WIN

SYMPOSIUM
2018
10th ANNIVERSARY

25-26 JUNE, 2018
PARIS, FRANCE

ABSTRACT
BOOK

GLOBAL IMPLEMENTATION OF PRECISION ONCOLOGY:

WIN'NING THE WAR AGAINST CANCER

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PRESIDENTIAL SESSION 1: The challenges of building real cooperation to implement precision oncology programs: a long and winding road

O1.1

The TAPUR family of clinical trials: building a global data sharing platform in Precision Oncology

Richard Schilsky

American Society of Clinical Oncology, Alexandria, VA, United States

Objectives: The TAPUR Study (https://clinicaltrials.gov/ct2/show/NCT02693535) is a phase II, prospective, non-randomized, multi-basket, pragmatic clinical trial that aims to identify signals of drug activity when Food and Drug Administration (FDA) approved drugs are matched to pre-specified genomic targets in patients with advanced cancer, outside of approved indications.

Methods: Patients eligible to participate in TAPUR are 12 years and older, with advanced, measurable or evaluable solid tumors, multiple myeloma or B cell non-Hodgkin lymphoma, ECOG PS 0-2 and acceptable organ function. Participants are matched to any of the sixteen FDA approved study drugs based on protocol-specified genomic inclusion and exclusion criteria. Genomic profiling from any Clinical Laboratory Improvement Amendments certified, College of American Pathologists accredited laboratory is acceptable. The treating physician selects the treatment from the available study therapies or consults with the TAPUR Molecular Tumor Board. Participants are placed into multiple parallel cohorts defined by tumor type, genomic alteration and drug. The primary study endpoint within each cohort is objective response or stable disease of at least 16 weeks duration.

Results: More than 1250 participants have thus far been registered and more than 875 treated with a TAPUR study drug. Cohorts examining the activity of palbociclib in patients with pancreatic and gallbladder or bile duct cancer harboring alterations in CDKN2A have closed to enrollment due to lack of activity. Fourteen cohorts, assessing pembrolizumab, palbociclib, olaparib, sunitinib, trastuzumab+pertuzumab, and others, have expanded to the second stage of enrollment due to preliminary activity. Other clinical trials based on TAPUR have now been launched in the Netherlands (DRUP study) and Canada (CAPTUR trial, https://clinicaltrials.gov/ct2/show/NCT03297606) and will soon be launched by the WIN Consortium. A data sharing protocol is in development to specify the objectives and processes for sharing data across the TAPUR family of studies.

Conclusion: The TAPUR family of studies will describe the efficacy and toxicity of the targeted drugs used outside of their approved indications when matched to a somatic genomic variant.

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No abstract available.

O1.5

The Future of the EU Framework Programmes for Research and Innovation

Cornelius Schmaltz

Head of Unit Health Research Strategy, Directorate General for Research and Innovation at the European Commission in Brussels, Brussels, Belgium

The European Union (EU) is the world's largest trading bloc with 500 million people in 28 countries and it is a global leader in research and innovation. With 7% of the global population, Europe accounts for 20% of global research and development investment and around one third of all high-quality scientific publications.

Horizon 2020 is the EU's current Research and Innovation programme with nearly €80 billion of funding available over 7 years (2014 to 2020) – in addition to the private investment that this money attracts. Its goal is to ensure Europe produces world-class science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. Roughly 10 billion of the total budget will be invested in health research and innovation.

Many of the biggest health-related issues are of a global nature and require a global solution. The possibility to attract international stakeholders, their expertise and resources into Horizon 2020 collaborations is helping to combine best skills and outcomes, and is delivering tangible results to patients in Europe and elsewhere.

Horizon 2020 is open to the world meaning that researchers from all over the world can participate in the calls and collaborate with European consortia (though eligibility for European funding is dependent on country status and bilateral agreements). International cooperation is a fundamental and inherent component of the Horizon 2020 health research programme, well placed to reach out to the best scientists and research laboratories in the world.

