meier analysis was used to estimate time to progression (TTP) and overall survival (OS). Hazard ratios (HR) and 95% confidence intervals (CI) were estimated within multivariable Cox models.

73% of males and 63% of females were overweight (BMI 25-29.9) or obese (BMI ≥30). With the exception of age, known prognostic factors (M stage, LDH, ECOG PS, gender, mutation status) did not differ by BMI. Median TTP and 3 yr OS were 4.1 mo and 37% respectively for lean (BMI 18.5 to <25), 6.5 mo and 46% for overweight and 6.6 mo and 47% for obese.

Overweight/obese, as compared to lean status, was modestly associated with improved OS [3-yr OS 46% vs 37%, HR and 95% CI: 0.7 (0.5-1.0); P=0.05)] and TTP [median 6.5 vs 4.1: 0.8(0.6-1.0); P=0.07] among all patients, but significant associations were only observed among men [median TTP 8.1 vs. 2.8 mo: 0.6 (0.4-0.9); P=0.01; 3-yr OS 43% vs. 32%: 0.6 (0.4-0.9); P=0.02;). No associations were observed among women (TTP Pinteraction = 0.06 and OS Pinteraction = 0.26).

Higher BMI is associated with improved outcomes in male MM patients treated with anti-PD1. This finding is similar to results observed in MM pts treated with targeted therapy. Mechanisms underlying this association are under investigation.

Regulation of immune checkpoint genes revealed by a melanoma Tumor Cancer Genome Atlas (TCGA) analysis – potential implications for improving immunotherapy

Raya Leibowitz-Amit, Jason Roszik, Dror Avni and Elizabeth Grimm

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Introduction

The interface between T lymphocytes and cancer or antigen presenting cells (C/APCs) is multi-faceted and complex. This interface, now designated 'the immunological synapse', comprises of both co-inhibitory and co-stimulatory transmembrane protein pairs ('checkpoint proteins') that all serve to modulate the signal transmitted to the T lymphocyte, leading to either activation, anergy or exhaustion. Monoclonal antibodies against co-inhibitory molecules at the synapse (termed 'immune checkpoint inhibitors') have anti-neoplastic activity in a wide range of cancers, and are already routinely used in clinic. Notwithstanding this major advancement, not all cancers and not all patients with a given cancer respond to the currently available checkpoint inhibitors. Extensive basic research now focuses on the regulation of checkpoint genes, and clinical research is currently underway to study the use of combinations of inhibitors or the use of agents that enhance co-stimulatory molecules at the immunological synapse. Micro-RNAs (miRNAs) are short intracellular RNA molecules known to be master regulators of gene expression. Here, our aim was to study the associations between a miRNA that was previously implicated in cancer and immune checkpoint genes.

Methods

Bioinformatic analyses of the expression of mRNAs and miRNAs from 451 samples was performed using the melanoma TCGA database. Correlation coefficients between the expression of mRNAs or mRNAs/miRNAs were calculated using the Spearman rho method. Survival analysis was performed using the Kaplan-Meier method. Potential 3'UTR binding sites of miRNAs were found using the web-based tool www.targetscan.org.

Results and future directions

Of 22 mRNAs of checkpoint genes assessed, the expression of 19 (PD1, PD1L, B7.1, B7.2, CTLA4, CD28, BTLA, CD40, CD40L, ICOS, ICOSL, LAG3, galectin-9, TIM-3, TNFSF 4&18, TNFRSF 4, 9& 18) was highly positively correlated, with Spearman rho correlation coefficients ranging from 0.33-0.81. These mRNAs code for both co-inhibitory and co-stimulatory proteins at both the T cell and the C/APC sides of the immunological synapse, suggesting that there is joint regulation on the expression of these checkpoint genes at the transcriptional level. The expression of the miRNA was also significantly positively correlated with the expression of eight of these checkpoint mRNAs (PD1L, PD1L, B7.1, ICOS, BTLA, LAG3, CTLA4, TNFRSF9), with correlation coefficients ranging between 0.2-0.28. This possibly indicates a joint, yet less-stringent, transcriptional regulation on the miRNA and the mRNAs. Bioinformatic analyses suggest that this miRNA may potentially target the 3'UTR of 6 of these mRNAs (PD1, PD1L, B7.1, ICOS, BTLA, TNFRSF9).

Data from 163 stage III melanoma patients with documented survival data, a high expression level of this miRNA and a low expression level of either B7.1, B7.2, CD28, ICOS, ICOSL, OX40, OX40L, TNFRSF18 or PD1L was each significantly associated with poor prognosis relative to all other expression groups. Eight of these nine mRNAs are co-stimulatory. It is tempting to speculate that aberrant co-regulation of the miRNA-mRNAs, leading to high levels of the miRNA and low levels of co-stimulatory checkpoint genes is associated with worse outcome, potentially as a result of 'immune evasion' due to decreased co-stimulation at the synapse.
We are now experimentally studying whether the miRNA targets the 3'UTR of checkpoint mRNAs and whether decreasing its expression with small interfering RNAs alters the expression of checkpoint genes and renders melanoma cells more immunogenic. Our research may have future implications for immunotherapy.

Genomic and immune heterogeneity contribute to differential responses to targeted therapy and immune checkpoint blockade in melanoma

Alexandre Reuben, Christine Spencer, Peter Prieto, Vancheswaran Gopalakrishnan, Mariana Petaccia De Macedo, Jiong Chen, Jason Roszik, Patrick Hwu, Lynda Chin, Michael Davies, Jianhua Hu, Michael Tetzlaff, Alexander Lazar, Ignacio Wistuba, Karen Clise-Dwyer, Brett Carter, Jianhua Zhang, Andrew Futreal, Padmanee Sharma, James Allison, Zachary Cooper and Jennifer Wargo

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Major advances have been made in the treatment of metastatic melanoma in the form of targeted therapy and immune checkpoint blockade. However, the majority of patients do not develop durable responses to these therapies, while several experience mixed responses - with one metastasis responding while another progresses. Recent studies have contributed to the growing appreciation for genomic and immune heterogeneity as a contributor to this phenomenon, though the relationship of these factors to treatment response has not yet been elucidated. Here, we first assessed radiographic responses in a cohort of 60 patients with synchronous melanoma metastases treated with targeted therapy or immune checkpoint blockade and identified heterogeneous responses in the majority of patients (>80%). Synchronous metastases from 15 patients were further analyzed by deep molecular profiling by whole exome sequencing (WES) and neoantigen prediction as well as immune profiling by flow cytometry, immunohistochemistry (IHC), T cell receptor (TCR) sequencing and gene expression profiling (GEP). Genomic analysis by WES demonstrated that synchronous metastases within a

given patient shared an average of 50% of mutations (NSEM) with the remainder being tumor-restricted branch mutations. We then performed in silico prediction of neoantigens and determined that more than 10% of those predicted to bind HLA with high affinity (IC50<100nM) were restricted to individual metastases within a given patient. Next, we analyzed the immune infiltrate by flow cytometry and determined that the majority of patients presented significant variability in the frequency of CD4 and CD8 T lymphocytes across synchronous metastases. Strikingly, TCR sequencing revealed that, on average, only 8% of T cell clones were detectable in both metastases within a given patient, suggesting that the vast majority of tumor-infiltrating T cell clones were restricted to individual metastases. Importantly, when evaluating molecular and immune correlates and response, we determined that responding lesions showed a higher frequency of infiltrating CD8 T cells, thereby providing insights into differential responses to therapy in melanoma. Functional studies are currently underway in order to evaluate the impact of genomic and immune heterogeneity on the anti-tumor immune response in melanoma.

KMT2A promotes melanoma growth by targeting the iNOS/COX-2/hTERT and NF-kB/CBP signaling pathways

Changlin Zhang, Wenbin Li, Tianze Liu, Yixin Li and Wuguo Deng

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Melanoma is an aggressive type of cutaneous malignancy. Although the inhibitors targeting BRAF and/or MEK pathways provide a therapeutic option for non-resectable melanoma driven by BRAF mutation, melanoma, especially metastatic melanoma, has still become one of the most threatening malignancies. Thus, discovering and identifying the exact molecular mechanisms regulating melanoma growth and the novel therapeutic targets for melanoma is urgently needed. In this study, we screened a siRNA library targeting 6024 human genes in human melanoma cells and identified KMT2A as a potential therapeutic target for melanoma. KMT2A was highly expressed in melanoma cell lines and tumor tissues of melanoma patients. Knockdown of KMT2A significantly inhibited cell viability, whereas KMT2A overexpression effectively promoted cell growth in various melanoma cell lines. Further mechanism studies showed that the KMT2A-mediated regulation of melanoma growth was through the modulation of the iNOS/COX-2, hTERT and NF-kB/CBP signaling pathways. Knockdown of KMT2A significantly inhibited the promoter activities and the expressions of iNOS, COX-2 and hTERT, suppressed the activation of a serial of upstream key proteins in NF-kB signaling pathway and the translocation of NF-kB from cytoplasm to nucleus. By contrast, overexpression of KMT2A up-regulated the expression of iNOS, COX-2 and hTERT and promoted the activation and nuclear transclocation of NF-kB in melanoma cells. Additionally, we found that KMT2A activated the iNOS, COX-2, hTERT and NF-kB signaling and melanoma growth by cooperating with the transcriptional co-activator CBP. CBP could interact with KMT2A and induced the acetylation of KMT2A. Inhibition of CBP by a CBP-specific siRNA or inhibitor significantly repressed the acetylation of KMT2A and NF-kB and their binding on the promoters of iNOS or COX-2 or hTERT, thereby inhibiting iNOS/COX-2/hTERT expression and melanoma cell growth. However, overexpression of CBP increased KMT2A acetylation and promoter binding and promoted iNOS/COX-2/hTERT expression and melanoma cell growth. Moreover, our in vivo results also showed that KMT2A promoted melanoma growth by targeting the iNOS/COX-2/hTERT signaling in a xenograft mouse model. Further analyses for the clinical samples also demonstrated that KMT2A expression was positively correlated with the expression of iNOS, COX-2 and hTERT in the tumor tissues of melanoma patients, and the high expression of both KMT2A and iNOS or COX-2 or hTERT was associated with the worse clinical TNM staging and poor prognosis in melanoma patients. Taken together, our results indicate that KMT2A up-

regulates melanoma growth by activating the iNOS/COX-2/hTERT and NF-kB/CBP signaling in human melanoma. Our study therefore provides new insights into understanding the regulatory mechanism of melanoma growth and suggests that the KMT2A signaling may be a potential therapeutic target for human melanoma.

A novel non-genotoxic p53 activator rallies NK cells via stress-inducible ligand expression on melanoma cells

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Dihydroorotate dehydrogenase (DHODH) is an enzyme expressed on the outer surface of the inner mitochondrial membrane and it plays a crucial role in de novo pyramidine synthesis. Inhibition of DHODH activity results in deficiency in pyrimidine ribonlecotides, upregulation of p53, cell cycle arrest and in certain cancer cell types, apoptosis. In vivo, tumors escape immune surveillance by altering antigen presenting molecules such as HLA class I and II and stress-inducible ligands MICA/B. Here we assessed whether DHODH inhibitor treatment affects the cell surface expression of HLA class I, II and MICA/B on ARN8 cells (human melanoma cell-line). Preliminary results obtained by a mass spectrometry analysis of ARN8 cells treated with one of our own DHODH inhibitors indicated upregulation of MICB expression. Accordingly, ARN8 cells treated with DHODH inhibitors upregulated MICA/B after 24 hours in culture. As expected, we observed an increased expression of intracellular p53. To test whether MICA/B expression was p53-dependent, we increased p53 expression levels using nutlin-3, a highly selective p53 stabilizing agent and observed that MICA/B expression was induced at 24 hours. We next tested the functional consequences of MICA/B expression on ARN8 cells. Natural killer (NK) and T cells express NKG2D and are activated when NKG2D binds its ligand MICA/B. We co-cultured human PBMCs with DHODH inhibitor treated ARN8 cells for 4 hours at several different effector: target cell ratios. NK cells expressed higher levels of the cytokine IFNg and the degranulation marker CD107a, when co-cultured with DHODH inhibitor treated ARN8 cells compared untreated groups. These data indicate that DHODH inhibitor treatment not only induces cancer cell death, but also upregulates MICA/B in a p53 dependent manner and activates NK cells. We are further characterizing the mechanistic details of this regulation, which can reveal potential therapeutic benefits.

Incidence and outcomes of central nervous system metastasis in metastatic melanoma patients treated with anti-PD1 therapy

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Background

Central nervous system (CNS) metastasis are common in patients with metastatic melanoma (MM) and represent a frequent site of treatment failure with current therapies. However, little is known about the incidence, characteristics and outcomes of CNS metastasis in MM patients treated with anti-programmed death-1 (PD1) drugs and in conjunction with more intensive local CNS treatment strategies.

Methods
Under an IRB-approved protocol, outcomes of MM patients treated with anti-PD1 at The University of Texas MD Anderson Cancer Center from January 2012 to February 2016 were reviewed. The association between development of CNS metastasis and overall survival (OS) was assessed using Cox regression analysis with time to CNS metastasis treated as a time-varying covariate.