The cooperation between the EU and the National Institutes of Health (NIH) has always been a strong asset in EU-US partnership. Both agencies have regular meetings to discuss policies and initiatives where cooperation may help getting innovations faster and more efficiently to patients. Another important element is an arrangement between the European Commission and the NIH: in recognition of the openness of the NIH's programmes to European researchers, any legal entity established in the USA is eligible to receive EU funding to participate in projects of Horizon 2020's health research programme'. Up to April 2018, there have been 106 participations of US entities in Horizon 2020 health research projects.

On 2nd May 2018, the European Commission rolled out its plans for the next 7-year EU budget for the period 2021-2027. The Commission proposed to allocate €100 billion to Horizon Europe, a huge boost to the next research and innovation framework programme. Beyond Horizon, there are other major funding programmes that will provide a significant stimulus to innovation, such as the VentureEU initiative to boost private investment and venture capital.

While Horizon Europe will continue funding excellent science, and tackle global challenges (such as health), it also aims to become a frontrunner in market-creating innovation: The Commission proposes to establish a full-scale European Innovation Council to offer a one-stop shop for high potential and breakthrough technologies, as well as for innovative companies with potential for scaling up.

The new programme will launch EU-wide research and innovation missions with bold, ambitious goals and strong European added value in areas to be defined with Member States, stakeholders and citizens. These could range from health challenges to clean transport or plastic-free oceans. The missions will encourage investment and participation across sectors and scientific disciplines to jointly crack a challenge. They should create synergies with research and innovation strategies at Member State, regional and local level.

PLENARY SESSION 2: The next frontier of therapeutics in newly diagnosed patients: standard of care or modern biomarker driven precision oncology?

O2.1

21st Century Medicine Is Transforming Healthcare and the Diagnosis and Treatment of Cancer

Leroy Hood

Institute of Systems Biology and Providence St. Joseph Health, Seattle, WA, United States

Systems medicine, the application of systems approaches to disease, places medicine at a fascinating tipping point—promising a revolution in the practice of healthcare. I will discuss how systems biology approaches have framed systems medicine and I will discuss some of the new systems-driven technologies and strategies that have catalyzed this tipping point. Moreover, five converging thrusts—systems biology, systems medicine, big data (and its analytics), the digitalization of personal measurements and patient-activated social networks—are leading to a proactive healthcare that is predictive, personalized, preventive and participatory (P4). I will contrast P4 healthcare, embodying 21st Century Medicine, with contemporary medicine and discuss its societal implications for healthcare. P4 healthcare has two central thrusts—wellness and disease.

I will also discuss our successful effort to introduce P4 healthcare into the current healthcare system with a 2014 P4 pilot program on scientific wellness—a longitudinal, high-dimensional data cloud study on each of 108 well patients (pioneers) over 2014. We started Arivale, a company focused on bringing scientific wellness to the consumer, in 2015 and already have about 4000 individuals enrolled. I will also discuss some preliminary results from the 108-person pioneer program and the Arivale studies. The preliminary results both with regard to data analyses and patient responses from these studies are striking.

Finally, I will discuss how 21st century medicine (scientific wellness, systems medicine and P4 medicine) are going to transform our approach to many different kinds of cancers.

O2.2
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No abstract available.

PLENARY SESSION 3: Disruptive concepts and clinical trials shifting the paradigm of Precision Oncology