Results
We identified 264 MM patients who received anti-PD1 treatment, including 74 (28%) who had CNS metastasis prior to the first dose of anti-PD1. With a median follow-up of 10.4 months (range 0-51.6) from the start of this therapy, 37 (19% of patients without prior CNS metastasis) developed.

CNS metastasis after the initiation of anti-PD1. Of those, 27 patients were diagnosed with CNS metastasis during anti-PD1 or within 90 days of treatment discontinuation, and 10 patients were diagnosed with CNS mets >90 days after last anti-PD1 dose. The majority of these patients were male (62%), their mean age at new CNS metastasis was 62 years (range 31-86), and most patients had a history of cutaneous primary (59%). Of the 26 patients who were tested for mutations, BRAF was identified in 8 (22%, V600E in 6 patients, V600K in 2 patients), NRAS in 5 (14%) and KIT in 6 (16%). 86% received at least one CNS directed treatment approach. 62% were treated with stereotactic radiosurgery, 11% received whole-brain radiation and 30% underwent surgery. Median OS from start of anti-PD1 was 34 months (range 0-51.6 months) for the whole anti-PD1 treatment cohort. Development of CNS metastasis while on anti-PD1 therapy was strongly significantly associated with death (HR 3.39, 95% CI 2.06, 5.59, $p < .0001$).

Conclusions
To our knowledge, this is the first report describing the incidence of CNS metastasis as an initial site of disease progression in MM patients treated with anti-PD1 and associated with worse OS, despite additional CNS directed therapy.

INFα-2b inhibitory effects on CD4+CD25 +FOXP3+ regulatory T cells in the tumor microenvironment of C57BL/6 J mice with melanoma xenografts

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Background
Regulatory T cells (Tregs), particularly the CD4+CD25+Foxp3+ Tregs, down regulate immunity and promote tumor cell growth by directly suppressing CD8+ and CD4+ T cells. Alternatively they can promote tumor growth by generating interleukin-10 (IL-10) and transforming growth factor β (TGFβ) in situ, which help tumor cells to evade the immune system.

Methods
In vivo tumor models were prepared via subcutaneous injection with a suspension of B16 melanoma cells into the left upper flank of C57BL/6 J mice. The mice were randomized into five groups: radiotherapy (RT),chemotherapy (CT), radiochemotherapy (RCT), Inteferon α (INFα) groups, and a control group. Flow cytometry was used to determine the Tregs levels in the

spleen and peripheral blood, and immunohistochemistry was performed to determine the expression levels of TGFβ and IL-10 in the tumor microenvironment.

Results

Tumor weight was significantly reduced in the CT or RCT groups (40.91 % and 41.83 %, respectively), while the reduction in tumor weight was relatively lower for the RT and IFNα groups (15.10 % and 13.15 %, respectively). The flow cytometry results showed that the ratios of CD4+CD25+Foxp3+ Tregs to lymphocytes and CD4+ cells in the spleen and in peripheral blood were significantly decreased after treatment with IFNα ($P < 0.05$). Expression of TGFβ and IL-10 in the tumor microenvironment in the CT and RT groups was higher compared with the control group($P < 0.01$), while the expression of TGFβ and IL-10 in the INFα group was not significantly different ($P > 0.05$).

Conclusions

The results show that INFα-2b inhibits cancer cell immune evasion by decreasing the levels ofCD4+CD25+Foxp3+ Tregs and suppressing the expression of TGFβ and IL-10 in the tumor microenvironment.

Defining the activation state of melanoma antigen specific CD8 cells as naïve/resting memory or effector cells

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Naïve tumor antigen-specific CD8 cells typically (with few exceptions) occur in very low frequency in blood, and do not secrete IFNg or Granzyme B (GzB). Effector CD8 cells, capable of cytolysis, secrete GzB in addition to IFNg, and due to clonal expansion occur in increased frequency in blood. Resting CD8 memory cells secrete IFNg but not GzB also occurring in increased frequency in blood. Such resting memory cells will re-express GzB within several days upon antigen re-encounter, converting into effector CD8 cells.

Using this basic features of CD8 cell biology, we performed IFNg and GzB ELISPOT assays to measure the frequency of melanoma antigen-specific CD8 cells secreting these analytes 24h and 72h after antigen stimulation. Tyrosinase (Tyr), gp100 and Melan/MART-1 antigen peptide pools were tested on PBMC of healthy donors and vaccinated melanoma patients. Of the above melanoma antigens only Tyr triggered relatively high frequency (~1/1000) CD8 cells at 24h ex vivo. At this time point these CD8 cells did not produce GzB yet, however they engaged in GzB production by 72h after antigen stimulation. Therefore, Tyr-specific CD8 cells in healthy controls are clonally expanded resting memory cells (IFNg +/GzB -) that can be reactivated to become effector cells (IFNg +/GzB+) within 72h. We are presently testing whether ex vivo (24h) GzB production is elicited in vaccinated melanoma patients by Tyr revealing the conversion of these memory cells into effector cells due to vaccination, or whether vaccinated subjects show evidence for immunological priming to the other melanoma antigens to which healthy controls do not seem to be primed, using this above time resolved IFNg/GzB approach.

Downregulation of C-FLIP enhance PD-1 blockade efficacy in B16 melanoma

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PD-1 blockade therapies fail to induce responses in part of melanoma patients, how to increase the objective response rate is very important. c-FLIP overexpression occurred frequently in melanoma and there was a relationship between its expression and the prognosis of the disease. We performed this research to investigate the relationship of c-FLIP expression with the efficacy of PD-1 blockade. We demonstrate that downregulation of C-FLIP enhance the PD-1 blockade efficacy in B16 melanoma tumor model. Knockdown of C-FLIP could down regulate the expression of PD-L1 in B16 and human melanoma cells which could reduce the apoptosis of T cells through PD-L1/PD-1 axis in tumor cells and lymph cells coculture system. Moreover, we found that downregulation of C-FLIP could recruit more T cells in tumor microenvironment. Our results imply that C-FLIP has the potential to predict the efficacy of PD-1 blockade.

Use of immune checkpoint inhibitors in the treatment of patients with melanoma and preexisting autoimmune diseases: A systematic review of case reports

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Background
Checkpoint inhibitors have dramatically increased the survival of patients with certain cancers such as melanoma. However, their use can be limited by frequent immune related adverse events (irAEs) that can be fatal. The exact mechanisms mediating the occurrence of irAEs are not completely understood. Genetic background of the host could play a role, since some individuals are more predisposed to autoimmunity than others, some who develop an irAE with a given drug do not have repeated toxicity with other inhibitors, and interestingly, many patients do not develop irAEs despite continued inhibition. Therefore, patients with preexisting autoimmune diseases were excluded from all clinical trials that led to approval of these novel agents. In clinical practice, checkpoint inhibitors are often not recommended for patients with autoimmune disease because of the concern of exacerbation of the underlying autoimmune condition, or susceptibility to severe irAEs. Recently, there are few sporadic case reports of patients with cancer and autoimmune diseases who have been treated with these novel agents after failure of other line of therapies.

Objective
We conducted a systematic review of all such reported cases to summarize the existing evidence in the literature on the use of checkpoint inhibitors in patients with melanoma and preexisting autoimmune diseases.

Methods
We searched Medline, EMBASE, Web of Science, PubMed ePubs, and the Cochrane Central Register of Controlled Trials (CENTRAL) through June 2016 with no restrictions. References cited in the included articles were also searched manually. We included case reports describing patients with melanoma and an established diagnosis of autoimmune disease before starting

treatment with the checkpoint inhibitors. We extracted data on patient's characteristics (age, gender, preexisting autoimmune disease, treatment given for the underlying disease, and whether the patients had active disease symptoms at the time of checkpoint inhibitor treatment), type of checkpoint inhibitors used, reported irAEs, how they were managed, and their clinical outcome, if discontinuation of immunotherapy was required, and if treatment rechallenge was reported.

Results

Forty seven patients with melanoma and preexisting autoimmune disease were found. More than 35% of the reported cases did not experience irAE or disease exacerbation, despite many patients having active autoimmune disease at the time of checkpoint inhibition. Those with exacerbation of the preexisting autoimmune disease, had manifestations similar to those occurring before checkpoint therapy, and none developed new disease features. Both exacerbation and de novo irAEs were easily managed with corticosteroids and few required more aggressive immunosuppressant. Resolution of irAEs was achieved in the majority of cases without the need for discontinuation of immunotherapy. Death was reported in only two cases who managed inappropriately.

Conclusions

Checkpoint inhibitors can be beneficial in cancer patients, despite coexisting autoimmune disease, under careful supervision. Nevertheless, because few patients have been treated and reported, additional studies are warranted to further establish the risk-benefit profile of this novel therapy in this population.

Pembrolizumab Expanded Access Program (EAP) in Taiwan for patients with progressive advanced melanoma after prior ipilimumab treatment

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Pembrolizumab, a programmed death 1 (PD-1) inhibitor, has been approved for treatment of advanced melanoma based on a significant survival improvement in patients who are refractory to other treatments and as the first line treatment. We evaluated the clinical activity of pembrolizumab in melanoma patients in Taiwan under the expanded access program. From December 2014 through June 2016, we enrolled patients with progressive unresectable stage III/IV melanoma, who have previously received ipilimumab and BRAF/MEK inhibitors if BRAF mutation. Pembrolizumab was given at 2 mg/kg every three weeks continuously until development of intolerable side effects or progression of disease per study protocol's designation. Data were collected from 23 advanced melanoma patients (15 cutaneous, 6 mucosal and 2 of unknown primary). 20 patients (61%) received at least 2 lines of treatment previously, and 6 patients (26%) with stable brain metastases were enrolled. Median overall survival (OS) was 6.7 months (CI 95% 4.6-8.8). 12-month OS was 38% and 15-month OS was 30%. Among 20 evaluable patients, 4 patients achieved a partial response, and 8 patients achieved a stable disease, with an overall response rate of 20.0% (CI 95% 0-39%) and clinical benefit rate of 60% (CI 95% 36-84%). The most common drug-related adverse events of any grade were rash (35%), pruritus (30%), and fatigue (30%). Two deaths were considered treatment-related; one cytokine storm and one ventricular tachycardia. Pembrolizumab's activity in melanoma as a second line immune checkpoint inhibitor (after prior exposure of ipilimumab)

was confirmed in Taiwan. Durable response, even in the previously heavily-treated patients, has been observed.

Prognostic relevance of baseline neutrophils and neutrophil-to-lymphocyte ratio in metastatic melanoma receiving anti-PD1 therapy

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Intro
Immunotherapy is a very effective modality of treatment for metastatic melanoma. Anti-PD1 or PDL-1 as single agents, or in combination with Anti-CTLA4 therapy, are achieving impressive long-term durable responses. Unfortunately, the majority of patients do not benefit from these therapy and biomarkers to better select the patients are lacking. Baseline absolute neutrophils and Neutrophil-to-lymphocyte ratio (NLR) are cheap and effective ways of measuring inflammation, and its prognostic role is well known before anti-CTLA4 therapy. We hypothesized if baseline absolute neutrophils and NLR are useful as prognostic markers before anti-PD1 therapy.

Methods
Prospectively collected data from 53 metastatic melanoma patients treated with Nivolumab 3mg/kg q14d in an EAP from a single Brazilian institution (AC Camargo Cancer Center) were analyzed. The absolute neutrophils count and NLR were calculated from baseline peripheral blood cell counts. We stratified patients according to dichotomized Neutrophils ≥ 7500 and NLR ≥ 5. Survival was calculated by Kaplan-Meier method and Cox proportional hazard model was used to test the impact of baseline neutrophils and NLR on progression-free (PFS) and overall survival (OS).

Results
The median follow-up was 6,8m. The median PFS for the entire group was 5,8m and the median OS was not reached (NR). Baseline neutrophils and NLR were significantly associated with outcome of nivolumab-treated melanoma patients, in terms of PFS and OS. For neutrophils ≥ 7500 vs. < 7500, the median PFS was 2,3m vs. 6,5m (p = 0,002; HR = 2,9; CI 1,08 – 7,77) and the median OS was 2,62m vs. NR (p = 0,03; HR = 3,77; CI 0,99 – 14). For NLR ≥ 5 vs. < 5, the median PFS was 2,3 vs. 9,8 (p = 0,001; HR = 3,22; CI 1,4 – 7,13) and the median OS was 3,5m vs. NR (p = 0,001; HR = 7,07; CI 2,2 – 22,5).

Conclusion
Although these findings need to be confirmed in a larger cohort of patients, baseline neutrophils and NLR may be a cheap and useful tool for assessing prognosis of patients using anti-PD1 therapy.

Cytotoxic T cell cooperation is required to overcome melanoma resistance to immunotherapy

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Immunological control of tumor progression requires the activation and expansion of tumor-specific cytotoxic T-lymphocytes (CTL), followed by an efficient infiltration of the tumor lesion. CTL eliminate tumor cells in an antigen and cell-contact dependent manner and based on in vitro evidence, lethal hit delivery is considered to be a rapid and binary yes/no process. Despite its significance for anti-tumor immune responses, the principles of CTL effector function and apoptosis induction within established tumors in vivo remain unclear. Using a collagen-based 3D organotypic assay and time-lapse microscopy we show that CTL effector function against melanoma cells is an inefficient process with a high failure rate which is rarely completed by a single CTL interaction, but requires a sequence of sublethal hits, delivered by multiple CTL. Optical reporters for CTL-induced damage (perforin-mediated Ca2+ influx and nuclear envelope rupture) confirmed the induction of sublethal damage to the cellular and nuclear membranes, and allowed to visualize serial CTL hits followed by repeated recovery of the melanoma cell. Quantification of CTL killing kinetics in three murine and human melanoma models and statistical modeling confirmed that apoptosis induction in melanoma is achieved by multiple additive CTL contacts and the accumulation of a death signal within the target cell ("additive cytotoxicity"). Thus, CTL effector function is not a binary process but instead depends on local CTL densities to deliver multiple hits and the integration of synergizing or interfering parameters which may tune damage versus recovery in the target cell. Additive cytotoxicity further implies CTL lethal hit delivery as a gradual and thus, tunable process, which is amenable for therapeutic targeting by increasing either the impact of single hits or their frequency.
In the tumor microenvironment in vivo, tissue structures can form barriers for CTL infiltration and may determine tumor resistance niches by limiting CTL access to tumor regions. Using intravital multiphoton microscopy, we mapped tissue topography together with CTL migration during adoptive immunotherapy of melanoma. Consistent with the in vitro data, serial engagements and tumor-cell apoptosis induction were confined to regions with high CTL density, which supported additive cytotoxicity. The highest frequency of serial engagements followed by near-complete melanoma cell elimination was reached in the invasive tumor front where moving tumor cells and CTL collided within the same pro-migratory tissue compartment, suggesting invading tumor cells as promising target for immunotherapy. To target tumor regions which were poorly infiltrated by CTL, we applied agonistic anti-CD137 antibody and visualized its immune-augmenting impact on the CTL effector phase in melanoma. CD137 (4-1BB) is a TNFR family costimulatory receptor which is expressed by activated T cells and other immune cells. CD137 ligation by agonist antibodies promotes immune cell proliferation, memory formation and enhanced effector function. Significant therapeutic effects of CD137-stimulation in solid tumor models have provided a rationale for ongoing clinical trials; however, underlying mechanisms remain unclear. Using intravital imaging, we provided direct evidence that stimulation of CD137 enhances the effector function of adoptively transferred CTL within the tumor lesion. The combined treatment increased CTL numbers in the tumor and prolonged CTL contacts with tumor cells which resulted in increased killing efficiency. It further sustained the effector function and viability of adoptively transferred CTL and consequently, enhanced tumor remission.
In conclusion, serial interaction of CTL with target cells and additive induction of death signal define the efficacy of CTL effector function which can be exploited by targeted therapy to increase both single contact efficacy and CTL cooperativity during anti-cancer immunotherapy.

Successful treatment of arthritis induced by checkpoint inhibitors with tocilizumab while preserving the anti-tumor immunity

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Background

Despite their clinical benefits, checkpoint inhibitors (CPI) are associated with immune-related adverse events (irAEs). Two percent of patients receiving CPI(s) develop arthritis (arthritis-irAE). The optimal treatment strategy for arthritis-irAE while maintaining anti-tumor immunity is unknown. Altered Th17 cells contribute to the development of many autoimmune diseases, including autoimmune arthritis. Interleukin (IL)-6 is critical in Th17 cell differentiation, and the anti-IL-6 receptor antibody tocilizumab is used to treat autoimmune arthritis. Notably, the role of IL-6 is minimal in the differentiation and function of Th1 cells, another CD4 T cell subset governing anti-tumor immunity. We recently reported a patient with metastatic melanoma whose Crohn's disease was well controlled with tocilizumab during CPI therapy, which induced complete remission of the melanoma. Here, we report the use of tocilizumab for two patients in whom arthritis-irAE was successfully controlled by tocilizumab while maintaining anti-tumor immunity.

Case 1

71-year-old man with a history of atrial fibrillation was diagnosed melanoma of the scalp in 1981 and treated with wide local excision. He had been well until August 2014, when melanoma recurred in the right parotid gland. He underwent right parotidectomy followed by radiation therapy. However, two new melanoma lesions were found in the left buttock, and ipilimumab was initiated. After the 4th cycle of ipilimumab, the patient developed severe fatigue and bilateral shoulder pain. He was referred to the rheumatology clinic in September 2015. Physical examination revealed mild lethargy without active synovitis. Rheumatology workup was unremarkable except for weakly positive rheumatoid factor. The symptoms responded well to prednisone but recurred shortly after prednisone was tapered off. Thereafter, we re-initiated prednisone 40 mg, but it caused severe atrial fibrillation. Tocilizumab was initiated in January 2016, and 2 months later, the arthritis got in remission and the prednisone was tapered off successfully. In August 2016, prednisone 5 mg was started due to persistent fatigue secondary to adrenal insufficiency. At present, he is doing well without fatigue, joint pain, or palpitations. Importantly, his melanoma remains in remission and he is not requiring any systemic anti-cancer therapy at this time.

Case 2

65-year-old man was diagnosed melanoma on the left elbow in December 2009 and treated with wide local excision followed by adjuvant interferon therapy. The melanoma recurred in the left ilium in May 2012. The patient received biochemotherapy plus radiotherapy, but two metastatic lesions were found, one in each humerus; the lesions were surgically removed, but in August 2014, three new lesions were found on the chest wall, right thigh and left hip. The patient received 4 cycles of ipilimumab over 9 weeks, but the lesions progressed; ipilimumab was stopped and pembrolizumab was initiated. One week after receiving the 2nd cycle of pembrolizumab, he developed generalized arthralgia and was referred to the rheumatology clinic. Physical examination revealed severe bilateral tenderness over the hands, knees, and shoulders, with mild effusion in the right knee. Rheumatology workup for autoimmune arthritis was negative. The symptoms responded well to prednisone 40 mg but the patient failed to taper it down to 20 mg. Tocilizumab was initiated in September 2015, and the arthritis improved

significantly. Prednisone was tapered off and the patient completed the 6th cycle of pembrolizumab. At present, the patient has bearable bilateral hand pain without active arthritis. Importantly, his melanoma remains stable and he is currently on treatment with an investigational therapy.

Conclusions

These two cases suggest that tocilizumab is an attractive option to treat irAEs in patients receiving CPI(s) while maintaining the anti-tumor effect of the CPI(s).

Lymphoma

The potential role of CD37 tetraspanin in adaptive immunity and sensitivity to PD-1 blockade in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B-cell lymphoma. After treatment with the standard immunochemotherapy rituximab-CHOP, 30-40% of DLBCL patients develop refractory or relapsed disease. PD-1/PD-L1blockade reconstituting antitumor immunity has been successful in Hodgkin lymphoma but not in non-Hodgkin lymphoma. It is critical for ongoing trials to identify subsets of patients with DLBCL sensitive to anti-PD-1 immunotherapies. CD37 (TSPAN26) is a member of the tetraspanin superfamily, widely expressed on normal and malignant matured B-cells and downregulated in plasma cells. CD37 form complexes with integrins, MHC-II and other tetraspanins on B-cells. It has been documented that CD37 plays important roles in T-cell–B-cell interactions, B-cell humoral response triggered by B-cell receptor cross-linking, and a balance between immune responses and tolerance. Interestingly, in a large cohort of DLBCL patients (n=527), we found that loss of CD37 expression in DLBCL predicts strikingly decreased overall and progression-free survival. PDCD1 gene expression was upregulated in CD37-negative ABC-DLBCL, and the costimulatory molecule ICOSLG was upregulated in CD37+ GCB-DLBCL. We further analyzed PD-1 and PD-L1 expression at the protein levels concurrently with immunomarkers for B cells, helper and cytotoxic T cells, macrophages, and NK cells using the multiplex technology, which confirmed that PD-1 levels were increased on both cytotoxic and helper T cells in CD37-negative DLBCL. These discoveries may suggest that CD37 is important for antitumor adaptive immunity, immune dysregulation plays an important role for poor clinical outcomes in DLBCL, and CD37-negative DLBCL may be sensitive to PD-1 blockade and/or ICOS agonists. In summary, loss of a B cell-specific tetraspanin CD37 was found to correlate with PD-1 overexpression in DLBCL clinical samples, and CD37 may serve as a novel biomarker for anti-PD-1 immunotherapy selection in DLBCL.

PD-L1 is Up-regulated by EBV-driven LMP1 Through NF-κB Pathway and Correlates with Poor Prognosis in Natural Killer/T-cell Lymphoma

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Background
Natural killer/T-cell lymphoma (NKTCL) is an EBV-associated, highly aggressive lymphoma. Treatment outcome remains sub-optimal, especially for advanced-stage or relapsed diseases. Programmed cell death receptor 1 (PD-1) and PD ligand 1 (PD-L1) have become promising therapeutic targets for various malignancies, but their role in the pathogenesis and their interactions with Epstein–Barr virus (EBV) in NKTCL remains to be investigated.

Materials and methods
Expression of PD-L1 was measured in NK-92 (EBV-negative) and SNK-6 (EBV-positive) cells by western blot, quantitative real-time PCR, and enzyme-linked immunosorbent assay,

respectively. Latent membrane protein 1 (LMP1)-harboring lentiviral vectors were transfected into NK-92 cells to examine the correlation between LMP1 and PD-L1 expression. Proteins in the downstream pathways of LMP1 signaling were measured in NK-92 cells transfected with LMP1-harboring or negative control vectors, respectively. PD-L1 expression on tumor specimens and serum concentration of soluble PD-L1 were collected in a retrospective cohort of patients with Ann Arbor stage I~II NKTCL and their prognostic significance were analyzed.

Results

Expression of PD-L1 was significantly higher in SNK-6 cells than in NK-92 cells, at both protein and mRNA levels. Expression of PD-L1was remarkably up-regulated in NK-92 cells transfected with LMP1-harboring lentiviral vectors compared with those transfected with negative control vectors. Proteins in the MAPK/NF-κB pathway were up-regulated in LMP1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induced significant down-regulation of PD-L1 expression in LMP1-expressing NK-92 cells. Patients with a high concentration of serum soluble PD-L1 (≥ 3.4 ng/ml) or with a high percentage of PD-L1 expression in tumor specimens (≥ 38%) exhibited significantly lower response rate to treatment and remarkably worse survival, compared with their counterparts. A high concentration of serum soluble PD-L1 and a high percentage of PD-L1 expression in tumor specimens were independent adverse prognostic factors among patients with stage I~II NKTCL.

Conclusions

PD-L1 expression positively correlated LMP1 expression in NKTCL, which was probably mediated by the MAPK/NF-κB pathway. PD-L1 expression in serum and tumor tissues have significant prognostic value for early-stage NKTCL.

TCL1-specific T-cell receptor gene transfer redirects T lymphocytes to display effective antilymphoma reactivity

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T-cell leukemia/lymphoma 1 (TCL1) oncoprotein is overexpressed in multiple forms of B-cell lymphoma. Previously, we demonstrated that TCL1-specific CD8+ T cells specifically and selectively lysed lymphoma cells, indicating that TCL1 is an excellent target for immunotherapy. In this study, we examined the feasibility of adoptive therapy using T cells transfected with an HLA-A*0201–restricted TCL1 71-79-specific T-cell receptor (TCR). Lentiviruslly transduced T cells recognized TCL1 71-79 peptide-pulsed but not control target cells. Furthermore, TCR-redirected CD8+ T cells lysed TCL1-overexpressing human lymphoma cells in an HLA-A*0201–restricted manner, but did not kill HLA-A*0201+ normal cells. In addition, we found certain mutations in the CDRs region of the TCR can improved the avidity of TCR. Collectively, our data demonstrate the feasibility of redirected T cell–based TCL1-specific immunotherapy for the treatment of human B-cell lymphoma.

αβ-Double Negative T cells (αβ-DNTs): Role on Tumor Surveillance and Relevance as Prognostic Factor of Clinical Outcome in Lymphoma and Solid Tumors

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The mutual interactions between the host immune system and cancer cells may either promote or control oncogenesis.Cancer immunotherapies remain viable approaches to sustain patient survival.However, positive clinical response of phase I/II clinical trials remains still to low.Double-negative T-cells (DNTs) showed to contribute specifically to anti-tumor in vitro activity against lymphoma and melanoma cells.They express either αβ or γδ TCR but lacking CD4,CD8,CD56 and are involved in immune regulation and tolerance acting as regulatory T-cells (Treg) and/or cytotoxic T cells.However no data are available on:The prognostic significance;The modulation during the therapy particularly after treatment with immunomodulating drugs;The correlation with the tumor burden to evaluate their role in tumor surveillance or progression.These findings has a strong clinical valence and might allow us to design new approaches of therapeutic strategy.

We aimed to evaluate the frequency and functional attitude of DNTs and their relative modulation following chemio-immunotherapy in lymph nodes (LN), bone marrow (BM) and peripheral blood (PB) of both lymphoma (Ly),Renal Cell Carcinoma (RCC) and Metastatic Melanoma (MM) patients (pts) in order to assess their potential role on clinical outcome and therapy response as well as tumor surveillance and progression.