O3.1

Combinations of monoclonal antibodies overcome resistance to drugs

Yosef Yarden

Weizmann Institute of Science, Rehovot, Israel

The modular structure of biological networks enhances their robust functioning and enables adaptation to changing environments. Likewise, the ability of networks to reduce the effects of perturbations, both internal (e.g., mutations) and external (e.g., pharmacological blockers), depends on negative feedback loops, which process a signal in a way that reduces output fluctuations. One important exemplification of these attributes of signal transduction networks is relevant to molecular oncology and cancer therapy. By referring to lung cancer and kinase inhibitors targeting mutant forms of the epidermal growth factor receptor (EGFR), my lecture will demonstrate these principles and their relevance to patient response to molecular-targeted drugs. EGFR mutations identify patients with lung cancer, who derive benefit from specific kinase inhibitors. However, most patients eventually develop resistance, due to secondary mutations, like T790M, or to adaptive mechanisms engaging alternative modules, such as HER3, HER2 and MET. Irreversible inhibitors like osimertinib inhibit T790M-EGFR, but the C797S third-site mutation and other mechanisms confer renewed resistance. Anti-EGFR antibodies recognize mutant EGFRs, hence might overcome resistance. Because several clinical trials found no survival benefit of a monoclonal antibody (mAb) to EGFR, we investigated this unexpected clinical observation. Employing T790M models, we discovered that prolonged exposure to an anti-EGFR mAb dramatically activates the mitogen-activated protein kinase (MAPK) pathway. The underlying mechanism involves enhanced transcription of HER2 and HER3, along with tyrosine phosphorylation of MET. To nullify these compensatory feedback loops we combined anti-EGFR mAbs with mAbs to HER2 and HER3. When tested in animals, the mAb mixture strongly inhibited drug-resistant lung tumors. These findings confirm that feedback loops can preempt application of mono-therapy, and propose a new combination treatment for tumors that progress under treatment with kinase inhibitors.

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O3.4

MyPathway study: a novel precision oncology multiple basket trial

Mary Beattie[1], Vaikunth Cuchelkar[1], John Hainsworth[2]

[1] Genentech, San Francisco, CA, United States
[2] Sarah Cannon Research Institute, Nashville, TN, United States

Purpose: Detection of specific molecular alterations in tumors guides the selection of effective targeted treatment of patients with several types of cancer. These molecular alterations may occur in other tumor types for which the efficacy of targeted therapy remains unclear. The MyPathway study evaluates the efficacy and safety of selected targeted therapies in tumor types that harbor relevant genetic alterations but are outside of current labeling for these treatments.

Methods: MyPathway (ClinicalTrials.gov identifier: NCT02091141) is a multicenter, nonrandomized, phase IIa multiple basket study. Patients with advanced refractory solid tumors harboring molecular alterations in human epidermal growth factor receptor-2 (HER2), epidermal growth factor receptor (EGFR), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), or the Hedgehog pathway (Hh) are treated with pertuzumab plus trastuzumab, erlotinib, vemurafenib, or vismodegib, respectively. In addition to these original study arms, MyPathway has added 3 therapies and 2 study arms. The BRAF arm added cobimetinib in combination with vemurafinib. A Cancer Immuno-Therapy (CIT) arm was added with atezolizumab treatment and an ALK arm was added with alectinib treatment. The primary end point is investigator-assessed objective response rate within each tumor-pathway cohort.

Results: Between April 1, 2014 and November 1, 2016, 251 patients with 35 different tumor types received study treatment. The efficacy population contains 230 treated patients who were evaluated for response or discontinued treatment before evaluation. Fifty-two patients (23%) with 14 different tumor types had objective responses (complete, n = 4; partial, n = 48). Tumor-pathway cohorts with notable objective response rates included human epidermal growth factor receptor-2–amplified/ overexpressing colorectal (38% [14 of 37]; 95% CI, 23% to 55%) and v-raf murine sarcoma viral oncogene homolog B1 V600-mutated non–small-cell lung cancer (43% [six of 14]; 95% CI, 18% to 71%).

Conclusion: Approved targeted therapy regimens in the MyPathway study produced meaningful responses when administered without chemotherapy in several refractory solid tumor types not currently labeled for these agents.

PLENARY SESSION 4: New concepts and therapeutics avenues in Precision Oncology

O4.1

No abstract available.

O4.2

No abstract available.