We collected PB and BM samples of 160 Ly pts at diagnosis and PB samples of 16 HD as controls.To evaluate the role of αβ-DNTs in tumor surveillance or progression we studied their interactions and functional correlation with the tumor burden in 20 fresh LN from pts clinically suggestive of Ly,52 RCC pts and 50 MM pts who received Ipilimumab (Ipi) as second line therapy.To study the effects of immunomodulating drugs we evaluated their frequency during Ipi treatement in MM pts at :1.Start of therapy;2. 3th infusion,3.Finish of therapy.The ontogeny, functional attitude and TCR clonality of DNTs were evaluated by: CD3,CD4,CD8,CD56,CD45,TCRαβ,CD45Ra,CD45Ro,CCR7,CD27,CD28,CD30,CD69,GITR,CD95,CD178,CD152,IFN-γ,TNF-α,perforin and granzymeB.Data were acquired by 8-colour flow cytometer and analysed using Kaluza software.Immune cells such as DCs,MDSCs and Treg was also detected to evaluate the correlation with DNTs.

We observed a significant decrease (p =0.006) of αβ-DNTs in untreated Ly pts (20.5 cells/ul ± 4.8) as compared with HD (31.3cells/ul ± 3.4) and their number seemed to be modulated during the follow-up.In HL pts the frequency of αβ-DNT was significantly increased as compared with other histotypes (p=0.005).Furthermore, the αβ-DNTs in LNs of Ly pts were significantly reduced as compared to RFH-LNs (p=0.006).Functional attitude study showed a more highly differentiated phenotype such as Temra in αβ-DNTs from cancer pts than controls.When evaluated in the other malignancies the αβ-DNTs were significantly decreased (p=0.001) in MM pts (13,49cells/ul±5,4) and RCC pts (14,75 cells/ul±2,1) as compared with HD and more interestingly when compared with Ly pts (p=0.001) given the greater immunological impairment of RCC/MM tumor burden.Furthermore,αβ-DNTs was significantly increased (p=0,048) in MM pts with ECOG performance status ≤1 as compared with >1.Finally, ex vivo expanded DNTs

acquired an immunomodulatory cytokine profile,characterized by the secretion of IFN-gamma and granzyme B,which are known as central components of anti-tumor immune responses supporting the hypothesis of αβ-DNT potential use in immunotherapeutic strategies.
Our data showed an inverse correlation between the frequency of αβ-DNTs and the tumor condition as well as that they could play an important role in both the development and the progression of lymphomas.

This first study compares the frequency of αβ-DNTs in three different pathologies correlating to tumor burden.In addition,it is likely that ex-vivo expanded DNTs exert an anti-tumor activity thus suggesting their possible use as a new strategy for adoptive immune-therapy.

Absence of Grail promotes CD8+ T cell anti-tumor activity

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T cell tolerance is a major obstacle to successful cancer immunotherapy; thus, it is of high priority to develop strategies to break immune tolerance. Here we report that expression of the E3 ubiquitin ligase Grail is significantly upregulated in CD8+ T cells infiltrated into transplanted lymphoma tumors and Grail-deficiency confers long-term tumor control. Importantly, therapeutic transfer of Grail-deficient CD8+ T cells was sufficient to repress established tumors. Mechanistically, loss of Grail enhanced anti-tumor reactivity and functionality of CD8+ T cells. In addition, Grail deficient CD8+ T cells exhibited increased IL-21R expression and hyper-responsiveness to IL-21 signaling as Grail promotes IL-21R ubiquitination and degradation. Moreover, CD8+ T cells isolated from lymphoma patients expressed high levels of Grail and lower levels of IL-21R compared with normal donors. Altogether, our data demonstrates that Grail is a crucial factor controlling CD8+ T cell function and is a potential target to improve CTL activity.

Dickkopf-3 (DKK3) regulates follicular cytotoxic T cells function in follicular lymphoma

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Dickkopf (DKK) family encodes secreted proteins and consists of four main members (DKK1, 2, 3, 4). DKK proteins contain s signal sequence and share two conserved cysteine-rich domains, while DKK3 contain a unique soggy (sgy) domain. DKK1, 2, and -4 regulate Wnt signaling, while DKK3 does not. DKK3 has been reported to suppress tumor cell proliferation in vitro. DKK3 mutant mice show changes in the frequency of NK cells, IgM, hemoglobin and hematocrit levels, as well as lung ventilation. Furthermore, DKK3-deficient mice display hyperactivity. Those data indicated DKK3 play as an immune modulator. However, the physiological function of this evolutionarily conserved molecule is unknown.

Recently study demonstrated that DKK3 expression enhanced in the tolerant CD8 T cells while not in activated T cells in mouse model. Further, by blocking DKK3 with antibody, T cells tolerance and proliferation can be restored. However, we found that DKK3 showed higher expression level in CXCR5+CD8+ T cells (also name follicular CD8 T cells) in human. The

CXCR5+CD8+ T cells also showed strong cytotoxic capacity by producing higher amount IFN-γ and TNF-α, as well as GZMA, K, M, and -H. Consistent with mouse model human CXCR5+CD8+ T cells showed less proliferation. We try to delineate the mechanism of DKK3 in regulating CD8 T cells developments by knockdown and knock-in DKK3 in primary T cells subsets. T cells proliferation, cytotoxic capacity, transcript regulation, and epigenetic regulation in inducible endogenous or knockdown DKK3 expression in CD8 T cells will be performed. In our previous study, we found DKK3 also participated Wnt and TGF-b signal pathways in CD8 T cells developments, primary proteins in these pathways will be also monitored. Further, blocking DKK3 in follicular lymphoma tumor filtrating cytotoxic T cells with antibody or small molecules will be screened.

To fully study DKK3 regulation in balance of memory and effective CD8 T cells maturation, we try to enhance novel strategy in adoptive T cells immunotherapy and novel vaccination.

LUNG

Circulating miRNAs in lung cancer are associated to pro-tumorigenic and immunosuppressive microenvironment

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Background
We previously reported the identification of diagnostic miRNA signatures in plasma samples of lung cancer patients detected by low dose computed tomography (LDCT) screening. Circulating miRNAs are released into the bloodstream by different mechanisms such as passive leakage from damaged cells or active secretion through extracellular-vesicles or protein complexes.

Methods
To evaluate the potential origin and the release of the 24 miRNAs of the diagnostic signature we analyzed their expression by real-time or digital PCR in both cells and conditioned medium (CM) from cancer cell and different cell types of the lung microenvironment. Lung tissues and cell-blocks were analyzed by miRNAs in situ hybridization (ISH). Modulation of miRNAs after in vitro treatments known to induce changes associated with cancer progression, in different cell types was assessed and correlated to changes observed in circulating miRNAs signatures.

Results
24-miRNAs analysis showed higher abundance in specific cellular components such as mir-145 in fibroblasts, mir-126 in endothelial cells, mir-133a in skeletal muscle cells or mir-451 and 142-3p in hematopoietic cells. Generally, tumor cells showed lower levels of miRNAs compared to bronchial epithelial cells. MiRNAs specific localization in lung tissue was confirmed by ISH. We observed that mir-451 is specifically expressed in lung interstitial alveolar walls while mir-126 by endothelial cells outside the tumor bulk; miR-145 is characteristic of fibroblast and muscle cells and miR-142-3p of hematopoietic cells, fibroblast and muscle whereas mir-21 is over-expressed in the tumor.

The analysis of miRNAs in CM showed that miRNAs secretion is correlated with cellular expression for most cell types (Pearson correlation range: 0.41-0.80). Interestingly, platelets and granulocytes were the components that mostly secreted miRNAs.

In vitro experiments showed that endothelial cells under hypoxic condition up-regulate mir-126 and that mir-145 was up-regulated and secreted in lung cancer-associated compared to normal fibroblasts. Interestingly, during conversion of T lymphocytes into T regulatory cells up-regulation of mir-15b, mir-19b and mir-320 was observed whereas mir-15b and mir-197 were up-regulated in the conversion of macrophages into M2 phenotype. Modulation of miRNAs in immune and stromal cells was consistent with up-regulation of the same miRNAs observed in plasma samples.

Conclusion

Our findings on the origin of circulating miRNAs support the conclusion that plasma miRNAs are heterogeneous and secreted by different cellular components of lung microenvironment rather than by tumor cells. In particular, we demonstrated that a pro-tumorigenic and immunosuppressive microenvironment contributes to the de-regulation of miRNAs observed in plasma of lung cancer patients.

N-myc interactor suppresses tumor growth by inhibiting targeting NF-κB/p300/COX-2 and PI3K/Akt signaling and predicted a better prognosis in human lung cancers

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N-myc and STAT interactor (NMI) binds to different transcription factors such as c-myc, N-myc, Sox-10 and STATs to regulate a variety of signaling mechanisms including DNA damage, cell cycle and epithelial-mesenchymal transition. However, the role of NMI in the regulation of lung cancer growth and progression remains poorly understood. In this study, we investigated the regulation of NMI on lung cancer growth and identified the underlying molecular mechanisms. NMI expression was found to be up-regulated in normal lung cells and tissues but down-regulated in lung cancer cells and tumor tissues. Overexpression of NMI suppressed lung cancer cell proliferation, colony formation and cell migration, which was mediated by inhibiting the expression of COX-2, phosphorylated PI3K/Akt, and MMP2/MMP9 in lung cancer cells. In contrast, knockdown of NMI by shRNA significantly promoted lung cancer cell growth and cell migration by up-regulating the NF-κB/COX-2 and PI3K/Akt signaling. Moreover, we demonstrated that NMI overexpression suppressed the p300-mediated acetylation of NF-κB and the binding of NF-κB and p300 on the COX-2 promoter, thereby inhibiting the transcription of COX-2 in lung cancer cells. The in vivo data further verified the NMI-mediated negative regulation of COX-2 expression and tumor growth in a lung cancer mouse model. Furthermore, tissue microarray immunohistochemical analysis of lung adenocarcinomas also demonstrated that NMI expression was negatively correlated with COX-2. Kaplan-Meier and multivariate survival analysis indicated that the patients with high levels of NMI had better prognosis and NMI was an independent prognostic factor for overall survival of the patients with lung adenocarcinomas. Collectively, our results indicate that NMI inhibits tumor growth through the simultaneous modulation of multiple signaling pathways and predicts a better prognosis in human lung cancer, suggesting that NMI may be a potential new tumor suppressor in human lung cancers.

Immune cell sub-populations as potential predictors of response to nivolumab in non-small cell lung cancer

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Introduction
Immune check-point inhibitors are becoming a cornerstone for the management of previously treated non-small cell lung cancer (NSCLC); however, consistent prognostic and predictive factors for this drug class are still lacking. Since these compounds enhance the immune response against tumor cells, it is possible that distinctive patterns in the circulating immune cell sub-populations might be associated with different responsiveness. Our aim was to determine whether variations in these populations might predict objective response to nivolumab in NSCLC.

Methods
Blood samples were collected and stored from patients receiving nivolumab (3 mg/Kg every 14 days) for previously treated advanced NSCLC within a single-institutional translational research study conducted in the San Martino Hospital – National Institute for Cancer Research, Genova, Italy (approved by the local ethical committee). Sample collection was performed before each administration for 4 consecutive cycles, followed by computed tomography (CT)-scan. The response evaluation criteria in solid tumors (RECIST) v. 1.1 and the immune-related response criteria (irRC) were employed; the observed responses were categorized as partial response (PR), stable disease (SD), and progressive disease (PD). Peripheral blood mononuclear cells (PBMC) were analyzed for the frequency of natural killer (NK) cells, B cells, and T-cells; the latter were further divided into CD8+ T cells, exhausted CD8+ T cells, CD4+ cells, and regulatory T cells (Tregs). The relative frequencies and the ratios between the sub-populations at each sample collection were compared with radiological response.

Results
Fifty-four patients were considered eligible; their demographic features were the following: median age= 70 years (44-85); male/female: 70%/30%; current or former smokers= 87%; non-squamous/squamous histology= 80%/20%. Patients achieving PR at the first RECIST assessment had a significant up-regulation of Tregs (CD4+ Foxp3+ CD39+ cells; p= 0.021), as well as a decreased CD8+/Treg ratio (p= 0.033) at the baseline sample. Conversely, patients experiencing PD at the first RECIST assessment had a significantly up-regulated CD8+/Treg ratio at cycle 2 (p= 0.029). Finally, patients experiencing PD at irRC had a higher proportion of activated T cells (PD1+ CD56+ CD3+) compared to the patients achieving SD and PR/CR (p= 0.018) at cycle 2.

Conclusion
The proportions of Tregs and activated T cells are apparently correlated with different responses to nivolumab according to RECIST and irRC. While the immunologic mechanisms at the basis of these findings have yet to be defined, further studies involving the predictive role of PBMC during treatment with immune check-point inhibitors for NSCLC are highly advised.

Intratracheal delivery of immunostimulatory oligonucleotides using biodegradable polyketal nanoparticles: effect on murine lung cancer

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Background
Lung cancer is the leading cause of cancer deaths in the US and is highly resistant to immune surveillance. Recent studies demonstrated that direct delivery of CpG oligodeoxynucleotides (ODNs) into tumors stimulates TLR9-expressing cells and overcomes the local immunosuppressive tumor milieu. Our strategy for treating lung cancer involves intratracheal CpG ODN administration using an engineered nanoparticle-encapsulated system to improve tumor targeting and pulmonary retention.

Methods
Biodegradable polyketal (poly-[1,4-phenyleneacetone dimethylene ketal] matrix) nanoparticles (MW, 4000; particle size, 200-600 nm, endotoxin contamination < 0.1 EU/mL determined by LAL test), which enable safe and efficient intratracheal administration of therapeutic agents, were used as ODN nano-carriers. ODNs were adsorbed onto the polyketal nanoparticles using the water-in-oil-in-water double-emulsion solvent evaporation method (polyketal-ODN conjugation ratio of 1:27-30, inclusion efficacy of ODN is approximately 80% determined by spectrophotometric analysis of fluorescent conjugated ODN-polyketal particles). In vitro study, polyketal-CpG ODN showed TLR9 dependently increase in cell proliferation and IL-12 production in murine splenocytes. In vivo study, intratracheal administration of polyketal-CpG ODN induced 1.5-2 times higher accumulation of macrophages, 5-10 times higher accumulation of lymphocytes, and 5-10 times higher production of IL-12 in the lungs analyzed by bronchoalveolar lavage fluid collected 2 days after instillation compared to those administered of same amount of free ODN. To evaluate their efficacy against lung cancer, we developed a fatal model mimicking primary lung cancer by intratracheal instillation of 106 Lewis lung carcinoma cells in C57BL/6 mice, resulting in peribronchial tumor formation with an approximately 22-day median survival. In this model, tumor nodules become associated with lymphoid structures known as tumor-induced bronchial associated lymphoid tissue (T-BALT), similar to that seen in human non-small cell lung cancer. Intratracheal delivery of free- or polyketal-ODNs was evaluated weekly, starting 7 days after implantation of carcinoma cells.

Results
Significant improvement in survival at 60 days was observed in the mice treated with polyketal-CpG ODNs (82%) compared with the mice treated with the same amount of free CpG ODNs (38%) or untreated mice (0%). The effect of polyketal-CpG ODNs was dose dependent in the 10-50 µg of ODN concentration range and less effective with systemic administration (12%). The effect was also abrogated in mice treated with the same amount of polyketal-non-CpG ODNs. Polyketal-CpG ODNs reversed the characteristic immunosuppression especially seen in T-BALT adjacent tumor micro-environment by i) decreasing the number of immunosuppressive Tregs and M2 macrophages and ii) increasing the number of tumoricidal CD8+ T cells and M1 macrophages.

Conclusion
Our polyketal-CpG ODN delivery method is an effective immunotherapy for lung cancer (Sato T, et al., Mol Cancer Ther 2015; 14: 2198).

Radiation Reduces B Cell Representation in TC-1 Mouse Lung Adenocarcinoma Cells

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Purpose
For radiotherapy and immunotherapy to be integrated into a combined protocol, it is imperative to understand how radiation affects immune function. This study investigated the impact of radiation therapy and/or anti-PD1 antibody on the systemic and regional immune responses in TC-1 mouse lung adenocarcinoma cells.

Materials and Methods
TC-1 mouse lung adenocarcinoma cells were injected s.c. into syngeneic mice at right thigh, which was in the radiotherapy field. When tumors were palpable, mice were randomly assigned into four groups: no treatment, radiotherapy alone (8 Gy x 2 fractions in consecutive days), anti-PD1 antibody alone, and combination of radiotherapy and anti-PD1 antibody. Mice were followed for tumor growth/regression. Systemic and regional immune responses were assessed in splenocyte and tumor drainage lymphocyte by identifying Th1, Th2, Treg, Tc, NK-T cells and B cells population via flow cytometry.

Results
Treatment with anti-PD1 antibody alone had no obviously detectable effect, but radiotherapy caused comparable tumor growth delay. Increasing Treg cell but decreasing B cell population was found in both tumor site and immune organ following local radiotherapy. The number of Th2 cells mildly increased in both tumor site and immune organ through radiotherapy and/or anti-PD1 antibody treatment.

Conclusions
The effect of radiation on immune function was complex. Radiation regulated immune responses by escalating Treg and Th2 cells but also de-escalating B cell representation in TC-1 mouse lung adenocarcinoma cells.

Cancer associated fibroblast conferred resistance to anticancer drugs in lung cancer

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With the development of targeted medicine directed at driver mutations in lung cancers, like epidermal growth factor receptor tyrosine kinase inhibitor(EGFR-TKI), lung cancer treatment has advanced into the era of molecular targeted therapy. However, complete clinical responses are rare, and drug resistance is still a big challenge to the treatment of patients. Many mechanisms regarding therapy resistance have been proposed. Besides cell-autonomous mechanisms, like EGFR T790M mutation, it is believed that tumor microenvironment plays an important role in drug resistance. Some studies have elicited stroma-mediated resistance to targeted agents in melanoma. However, in lung cancers, the impacts of stromal cells on targeted agents and chemotherapy still remain unclear. This study aimed to study the effects of cancer associated fibroblast(CAF), which is abound in tumor stromal, on the resistance of EGFR-TKIs and chemotherapy drugs in lung cancer.

We collected primary CAF cells via fresh tumor tissues, then co-cultivated together with EGFP-labelled cancer cells in 384-well plates. Cancer cells were treated with drugs the day after plated. EGFP fluorescence was read 3 days later, using the Synergy H4 Hybrid Microplate Reader (BioTek). A fluorescence microscope (Olympus IX51) was used to document bright-field and EGFP images on day 4. The drug effect on each cancer cell line in the presence or absence of CAF was calculated by normalizing the number of cells after 3 days of treatment to the number of cells present in the dimethylsulphoxide (DMSO) control wells. The CAF-mediated effects on drug resistance were calculated by subtracting the 'without CAF' drug effect from the 'with CAF' drug effect.

CAF cells obtained from fresh tumor tissues were all confirmed by immunofluorescence in order to eliminate the contamination of epithelial and endothelial cells. These cells showed high expression of PDGFR-α and α-SMA, but little-to-no expression of CD31, marker of endothelial cell, and AE1-AE3, marker of epithelial cell. Thus, high purity of fibroblasts was assured. We chose two lung adenocarcinoma cell lines, PC-9 and HCC4006, which harbored EGFR mutations of exon 19 deletion. CAF-mediated resistance to anticancer drugs, including gemcitabine, docetaxel, erlotinib, and WZ4002, was characterized in five CAF cells. One out of five CAF cells conferred resistance to all four anticancer drugs on PC-9 cell lines. And the phenomenon was particularly marked for EGFR-TKIs compared with conventional cytotoxic chemotherapy. For HCC4006 cell line, however, the CAF-mediated resistance was observed for WZ4002 and gemcitabine in one CAF cell. Based on the results, there was evidence of CAF-mediated resistance to conventional chemotherapy drugs and targeted agents in lung cancer. However, the resistance phenomena were quite variable among different patients. That may explain why patients had different clinical outcomes when treated with same therapeutic regimen. Mechanisms underlying drug resistance mediated by CAF need further researches to uncover so that new therapeutic strategy for drug resistance in lung cancer can be developed.

Nivolumab in Advanced Non-Small Cell Lung Cancer: a Comparison among Different Radiological Criteria for Assessing Response

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Background

In the last few years, immune check-point inhibitors have become part of the management of non-small cell lung cancer (NSCLC); the most appropriate radiological method for evaluating the responses to these new molecules has not been defined yet, due to their peculiar mechanism of action. The aim of our study is to compare different radiological evaluation criteria for a subset of patients receiving nivolumab for advanced NSCLC.

Methods

Patients with pre-treated advanced NSCLC have been enrolled in a single-institutional translational research study in the San Martino Hospital – National Institute for Cancer Research, Genova, Italy, to receive nivolumab (3 mg/kg every 14 days). Radiological evaluation with computed tomography (CT) was performed at baseline and after every 4 administrations; we compared response evaluation criteria in solid tumors (RECIST 1.1), immune-related response criteria (irRC), World Health Organization (WHO) criteria and immune-related RECIST

(irRECIST), which are recently proposed based on the original RECIST. The concordance among the different criteria was determined with Cohen's kappa coefficient (K).

Results
Fifty-two patients were eligible: median age= 70 years (44-85); male/female: 70%/30%; current or former smokers= 87%; non-squamous/squamous histology= 79%/21%; median number of cycles= 6 (4-29). At the first evaluation: 4 patients had partial response (PR) (7.7%), 19 had stable disease (SD) (36.5%) and 29 had progressive disease (PD) (55.8%) according to RECIST 1.1; 3 patients had PR (5.8%), 23 had SD (44.2%) and 26 had PD (50%) according to irRC; 3 patients had PR (5.8%), 20 SD (38.5%) and 29 PD (55.8%) according to WHO; 4 patients had PR (7.6%), 24 SD (46.2%) and 24 PD (46.2%) according to irRECIST. At the best response: 9 patients had PR (17.3%), 14 had SD (26.9%) and 29 had PD (55.8%) according to RECIST 1.1; 8 patients had PR (15.4%), 19 had SD (36.5%) and 25 had PD (48.1%) according to irRC; 7 patients had PR (13.5%), 17 had SD (32.7%) and 28 had PD (53.8%) according to WHO; 11 patients had PR (21.2%), 18 had SD (34.6%) and 23 had PD (44.2%) according to irRECIST. The concordance between first evaluation and best response was good for all the criteria (K ranging from 0.783 to 0.839); the concordance between irRECIST and irRC was high (K= 0.828) and RECIST 1.1 had a good concordance with irRC (K= 0.734), irRECIST (K= 0.767), and WHO (0.766).

Conclusion
In our analysis, the different response assessment methods were generally concordant. Since response is more easily assessed with irRECIST than with irRC, the former might be proposed as an appropriate method of response evaluation.

Metachronous Second Malignancy after Treatment of Limited-Stage Small-Cell Lung Cancer: Incidence and Survival

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Purpose
Extended survival outcomes for many types of cancer because of the ability to detect disease early and because of improvements of treatment modalities with supportive care come with an increased risk of developing a metachronous second malignancy (MSM). Some patients with limited-stage small cell lung cancer (SCLC) have lived long enough to manifest MSM despite an overall poor prognosis. Multiple genetic mutations as well as under immune suppressed status after chemoradiotherapy may have relation to develop MSM. We retrospectively evaluated the incidence of MSM, the factors potentially being associated with the development of MSM and survival outcomes after treatment for limited-stage SCLC.

Methods and Materials
Inclusion criteria were having a diagnosis of limited-stage SCLC and having received ≥45 Gy radiotherapy and chemotherapy at a single institution from 1985 through 2012. MSM was defined as a tumor of a different histologic type occurring more than 2 years after the diagnosis of primary SCLC. OS was calculated by using Kaplan-Meier estimators, and the log-rank test was used to compare the Kaplan-Meier curves. Sex, race, smoking history, tumor histology, and receipt of radiotherapy, chemotherapy, and prophylactic cranial irradiation (PCI) for SCLC were evaluated as potential predictors of MSM development by using Fisher's exact test and the

Wilcoxon rank-sum test. Results: Of 704 patients identified, 163 were excluded (32 for lack of follow-up, 48 for having SCLC as MSM, 37 for having non-melanoma skin cancer as MSM, and 46 for MSM arising within 2 years after SCLC diagnosis). Among the 541 patients analyzed, 15 (2.8%) developed metachronous MSM (8 adenocarcinoma, 5 squamous cell carcinoma, 1 sarcoma, and 1 acute myeloid leukemia). All 15 patients who developed MSM had achieved complete response after SCLC treatment. OS was longer for patients with MSM than for patients with no other malignancies and no SCLC recurrence, with 10-year rates of 61.9% (95% confidence interval [CI] 30.0%-82.6%) and 29.9% (95% CI 21.5%-38.6%), respectively (P=0.03). Among patients with MSM, OS time from MSM diagnosis may have been longer than OS time after SCLC recurrence among patients with no other malignancies but recurrence, with 10-year rates of 38.0% (95% CI 9.7%-67.0%) and 12.4% (95% CI 5.7%-21.7%), respectively (P=0.08). Patients diagnosed with SCLC in 2000 or later were more likely to have had MSM, whereas patients diagnosed SCLC before 2000 were more likely to have had recurrent SCLC (P<0.001). Patients who received concurrent radiochemotherapy were more likely to have had MSM than patients who received sequential radiochemotherapy (P = 0.001). Number of smoking pack-years was not linked with the development of MSM (P=0.077), and never smoking, stopped smoking, and never stopped smoking were also not linked with development of MSM. Patients who were alive at last follow-up were at greater risk of having MSM compared with patients who had died at last follow-up (P<0.001). Among the 15 patients with MSM, the MSM was acute myeloid leukemia in 1 and limited in the other 14 patients.

Conclusions

Long-time survivors after treatment for SCLC should be made aware of the risk of MSM and the necessity of follow-up, even when the SCLC responds well to treatment. Early detection of MSM might be necessary to improve prognosis among long-term survivors of SCLC. Although the number of patients who developed MSM in this study was small, these patients could have been immune-suppressed by the chemoradiotherapy. The development of more effective treatments including immunotherapy for limited SCLC will enhance the importance of MSM, and further analysis is warranted to establish the optimal strategies for diagnosing or treating MSM in such cases. Immunotherapy and chemoradiotherapy for limited-stage SCLC is being investigated in a phase I/II study (PI: J Welsh).