O4.3

Precision oncology approaches for immunotherapy

Eric Rubin

Merck Sharp & Dohme, Philadelphia, PA, United States

Recent advances in immunotherapy have transformed the lives of many cancer patients. However, not every patient benefits, and there is a great need to identify patients most likely to respond to immunotherapy when administered as monotherapy, as well as to identify optimal combinations for specific patients. While testing lung cancer patients for tumor expression of programmed death ligand-1 was the first example of an FDA-approved precision approach for immunotherapy, several other biomarkers are under investigation as being predictive and/or prognostic for clinical outcomes in patients receiving immunotherapy. In addition, testing patients for tumor microsatellite instability/mismatch repair deficiency yielded the first tissue-agnostic FDA approval, with the indication based on a biomarker characteristic rather than tumor histology. Given that no diagnostic test is completely accurate, the clinical utility of new and existing predictive biomarkers must be considered in the context of both emerging investigational treatments as well as standard therapies. Furthermore, given that similar potentially predictive tests are often pursued concurrently but independently by multiple pharma and diagnostic companies, ongoing collaborative efforts should be continued in efforts help to standardize these tests, and to provide information about their comparability.

Early development in oncology – are the MTD and monotherapy efficacy still the best objectives?

Giorgio Massimini

Merck KGaA, Darmstadt, Germany

Background: Phase 1 clinical trials in oncology usually enroll advanced disease patients without other available treatment due to the drugs higher toxicity and aim to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D). This approach is justified by the safety risk with cytotoxics, and resulted in the successful development of a number of drugs.

Material and Methods: Recently, phase 1 trials from Merck KGaA have incorporated other parameters than safety to define the RP2D, and more and more trials include pharmacodynamics and pharmacokinetics (PK), or a combination of the safety and pharmacodynamic and PK, to determine the RP2D. [Falchook et al. ESMO 2014[1] and Tsimberidou A. ESMO 2017[2]]. Early development studies often include expansion cohorts, de facto full phase 2 studies with efficacy end-points, allowing to move from indiscriminate targeting cancer cell to focus on specific cells and therefore selected malignant diseases or subpopulations of the malignant disease. Because of the heterogeneity of solid tumors, not dependent from a single (and constant) driving mutation, rarely a single treatment agent is fully effective. The number of complete responses observed in most of the solid tumors is indeed low. This has led to a number of combination approaches, potentially endless, implying a number of combinatory phase 1 and phase 2 studies.

Results: Despite efforts to speed up the development of such combinations, these phase 1 studies are cumbersome and still linked to the concept of MTD [Heist et al. ASCO 2013[3]], while expanding each of combinations in exploratory cohorts results in a large sample size addressing only partly the synthetic lethality potential of the combination(s).

Conclusions: To address this inefficiency, the WIN Consortium is developing a new concept that would allow the combination of new drugs with a known target in a non-competitive space on the basis of the presence of the target, targets combinability, drug compatibility and safety profile of each drug to achieve synthetic lethality. The proposed design and its advantages will be discussed.

1.   *Falchook, G.S. et al. EJC, Volume 47, S158.*

2.   *Tsimberidou A. et al. Ann of Onc (2017) 28 (suppl_5): v122-v141.*

3.   *Heist RS, et al. JCO 2013 31:15_suppl, 2530-2530.*

Celebration of the life and achievements of Professor Thomas Tursz

PLENARY SESSION 5: Challenges of the combination of targeted therapies

O5.1

No abstract available.

O5.2

The 'greatest hits' of precision oncology: the view from the newsroom

Bernadette Toner

GenomeWeb, New York, New York, United States

There have been undeniable advances in the field of Precision Oncology over the last several years. New therapeutic and diagnostic approaches, such as immunotherapy and liquid biopsy, as well as recent regulatory and reimbursement policy changes in the US, are important steps toward broader adoption of individualized cancer treatment for all patients. While it is worth highlighting and even celebrating these advances, there is a risk that focusing solely on the "good news" in Precision Oncology can lead to a distorted sense of the field's progress as it relates to typical cancer patients. News organizations play a vital role in communicating scientific advances to the public, but must ensure that they include the necessary context to help readers understand the true significance of any one milestone. This talk will address the challenges news organizations face in balancing hype against reality when covering the rapidly advancing field of Precision Oncology, and will also discuss the need for a framework for defining "success" in the field.