Reprogramming Lung Tumor Microenvironment by Targeting Cytokine Network as a Preventive and Therapeutic Strategy for K-ras Mutant Lung Cancer

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Lung cancer is the leading cause of cancer mortality worldwide, and cigarette smoke is by far the most common cause of it. Activating mutations of K-ras are the most common oncogenic alterations found in lung cancer (30%), which are heavily associated with tobacco exposure, and poor prognosis. Unfortunately, our knowledge of the molecular mechanisms mediating/affecting K-ras induced lung tumorigenesis and growth is substantially deficient in comparison to that of lung cancers with other types of mutations (e.g. EGFR). Moreover, pharmacologic attempts directly targeting K-ras have thus far failed; therefore, alternative strategies targeting downstream effectors of K-ras and K-ras associated tumor microenvironment may be a more effective approach at inhibiting effects of this oncogenic pathway. Obtaining such knowledge is a main focus of research in my group. Furthermore, because of the persistent risk among former smokers, and increased diagnosis of early stage

lung cancer with low dose CT screening, personalized strategies targeting pathways which stop the progression from pro-tumor chronic lung diseases (e.g. chronic obstructive pulmonary disease, COPD) and early stage lung cancer to advanced lung cancer would also be valuable a resource, which we seek to determine in my laboratory as well. It is now apparent that the numerous cytokines and growth factors released during inflammation can influence carcinogenesis. Importantly, these cytokines are released by both immune and non-immune cells (e.g. the epithelial/tumor cells). Studies published by my group have shown essential roles and functional significances of a proinflammatory cytokine network (IL-6, IL-17, and TNF) and its respective transcription factors (STAT3, and NF-kB) during K-ras mutant lung tumorigenesis and its promotion by COPD-type airway inflammation. We are currently dissecting the related mechanisms and further entertaining pharmacologic targeting of this network. By addressing novel questions with our state-of-the-art research collaborative, we aim to delineate specifically tailored targets that could ultimately be manipulated in order to provide an alternative path for preventive and therapeutic benefit in patients at high risk for lung cancer (smokers with COPD), and also patients with a diagnosis of K-ras mutant lung cancer in combination with conventional chemotherapy, immune check point blockade or other targeted therapies (e.g. MEK inhibitor). These studies could also help us to develop a panel of clinical predictive and prognostic markers to identify responders and nonresponders, and to explore mechanisms of susceptibility or resistance to targeted therapy in these patients.

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Expression of syndecan-1 (CD138) in pulmonary lymphoepithelioma-like carcinoma is correlated with early stage and good prognosis

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Objective
Pulmonary lymphoepithelioma-like carcinoma is a rare type of carcinoma characterized by poorly differentiated morphology admixed with marked lymphocyte infiltrated and is more common in South-East Asia with Epstein-Barr virus infection. Little is known about the association between lymphocyte infiltration and prognosis and the expression of Syndecan-1 (CD138) in pulmonary lymphoepithelioma-like carcinoma has never been studied.

Methods
A total of 54 surgically treated pulmonary lymphoepithelioma-like carcinoma patients were included. Paraffin-embedded tumor sections were subjected to immunohistochemistry and analyzed for (i) immunophenotype and amount of tumor-infiltrating T lymphocytes (CD3, CD4, CD8), (ii) Syndecan-1 (CD138) expression by lymphocytes and tumor cells respectively. The results were correlated with the clinicopathologic characteristics as well as survival outcomes.

Results
Syndecan-1 (CD138) expression by tumor cells in patients with advanced clinical stages was significantly lower than those without. There were 13 cases, 26 cases and 13 cases demonstrated strong positive, median positive and weak positive respectively. 2 cases were

identified as negative. Multivariate logistic regression analysis indicated that number of metastatic lymph node was an independent factor to patients' clinical stage.

Conclusion
The high protein expression of Syndecan-1 (CD138) in pulmonary lymphoepithelioma-like carcinoma was associated with early disease and good prognosis.

Repeated observation of immune gene sets enrichment in women with non-small cell lung cancer

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Background
There are different biological and clinical patterns of lung cancer (LC) between genders indicating intrinsic differences, leading to increased sensitivity to cigarette smoke induced DNA damage, mutational patterns of KRAS and better clinical outcomes in women. In despite of the great advances in the knowledge of the genomic landscape of lung cancer, it is not explored the molecular differences regarding to gender. Our aim was to evaluate differentially enriched gene sets between women and men.

Methods
We evaluated 05 public databases containing gene expression values from NSCLC patients: GSE50081 (HG-U133_Plus_2; n=81 samples), GSE47115 (Illumina HumanHT-12 WG-DASL V4.0 R2; 16 samples), GSE10072 (HG-U133A; n=71 samples), GSE32863 (Illumina HumanWG-6 v3.0; 116 samples), GSE7670 (HG-U133A; n=52 samples). In each dataset, expression levels were log2 transformed and median centered. We performed the Gene Set Enrichment Analysis (GSEA) to find differences between the genders. Each dataset was analyzed individually. Since the smoking status is the main confounding factor, datasets were divided in cohorts of smokers and non-smokers (and healthy tissues by smoking status when it was included in the dataset). Cases with unknown smoking status and former smokers were excluded from the analysis. We use the Gene ontology biological process terms to find similar enriched pathways between cohorts, 1454 gene sets named by gene ontology terms were examined. We consider a gene set enriched when at least a cohort had a p-value<0.05 and the observation was repeated in other datasets with a p-values <0.08 (statistical trends).

Results
The analysis showed repeated observation of immune genes enrichment in women; "Immune system process", "immune response", "defense response", "cellular defense response" and "regulation of immune system process" were the gene sets most over-represented while APOBEC3G, APOBEC3F, LAT, CD1D and CCL5 represented the top-five core genes. Characterization of immune cell composition with the platform CIBERSORT showed no differences between genders; however, there were differences when tumor tissues were compared to normal tissues. Our results suggest different immune responses in NSCLC between genders that could be related with the different clinical outcome.

Conclusion
Biological differences of lung tumors among genders should be deeply explored in order to improve the immunotherapeutic approaches. Our study provides evidence of biological differences of NSCLC between genders and the basis for the distinct clinical outcome.

PD-L1 checkpoint blockade in combination with MEK inhibition reduces lung tumor growth

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths due to late stage disease presentation, metastasis, and resistance to conventional treatment, suggesting a necessity for targeted therapies. Approximately 30% of patients with lung adenocarcinoma possess an activating KRAS mutation, which currently lacks any pharmacological drugs that can specifically and effectively target the oncogenic protein. Extensive work with the KrasG12D;p53R172H (KP) murine model of lung adenocarcinoma and the derived KP cell lines identified a subpopulation of cells within heterogeneous tumor tissue that undergo an epithelial-to-mesenchymal transition (EMT), which is regulated by a double-negative feedback loop between Zeb1 and the miR-200 family. Immunologic, proteomic, and genetic profiling revealed that mesenchymal cells suppress the activity and proliferation of CD8+ T-cells in the tumor microenvironment through an EMT-dependent upregulation of PD-L1. Treatment of tumors with anti-PD-L1 reduced mesenchymal tumor growth with no effect on epithelial tumor proliferation, but ultimately developed resistance. Separate proteomic profiling of the epithelial tumors showed an increase MEK/ERK compared to mesenchymal tumors. Combinatorial treatments of syngeneic epithelial or mesenchymal KP tumors with an anti-PD-L1 antibody and a MEK inhibitor demonstrated an additive effect on reducing tumor growth compared to either single agent therapy. The decrease in tumor growth correlated with an increase in CD8+ T-Cell infiltration in epithelial and mesenchymal tumors but only mesenchymal tumors exhibited decreased exhausted T-Cell markers with dual treatment. Future studies will aim to perform co-clinical trials on autochthonous murine models with the combination therapy, analyze human patient samples to correlate expression of MAPK signaling molecules with PD-L1 and EMT markers, and elucidate additional therapeutic targets to combine with anti-PD-L1 treatment.

Development of a novel anti-cancer immune therapy using the synthetic oligonucleotide containing poly-G motif.

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Synthetic oligonucleotides containing specific sequences have an immunomodulatory effect. It is highly expected to develop to a novel anti-cancer immunotherapy with these effects. We showed that a novel poly-G ODN has an anti-tumor immune effect in the tumor-bearing murine model. This effect of poly-G ODN was mediated through T cells in a TLR9 independent manner. Poly-G ODN directly induced the phosphorylation of Lck, thereby enhancing the production of IL-2 and T cell proliferation. Meanwhile, it was suggested that the immune modulation by poly-G

ODN against cancer was different in human. We identified poly-G ODN induced T cell proliferation and IFN-gamma production through the enhancement of monocyte maturation in human mononuclear cells isolated from peripheral blood or malignant pleural effusion among lung cancer patients. Further studies have been continued for the establishment of a novel anti-cancer immune therapy with poly-G ODN.

CD38 as a novel immune checkpoint and a mechanism of resistance to the blockade of the PD-1/PD-L1 axis

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Lung cancer is a leading cause of cancer-related mortality. Although immune checkpoint inhibitors including PD-L1 blockade provide significant clinical benefit for patients with lung cancer, effective clinical use of these agents is encumbered by a high rate of resistance. Thus, successful prevention of lung cancer mortality requires a thorough understanding of the biological process of resistance. To our knowledge, there are only a few studies reporting the mechanisms of resistance to PD-L1 blockade so far. The mechanistic basis remains poorly defined.

Here we have explored the resistance mechanisms using pharmacological and genetic approaches in immunocompetent murine models of lung cancer. Mice bearing mutant K-ras and p53+-derived tumors (KP tumor) or non-mutation detected tumors (Lewis lung cancer) were treated with anti-PD-L1 antibody until tumors demonstrated therapeutic resistance. The molecular and immune profiles of the tumor microenvironment were evaluated. We identified the up-regulation of CD38 on tumor cells as well as enrichment of CD38highTregs and CD38highMDSCs in tumor as the markers of treatment resistance. We observed the same resistance mechanism caused by CD38 in PD-L1 KO mice bearing PD-L1 KO Lewis lung tumors edited with the CRISPR/Cas9 system. Furthermore, by manipulating CD38 on a panel of lung cancer cell lines, in vitro and in vivo data demonstrates that CD38 inhibits CD8+ T cell proliferation, antitumor cytokine secretion, and tumor cell killing capability. To test whether CD38 blockade might be therapeutically efficacious to anti-PD-L1 resistance, we applied the combination therapy of anti-CD38 and anti-PD-L1 in mice and demonstrated dramatic therapeutic benefit on primary tumor growth and metastasis. More importantly, to extend these results and determine the applicability to patients with lung cancer, we analyzed specimens from 259 patients and found that 18.5% of cases exhibited positive staining for CD38 on tumor cells, showing a great potential benefit for treating lung patients. The results suggest that targeting this novel immune checkpoint upregulated in the context of PD-L1 therapy may broaden the benefit of PD-L1/PD-1 axis blockade for lung cancer treatment.

Theranostic Probes for Targeted Immunotherapy

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Objective
The overall purpose of this study is to produce a bispecific aptamer-antibody hybrid probe for immunotherapy able to generate an immune response solely at the tumor site.

Abstract
Current cancer therapies are not effective in significantly prolonging survival. While immunotherapy has been paradigm changing, immune related adverse events such as autoimmune diseases, hypophysitis and hepatotoxicity are widely reported.
We propose to use an aptamer, which are short oligonucleotide sequences that specifically bind their target. Epidermal Growth Factor Receptor (EGFR) is a surface glycoprotein, which is overexpressed in lung cancer cells w.r.t. the normal lung tissue, and provides a target for diagnostic and therapeutic purpose. The EGFR aptamer will be conjugated to a PD-1 antibody, known to restore T cells function in the tumor environment and approved for systemic immunotherapy in lung cancer.

The conjugation is performed in mild conditions and does not influence the biological function of the antibody or the aptamer.

This approach can be easily applied for different cancers to provide targeted immunotherapy by using the appropriate tumor antigen.

Combining immune checkpoint targeting and DNA damage repair (DDR) targeted therapy in small cell lung cancer (SCLC)

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Background
Small cell lung cancer (SCLC) is a highly aggressive disease for which standard treatment remains virtually unchanged since the 1980s. SCLC has a relatively immunosuppressed phenotype with relatively low levels of infiltrating T-cells and evidence of reduced antigen presentation. Only a minority of SCLC patients responds to programmed cell death protein 1 (PD-1) or programmed death ligand 1 (PD-L1) inhibitors as monotherapy. Therefore, though the clinical data is promising, there is a strong need to develop strategies to enhance the efficacy of immunotherapy in SCLC. Our group previously discovered that DNA damage repair (DDR) protein, checkpoint kinase 1 (CHK1), is overexpressed in SCLC and that CHK1 inhibitors have activity in preclinical models of SCLC. Based on data from others and our group we hypothesize that tumor associated neoantigen (TAA) expression is suppressed in SCLC by several mechanisms, including DDR machinery and that targeting CHK1 can enhance antitumor immunity and response to immune checkpoint targeting.