O5.3

Patients' access to Precision Oncology

Francesco De Lorenzo, Isabelle Manneh-Vangramberen

European Cancer Patient Coalition, Brussels, Belgium

Access to precision oncology requires access to innovative treatments, however, there are many inequalities and barriers within Europe that must be overcome.
The Joint Action on Cancer Control (CanCon) was a common effort between 17 countries, organisations, and experts. ECPC was the only partner patient organisation and a co-author of the "European Guide on Quality Improvement in Comprehensive Cancer Control". This Guide has recommendations for countries to harmonise National Cancer Plans.

In order for precision oncology to become a reality, there is a need for more professional health providers, laboratories, and multi-disciplinary centres. ECPC is monitoring the implementation of these resources by countries in line with National Cancer Plans.

ECPC succeeded in working with the European Commission to develop a proposal for EU HTA cooperation. This proposal will strengthen regulatory cooperation, utilise early scientific advice, increase clinical assessments' speed, and accelerate access to innovative medicines. Once it passes through the European Parliament, the Council must approve this proposal.

Innovation is linked to sustainability. CanCon's Policy Paper on Enhancing the Value of Cancer Care Through a More Appropriate Use of Healthcare Interventions has recommendations to improve access by updating obsolete procedures and eliminating waste to find funds for innovation. Patients should be at the centre of this process, and should be treated in centres of excellence. The European Reference Networks are a good model for harmonisation of treatment that demonstrates implementation of the Cross-border Healthcare Directive. In order to ensure the sustainability of the healthcare system, better access to biomarker molecular testing is

needed. Biomarker molecular testing is critical to identify the people who may benefit from different types of cancer treatment and avoid treatment-related toxicity.

ECPC is one of the stakeholders in the Innovative Partnership Action Against Cancer (iPAAC) Joint Action. Partners from across Europe will implement innovative approaches to cancer control. ECPC is involved in the work package dedicated to genomics, which will develop practical guidance on successfully integrating genomics in the health system.

POSTER SESSION 1: Bioinformatics

P1.1

Machine-learning algorithm applied to clinical trial and molecular profile data predicts outcome of cancer treatments

Alexander Schperberg[1,2], Amelie Boichard[3], Stephane Richard[1], Razelle Kurzrock[3]

[1] *CureMatch, Inc., San Diego, CA, United States*
[2] *University of California Los Angeles, Mechanical Engineering, San Diego, CA, United States*
[3] *University of California San Diego Moores Cancer Center, La Jolla, CA, United States*

Background: Predicting clinical outcome for cancer therapies is difficult due to the diversity of cancer histologies and the complex biological network of genetic and environmental factors. Machine-learning algorithms excel at pattern recognition and have shown success at identifying correlations in the precision oncology landscape. In this study, we trained our machine-learning algorithm on a dataset incorporating clinical trial and molecular profile information in order to predict outcomes of different drug regimens for cancer therapy.

Materials and methods: We retrieved 395 clinical trials from ClinicalTrials.gov and 939 molecular profiles from the Cancer Genome Atlas with advanced (stages III-IV) colorectal adenocarcinoma, melanoma, pancreatic adenocarcinoma or lung cancer (adenocarcinoma or squamous cell carcinoma). For each intervention arm, a dataset with the following attributes was curated: line of treatment, number of drugs, pharmacology class, disease, mutation status of the population, probability of drug sensitivity (percentage reflecting the status of genomic, transcriptomic, and proteomic biomarkers in the population of interest), and outcome. The nonparametric Spearman correlation (r_s) was used to correlate predicted and actual outcome. Predicting the best treatment arm in randomized trials was evaluated by a z-score using the binomial probability test.

Results: A total of 544 progression-free survival (PFS) and 451 overall survival (OS) data points were used as training sets to build a random-forest decision model, providing correlation coefficients of 0.82 and 0.70 respectively. A total of 165 PFS and 113 OS data points were used as validation sets. For both PFS and OS validation sets, the Spearman correlation (r_s) between predicted and actual outcome was statistically significant (PFS: r_s=0.879, OS: r_s =0.878, $P<0.0001$). The better outcome arm was predicted in 81% (PFS: 62/77) (z=5.24, $P<0.0001$) and 71% (OS: 37/52) (z=2.91, P=0.004) of randomized trials.

Conclusions: Derived from clinical and biologic data points, our machine-learning algorithm was able to predict the better outcome arm in the majority of randomized trials. Our model may be exploitable as a tool to optimize clinical trial design with pharmaceutical agents.

Epigenetic background for cell transformation: new paradigm of carcinogenesis and new approach to cancer treatment

Volodymyr Halytskiy

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kiev, Ukraine

The current paradigm considers carcinogenesis to be a result of mutation, following activation (initiation and promotion, respectively) of cell oncogenes. However, oncogene abnormalities are not always revealed in tumor cells. At the same time, tumor growth is associated with widespread shifts in non-coding RNAome, esp. in microRNA (miRNA) pattern.

Our recent researches have shown that tumor-related shifts in miRNAome can lead to complex disorganization of all molecular mechanisms responsible for cell normality – DNA repair, cell adhesion and polarity, contact inhibition, apoptosis and anoikis. Abnormalities in miRNAome affect the DNA methylation and chromatin remodeling machinery and, therefore, can reset the epigenome. Shifts in miRNAome can be also responsible for the cell survival, uncontrolled proliferation, detachment, epithelial-mesenchymal transition, stemness acquisition, tumor microenvironment development, angiogenesis induction, invasive growth, evasion of immune response, resistance to radio-, chemo- and immunotherapy.

It is expected that mutation of oncogenes (as well as other coding genes, important for transformation) is not the first, but the resulting and even optional event in carcinogenesis. We hypothesize that prototype of future abnormalities appears at first at the epigenetic level, whereas mutations only consolidate these changes and underlie the tumor progression. The somatic hypermutation process is probably launched after the reactivation of the AICDA gene encoding the cytidine deaminase AID. On the contrary, tumors have indolent course and favorable prognosis if AID cannot be reactivated. For instance, marginal zone B-cell lymphomas progress slowly because they arise from memory B-cells and express high level of miRNAs miR-155 and miR-150, binding sites of which are revealed in transcript of AICDA gene.

Despite contemporary attempts to elaborate universal drugs for more or less wide range of tumors, future strategy should include: 1) high-throughput deciphering of the epigenome and miRNAome shifts in each tumor case, 2) in silico choice of the most adequate drugs and individual treatment plan, 3) real-time monitoring whether treatment leads to normalization of the signaling pathways decisive for tumor resistance, surviving and growth.

P2.1

The role (TOPO IIA) as a predictive factor for response to neoadjuvant anthracyclines based chemotherapy in locally advanced breast cancer

Mohamed Gamea

Aswan University, Aswan, Egypt

Background: Topoisomerase II-α is a molecular target of anthracyclines; several studies have suggested that topoisomerase II-α expression is related to response to anthracycline treatment. The objective of this study was to evaluate if topoisomerase II-α overexpression predicts response to anthracycline treatment in locally advanced breast cancer patients.

Material and Methods: This prospective study included 50 patients with primary non metastatic locally advanced breast cancer according to American Joint Committee For Cancer Staging(T3-4;N0-3)were treated between January 2012 and Jaune 2012 at Clinical Oncology Department, Tanta University Hospital. Topoisomerase II-α, HER2, estrogen receptor (ER) , progesterone receptor (PR) expression and KI-67 were evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded breast tumors from 50 patients presenting with locally advanced breast cancer.