Results

In SCLC models, inhibition of CHK1 by genetic knockdown and small molecule inhibitor (LY2606368) treatment induces DNA damage as demonstrated by increased γ-H2AX levels. We also observed increased protein levels of immune checkpoint ligand, PD-L1, following pharmacologic inhibition with LY2606368. We next tested whether co-targeting CHK1+PD-L1 enhances the anti-tumor effect in immune-competent B6129F1 flank xenograft SCLC model. Mice were injected with TKO.mTmG cells harboring conditional deletion of *Trp53, Rb1* and *p130*. When the tumor volume reached 120mm^3, mice were treated with either IgG (control), LY2606368 (10mg/kg, 2/7), anti-PD-L1 (300ug, 1/7) or combination of LY2606368 and anti-PD-L1 antibody. Single agent treatment with anti-PD-L1 antibody did not cause tumor regression in these models with T/C ratio=0.93 ($p<0.001$) at Day 21. Treatment of single agent LY2606368 with a sub therapeutic dose of the drug, significantly delayed tumor growth in these models with T/C=0.31 ($p<0.001$) at Day 21. However, 3 out of 5 mice treated with anti-PD-L1+LY2606368 had a complete tumor regression within 15 days of treatment with T/C=0.05 ($p<0.001$) at Day 21. The tumors were collected at the end of 21 days for RPPA analysis, RNA sequencing and flow cytometry to characterize tumor-infiltrating immune cells post treatment with the single agents or the combination.

Discussion

SCLC has an immunosuppressed phenotype (despite a high mutational burden); contributed by mechanisms other than the PD-1/PD-L1 pathway. This study shows that targeting CHK1 (by genetic knockdown and pharmacological inhibition) leads to increased DNA damage and increased expression of immune checkpoint ligand, PD-L1. Combining CHK1 and PD-L1 targeting significantly enhanced the effect of PD-L1 antibody leading to tumor regression in an immune competent SCLC model. Biomarker analyses from these models are ongoing to confirm the expression of immune markers. PD-L1 inhibitors as monotherapy lead to objective responses in only a minority of SCLC patients. CHK1 inhibitor, LY2606368 is currently in clinical trial for SCLC patients. The complementary modes of action of the two promising modalities, immune checkpoint targeting and CHK1 inhibition, suggest intriguing possibilities for therapeutic synergy with combination treatment and warrants further clinical investigation.

Other Hematologic Malignancies

Role of cell source and graft composition in haploidentical transplantation using post-transplant cyclophosphamide

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Introduction
Cell source and graft composition are known to have a prognostic role in in allogeneic hematopoietic cell transplant (HCT). Currently, there are no data in the setting of haploidentical-HCT with post-transplant cyclophosphamide (PT-Cy).

Patients and methods
One-hundred patients undergoing haplo-HCT with PT-Cy from September 2011 to November 2015 were included in this multicenter retrospective analysis. Bone marrow (BM) was used as graft source in 25 patients and peripheral blood stem cell (PBSC) in 75 patients. The only differences between the two groups were disease type (lymphoid malignancies 76% vs 57%, p=0.02) and conditioning regimen (myeloablative: 100% versus 30%, p<0.01).Graft cellular subsets analysis was performed by means of flow cytometry (CD34, CD3, CD4, CD8, CD56, CD19, CD38, CD31, CD25, CD45RA, CD95, CD127, CCR4, CCR6, CCR7).

Results
For the whole cohort, neutrophil and platelet engraftment cumulative incidences at day +30 were 97% (95% CI: 91-99) and 64% (95% CI: 53-73), with no differences between BM or PBSC. Grade II-IV and grade III-IV acute GVHD cumulative incidences at day +100 were 36% (95% CI: 26-48) and 10% (95%CI: 5-18), respectively. Acute GVHD cumulative incidence was significantly lower for BM grafts [16% (95% CI: 5-33) vs 43% (95% CI: 31-54), p=0.02], but no differences were observed regarding grade III-IV acute GVHD [8% (CI 95%: 1-24) vs 11% (CI 95%: 5-20), p=0.73]. Chronic GVHD cumulative incidence at 18 months was 23% (95%CI: 15-32) with no difference between BM or PBSC. With a median follow up of 16.5 months (range 0.8 - 40.6), the 18-months PFS and OS were 43% (95%CI: 32-54) and 63% (95% CI: 52-73), respectively. NRM and RI/POD at 18 months were 18% (95%CI: 11-26) and 38% (95%CI: 27-49).

Multivariable analysis was performed using patient (age, patient gender, HCT-CI, DRI) and transplant characteristics (donor gender, donor-recipient relation, graft source). A DRI>1 was the only factor associated with higher RI/POD (HR 3.45, 95% CI: 1.4-8.2, p<0.01), lower PFS (HR 2.77, 95%CI: 1.4-5.3, p<0.01), and lower OS (HR 2.33, 95%CI: 1.1-4.9, p=0.02). Graft source did not influence survival outcomes.

For the BM group, univariable analysis showed that CD4 graft count >20x10^6/kg (median) was associated with a prolonged PFS [60% (95%CI: 28-81) vs 0%, p<0.01] (figure 1), a trend toward a decreased NRM [0% vs 27% (95%CI: 5-56), p=0.05] and a prolonged OS [92%(95%CI: 57-99) vs 58% (95%CI:27-80), p=0.05]. Among the CD4 subsets analyzed (activated, central memory, effector, effector memory, memory stem cells, regulatory, Th17), naïve T cells >8.5x10^6/kg (median) (CD3+/CD8-/CD4+/CCR7+/CD45RA+/CD95-) and recent thymic emigrants >6.9x10^6/kg (median) (RTEs, CD4+/CD31+/CD45RA+) were the only

phenotypes associated with a better PFS and OS (p=0.02 and p=0.04 respectively). For the PBSC group, CD8 graft count > 75 x10^6/kg (median) was associated with a lower NRM [3% (95%CI: 0-12) vs 16%(95%CI:6-30), p=0.01]. No associations were found regarding graft cell composition and incidence of acute or chronic GVHD for both BM and PBSC groups.

Conclusions

We did not observe significant differences in survival outcomes based on graft source. Patients receiving BM grafts developed less grade II-IV acute GVHD, but grade III-IV acute GVHD incidence was similar between the two groups.

For the BM group, a higher CD4 count was predictive of better PFS, TRM and OS, as reported with standard GVHD prophylaxis and matched donors. Interestingly, of the CD4 population, only naïve T cells and RTEs were associated with an improved PFS and OS. A protective effect of CD8 cell count was observed in the PBSC group. This is similar to the non PT-Cy setting where higher CD8 cells are associated with better survival outcomes. These data should be validated in a prospective analysis to guide the choice of cell source in the haplo PT-Cy setting.

Necroptosis as a prognostic marker and possible target in cancer

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Recently, a novel cell death pathway was identified and named necroptosis. This cell death type resembles the accidental necrosis regarding its morphology and the release of the cytoplasmic contents following the plasma membrane rupture. On the other hand, its induction and execution are biochemically controlled, and it involves the activation of the RIPK3 kinase which, in turn, activates MLKL, the effector molecule of this cell death type. The sensitivity to necroptosis of a given tissue or cell type is correlated to the expression levels of these molecules. To date, little is known about the mechanisms that modulate the expression of RIPK3 and MLKL despite the evidence that their levels vary upon different pathophysiological settings, like ischemia-reperfusion, viral infection and cancer. In our current project we are evaluating RIPK3 and MLKL expression levels as prognostic markers for overall survival and response to treatments in colorectal cancers. Also, we are investigating in vitro means of regulating RIPK3 and MLKL expression levels to sensitize cells to necroptosis. Finally, our preliminary results have identified compounds that are able to directly activate MLKL in the absence of RIPK3; their therapeutic potential will be examined in conditions wherein RIPK3 expression is absent, such as breast cancer, leukemias and melanoma. Currently, we are looking for collaborators to expand our research specially with melanoma and hematologic malignancies.

Tapping CD4 T cells for cancer immunotherapy

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T-cell based immunotherapy is an attractive treatment for advanced cancer.
Whereas cellular immune responses against tumour have typically been attributed to CD8 T cells, CD4 T cells play a critical role in tumour elimination and the priming and maintenance of CD8 T-cell responses. Recent findings have highlighted new opportunities for CD4 T cells in cancer immunotherapy.

From patients that clinically benefitted from treatment with cancer vaccines targeting antigens such as hTERT, survivin and frequent neoantigens such as frameshift mutated TGFβRII we have isolated CD4+ T cells reactive against tumour antigens.

Strong T-cell responses against the vaccine or unrelated cancer antigens suggesting epitope spreading correlated to enhanced survival and/or tumour regression in late stage cancer patients.

These HLA class II restricted T-cell clones recognised target cells loaded with long peptides or protein and for some CD4+ T cell clones we could also show direct tumour recognition.
TCRs were expressed in expanded third-party T cells by mRNA electroporation or retroviral transduction and tested for functionality. Both CD8+ and CD4+ T cells expressing the TCRs produced TNF-α, IFN-γ and had the capacity to kill following co-incubation with their targets.
Selecting highly functional CD4+ T-cell clones reactive against tumour-associated or -specific antigens from patients with clinical responses after immunotherapy treatment is a successful method for identifying highly functional HLA class II restricted TCRs for adoptive immunotherapy.

The use of HLA class II-restricted TCRs may be of therapeutic value both in hematopoietic malignancies and in melanoma where tumour cells often express HLA class II.
Furthermore, combining HLA class I- and class II-restricted TCRs for T-cell redirection may provide a more potent therapeutic effect in adoptive T cell therapy.

A Universal Killer T-cell for Adoptive Cell Therapy of Cancer

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T cell-based therapy has generated remarkable remissions in hard-to-beat cancers and represents a large part of innovations in immunotherapy. Adoptive T-cell transfer (ACT) is a labour intensive method in terms of logistics and mainly depends on the quality of the patient's T cells. To overcome these obstacles we have developed a universal cell line for TCR expression by modifying the FDA-approved NK cell line, NK-92. Advantages of using this cell line is that it is easy to expand and maintain in culture, it has retained its killing capacity and can readily be genetically engineered. However, tumour cell recognition by NK-92 is not specific. This can be

controlled by introducing an antigen receptor, such as a chimeric antigen receptor (CAR) or, as in the current work, a TCR. We herein present evidence that NK-92 can be modified to become a T cell-like lymphocyte. Complementing the inherent killing activity of the NK cells with the specific targeting of cancer antigens through TCR could represent a perfect combination to prevent tumor escape. We named this novel cell line UK-92, for Universal Killer derived from NK-92, and showed that UK-92 expressing a therapeutic TCR conserved the binding capacity to cognate pMHC. Phosphoflow cytometry was used to verify that the introduced TCR in UK-92 would mediate intracellular signaling upon crosslinking or by cognate pMHC binding. Our data show that both early and late TCR signalling players were activated in a TCR-specific manner (anti-CD3/anti-CD28 stimulation) and further in a pMHC specific manner upon specific TCR binding. Finally, functional assays using both TCR isolated from CD8 and CD4 T cells demonstrated that UK-92-TCR could be stimulated in a pMHC-specific manner and, importantly, could kill target cells in a pMHC –specific manner. We have now shown in vitro that UK-92 cells are as specific and potent as redirected T cells to kill target cells. With the use of UK-92 as a universal killer cell line this technology, if confirmed efficient in vivo, is expected to dramatically reduce ACT production time. In addition the use of UK-92 will bypass other challenges related to the use of autologous patient T cells.

HSP90 inhibitors specifically target FLT3-ITD-driven AML and bypass TKI resistance

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Activating mutations in the gene encoding the receptor tyrosine kinase FLT3, particularly internal tandem duplications in the receptor's juxtamembrane domain (FLT3-ITD), are the most common lesions found in acute myeloid leukemia (AML) and are associated with inferior clinical outcome. The cellular pathways that support FLT3-ITD-driven cell proliferation and survival remain poorly understood. We systematically screened primary patient samples against a library of nearly 400 anticancer compounds, and discovered that FLT3-ITD-expressing AML blasts are highly sensitive to HSP90 inhibitors such as ganetespib. In addition, HSP90 inhibitors specifically sensitize FLT3-ITD-expressing bone marrow-derived cells to TKIs, whereas cells derived from healthy donors are unaffected. HSP90 inhibitors also preferentially eradicate a population of patient-derived FLT3-ITD+ AML cells expressing leukemic stem cell markers. Taken together, our study reveals provides a rationale for treatment of this form of AML with HSP90 inhibitors.

Deciphering the Role of Metabolic Reprogramming in the Immuno-Modulation of the Leukemic Microenvironment

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Background

Post-therapy recurrence of leukemia remains an unmet clinical problem, especially in adult acute lymphocytic and myelocytic leukemias (ALL and AML). Current advances in cancer immunotherapy have emerged from increased understanding of the immune-suppressive mechanisms that cancer cells deploy to avoid immune responses. However, the mechanisms

through which leukemic blasts create an immune-suppressive niche in the bone marrow are poorly understood. Therefore, deeper understanding of the factors responsible for immune evasion by dormant hematopoietic malignancies is necessary for the development of more efficacious immunotherapeutic treatment modalities in leukemia. Previous studies have shown that leukemic cells are highly oxidative, and rapidly deplete the oxygen tension in the bone marrow microenvironment, which induces hypoxia. It is through this upregulated reliance on oxidative phosphorylation (OxPhos) that leukemic cells generate the intracellular energy and metabolic intermediates necessary to promote tumor growth. In this study, we aim to decipher the role that metabolic reprogramming of leukemic blasts plays in modulating the bone marrow microenvironment, particularly the impact of leukemic hypoxia on the anti-leukemia immune response.

Hypotheses

1) The metabolic reprogramming of leukemic blasts creates localized hypoxic niches in bone marrow that become excluded from surveillance by CD8 T cells, thereby promoting tumor escape from immunotherapy and survival in bone marrow. 2) Inhibiting oxidative metabolism will alleviate leukemic bone marrow hypoxia, restore the anti-leukemia immune surveillance in the bone marrow and synergize with immunotherapies.

Specific Aims

1) To determine the extent of leukemia cell dependence on OxPhos for energy generation, and characterize biomarkers of leukemia cell response to a novel inhibitor of oxidative phosphorylation. 2) To characterize the hypoxic niches that leukemic cells occupy during disease progression and to test the hypothesis that inhibition of OxPhos can reverse hypoxia and HIF-1α expression. 3) To characterize the spatiotemporal dynamics of leukemia infiltrating T cells during disease progression, and determine the impact of hypoxia, OxPhos, and OxPhos inhibition on T cell function inside the bone marrow.

Methods Overview

We will utilize cutting edge intravital imaging techniques (including multi-photon microscopy and confocal microscopy) in our unique portfolio of fluorescent reporter mice (which allow for co-visualization of endogenous T-cell subsets), to ascertain the impact of metabolic reprogramming on leukemia development and immune modulation. We will be the first laboratory in the world to directly map and visualize oxygen tension in the bone marrow in vivo, using the lifetime of two-photon excited phosphorescence emitted by a state-of-the-art two-photon enhanced dendrimeric oxygen sensor. Moreover, we will employ a novel OxPhos inhibitor to determine whether reversing the metabolic reprogramming of leukemia will normalize the bone marrow microenvironment, and restore the anti-cancer immune response.

Significance and Innovation

The prognosis for patients with relapsed leukemia is poor, and recurrent disease is often not amenable to current treatment regimens. Therefore, the identification of novel therapeutic agents is necessary to improve patient outcomes. The proposed studies will, for the first time, shed light on the role that metabolic reprogramming (particularly a heightened reliance on OxPhos) plays in the profound immune suppression which characterizes the leukemic microenvironment. In addition, our experiments will help determine whether a novel OxPhos inhibitor is a promising drug candidate for further clinical development.

Exploring mechanisms associated with loss from immune surveillance during early progression from smoldering multiple myeloma to symptomatic multiple myeloma

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Multiple myeloma (MM) is a plasma cell malignancy that is preceded by monoclonal gammopathy of unknown significance (MGUS) and smoldering multiple myeloma (SMM). Although treatment outcomes over the past decade have improved dramatically, there is still no established curative therapy. Intervening earlier in the disease process could be an important aspect to establish a curative blueprint in MM. However, identification of the fraction of patients with asymptomatic monoclonal gammopathies who have a significant risk of progression to symptomatic MM is needed to initiate therapy before end-organ damage ensues.
We hypothesized that loss of immunosurveillance leads to early progression from SMM to symptomatic MM. Toward this end, we have initiated comprehensive immunophenotyping of bone marrow samples to analyze the frequency and functional status of various immune cell subsets and immune markers, which have the potential to activate or inhibit an effective immune response. Markers analyzed include T cell activating receptors (CD134, CD137, CD40L, ICOS, GITR), NK cell activating receptors (NKG2D, NKp46, NKp44, NKp30, CD226), NK cell activating ligands (MICA/B, ULBP1, ULBP2, ULBP3, CD112, CD155), T/NK inhibitory receptors (PD1, LAG3, TIM3, CD244, CD160, BTLA, CD200R, CTLA4, TIGIT), NK cell inhibitory receptors (KIR2DL1/L2/L3/S1/S2), T/NK inhibitory ligands (PD-L1, PD-L2, B7-H3) and markers of immune cell subsets including naïve, effector, and memory T cells, regulatory T cells, NK cells, macrophages, and myeloid derived suppressor cells. We observed that BTLA (50 fold, $P<0.01$) and CTLA4 (48 fold, $P<0.01$) were overexpressed on marrow-infiltrating T cells at diagnosis in patients with SMM that progressed to symptomatic MM within 1 year compared to patients with SMM that did not progress. Detailed results from this analysis will be presented at the meeting.

Early changes in intracellular signalling networks of acute myeloid leukaemia in response to chemotherapy

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Background
Acute myeloid leukemia (AML) is an aggressive hematological malignancy, characterized by a rapid proliferation of immature blast cells that suppress the normal function of the bone marrow. The heterogeneity of the disease gives variable survival outcomes, ranging from below 10 % to above 80 %, depending on biologic subtypes.

The current therapeutic approach in AML in patients eligible for treatment with curative intent has remained relatively unaltered the last 30 years, consisting of an aggressive chemotherapy regimen of an antracycline in combination with cytarabine (Ara-C).

An immediate response of chemotherapy in intracellular signalling networks can be detected within minutes in vitro. Whether this can be detected in the peripheral blood of leukemic patients receiving chemotherapy is not well studied.

To explore the immediate chemotherapy response in vivo, we performed a high dimensional detection of intracellular signalling networks, by applying a 36-antibody panel of surface and phosphoprotein markers on peripheral blood samples of 30 patients with de novo AML undergoing initial chemotherapy.

Experimental design
Peripheral blood samples were obtained from 30 newly diagnosed AML patients admitted to Oslo University hospital, Rikshospitalet and Haukeland University hospital, Norway. All patients received standard induction therapy with idarubicine 12 mg/m2 and cytarabine 200 mg/m2 in a 3 + 7 regimen according to the trial HOVON SAKK AML 102 and 132 (1). Sampling was performed before start of chemotherapy treatment, four hours and twenty-four hours after treatment onset. Blood samples were brought directly to the laboratory for immediate fixation. We established an antibody panel of 36 markers, containing 21 surface- and 15 phosphoprotein markers. The antibody panel was designed using clinically established AML phenotype markers, and phosphoprotein markers characteristic for AML signalling networks (2).
Fluorescent-based flow cytometry has limitations in multi-parameter analysis due to spectral overlap of fluorescent markers. To capture the complexity of AML we applied cytometry by time of flight (CyTOF) that uses stable metal isotopes as reporters, instead of fluorophores. This significantly increases the number of parameters that can be measured per cell (3). The intracellular signalling response will be correlated to mutational analysis applying TruSight myeloid sequencing panel and clinical data including therapy response, white blood cell count and survival.

Discussion
To improve disease management of AML, an early treatment response assessment could prove valuable. Signs of therapy resistance could possibly lead to treatment alteration, preferably at an early time-point. The intracellular response to induction chemotherapy in AML, which is likely to be immediate, remains to be studied in vivo. We hypothesise that the immediate intracellular response, at the single cell level, may reveal a signature of response in the individual patient undergoing chemotherapy (4),(5).

NK cell dysfunction in CLL is associated with poor prognosis and is mediated through SHP-1

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The evasion of cancer cells from the so-called immunosurveillance may occur due to selection of nonimmunogenic tumor cell subsets or by active suppression of the immune response by cancer cells. A number of studies have reported defective T cell function in chronic lymphocytic Leukemia (CLL). Gene expression studies have shown alteration in profiles of T cells in patients with CLL with upregulation of checkpoint molecules such as PD-1. Moreover, T cells in patients with CLL are functionally defective and show impaired immunological synapse (IS) formation.

Although a number of studies have suggested that NK cells may also be compromised in CLL, only limited data are available on the possible mechanisms underlying these abnormalities. The aim of this study was to analyze the global phenotypic and functional profiles of highly purified PB NK cells from CLL patients compared with age-matched healthy donors to examine the mechanisms of NK defect in CLL.

We performed a comprehensive characterization of NK cell phenotype and function in primary peripheral blood samples collected from 52 untreated patients with CLL and 20 healthy controls using 14-color multiparameter flow cytometry. The functional studies included intracellular cytokine flow cytometry to assess IFNγ and TNFα production, CD107a degranulation and 51Cr release assay in response to K562 targets. We observed that patients with CLL could be broadly divided into 2 groups based on their NK effector function: those with relatively normal NK function (48%), and those with significantly impaired NK cytotoxicity (52%). Impaired NK cells function was significantly associated with markers of poor clinical prognosis, such as ZAP70 expression (52% in the dysfunctional group vs. 16% in the normofunctional group; p<0.0001), IgVH mutational status (56% unmutated vs. 12%;P<0.0001), Rai stage (30% in stage III-IV vs. 8%;P=0.0007) and TP53 mutation (26% vs. 4%;P<0.0001). Moreover, NK cell dysfunction was associated with a higher rate of secondary malignancies (26% vs. 8%, mostly squamous cell carcinoma and melanoma;P=0.0004). However, we did not find any significant differences in the expression of the major activating (CD16, NKG2C, NKG2D, DNAM, NKp30, NKp44, NKp46) and inhibitory (NKG2A, KIR) receptors in patients with dysfunctional vs. those with normal NK cell function and healthy controls.

To understand the underlying mechanisms for NK cells dysfunction in CLL, we studied the major inhibitory signaling pathways in NK cells including the TGF-β/SMAD pathway, IL-10/STAT3 pathway and the Src homology region 2 domain-containing phosphatase-1 (SHP-1) pathway. While there were no significant abnormalities in the TGF-β/SMAD and IL-10/STAT3 pathways, we found constitutive phosphorylation and activation of SHP-1 in NK cells in 6/6 CLL patients tested in the dysfunctional group. In contrast SHP-1 was not active in NK cells in 5/6 CLL patients with normal NK cell function and in 6/6 healthy controls. SHP-1 is known to mediate inhibition of NK cell function, preventing activation of stimulatory signaling cascades. Thus, we next tested if inhibition of pSHP-1 with a selective SHP-1 inhibitor could reverse NK dysfunction. Ex vivo treatment of NK cells from 3 CLL patients in the dysfunctional group with a SHP1 inhibitor overnight (1μM of NSC 87877, Millipore) resulted in significant improvement in NK effector function, as assessed by response to K562 targets, including IFN-γ (11% vs. 24% ;P=0.003) response and CD107a degranulation (5% vs. 26% ;P=0.03) but not TNF-α (1.1% vs. 1.7%;P=0.4).

In conclusion, dysfunction of NK cells in CLL is associated with poor prognosis and increased risk of secondary malignancies. This can be attributed to the constitutive activation of the phosphatase SHP-1. Due to their crucial role in anti tumor immune response, restoring NK cell function by inhibition of SHP-1 can improve anti tumor immune surveillance, reducing the rate of complications and improving prognosis.

Investigating the suitability of standardized Euroflow flow cytometry panels for the characterisation and diagnosis of chronic lymphocytic leukemia/Small lymphocytic leukaemia (CLL/SLL) at Tygerberg Academic Hospital (TAH), South Africa.

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Background

Inadequate or non-existent national cancer registries coupled with limited diagnostic capabilities adds to unreliable incidence statistics of haematological malignancies in African countries. It is crucial to collect malignant disease data that can be used for statistical research in an efficient and reproducible manner within the South African (SA) setting. Reproducibility can be facilitated by standardisation of diagnostic techniques which would increase diagnostic turnaround time and subsequent treatment. The aim of this study is to evaluate the use of an expanded immunophenotypic and molecular panel in improving the understanding of CLL incidence, pattern and prognosis in the TAH catchment areas of SA.

Methodology

CLL/SLL patients will be recruited at TAH, Cape Town. Biospecimens will be prepared and analysed on the Beckman Coulter Navios flow cytometer using Euroflow standardised protocols. B-cell chronic lymphoproliferative disorders (B-CLPD) will be detected using Euroflow lymphoid screening tube (LST) immunophenotyping panel including CD20, CD4, CD45, CD8, smlgλ, CD56, smlgκ, CD5, CD19, TCRγσ, smCD3 and CD38. Tube 2 of Euroflow B-CLPD panel, combined with LST, will identify CLL from other B-CLPD and will include CD20, CD45, CD23, CD10, CD79b, CD19, CD200 and CD43. Immunophenotypic profiles from CLL positive patients will then be stored in a database using the compass tool of the Infinicyt™ FC software.

Results

Based on the samples that have already been analysed in comparison to the National Health Laboratory Service of South Africa (NHLS) flow cytometry results, it appears that the Euroflow flow cytometry panels are suitable for the diagnosis of CLL for the African population. The study is still ongoing and the setting up of the CLL immunophenotypic database for the South African population is underway.

Discussion

The compass tool of the Infinicyt™ FC software allows for the creation of different disease group databases thus allowing for faster differential diagnoses of new case studies by comparison with the reference disease group database. Multicolour FC protocols (Euroflow 8-colour) have higher sensitivity and specificity, which in turn would lead to faster diagnosis and treatment turnaround time.