Results: Tumors from 50 patients, 45 (90%) showed topoisomerase II-α overexpression, patients 34 (68%) for ER positive, 32 (64%) for PR positive and 10 (20%) for HER2 overexpression and 16 (32%) for high KI 67. Significant correlation between clinical and pathological response with topo IIA, HER2 and KI-67. p value (≤ 0.001), (0.005) and (0.015) respectively.

1-Responders :

- Clinical (CR): 3 patients had co-expression of topo II and HER2, hormonal receptor negative and high KI-67.
- Clinical(PR):43 patients majority of them had topo IIA overexpression .fig(9-10)

2-Non responders :

4(8%) patients all had negative (TOPOII/HER2), low KI-67and 2 had hormonal receptor positive and another 2 had hormonal receptor negative.

Conclusions: Our data support a correlation between topoisomerase II-α expression in locally advanced breast cancer patients and improved clinical benefit with neoadjuvant anthracyclines based therapy.

Circular RNA profile and bioinformatics analyses reveal that circOSBPL10 is upregulated and as a proliferative factor in gastric cancer

Jie Chen, Jinggui Chen, Ziwen Long, Jianghong Wu, Ye Zhou, Guangfa Zhao, Yingqiang Shi, Yanong Wang

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Despite many advances in the diagnosis and treatment of gastric cancer (GC), the prognosis of patients with GC remains poor. Circular RNAs (circRNAs), a new star of the non-coding RNA network, have been identified as critical regulators in various cancers. There is increasing evidence that circRNAs represent a class of widespread and diverse endogenous RNAs that may regulate gene expression. Here, we aimed to determine the circRNA expression profile and investigate the functional and prognostic significance of circRNA in GC.

Materials and Methods: In this study, we investigated the expression profile of circRNAs in three GC samples and paired adjacent normal tissues using ribo-minus RNA sequencing and a bioinformatics analysis. Furthermore, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to identify circRNA candidates. Molecular and cellular techniques were used to explore the biological function and mechanism of circRNA in GC cells. The prognostic significance was analyzed using the Kaplan-Meier method and the Cox proportional hazards model.

Results: We first characterized circular RNA transcripts using RNA-seq analysis of ribosomal RNA-depleted total RNA from three paired normal and cancerous gastric tissues. In all, 15623 distinct circRNA candidates were found in these tissues and at least 5500 distinct circRNAs are differently expressed in GC tissues compared with matched normal tissues. We further characterized one abundant circRNA derived from the OSBPL10 gene, termed circOSBPL10. The expression of circOSBPL10 is often upregulated in GC tissues and the silencing of circOSBPL10 significantly inhibits gastric cancer cell growth, invasion and migration. Furthermore, the level of circOSBPL10 was observed as an independent prognostic marker for overall survival and disease-free survival of patients with GC.

Conclusions: Our study revealed the circular RNA profile of GC tissues and characterized a differentially expressed circRNA derived from the OSBPL10 gene. CircOSBPL10 may serve as a new proliferative factor and prognostic marker in gastric cancer.

Figure legends

Fig. 1. Identification of circular RNAs by RNA-seq analyses in gastric cancer

Fig. 2. The characteristics of the circular RNA circOSBPL10 in gastric cancer cells
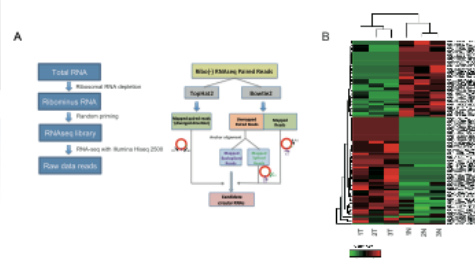

Fig. 3. Silencing of circOSBPL10

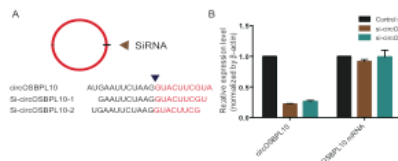### Fig. 4. Silencing of circOSBPL10 inhibits the proliferation of GC cells and suppresses GC cell migration and invasion
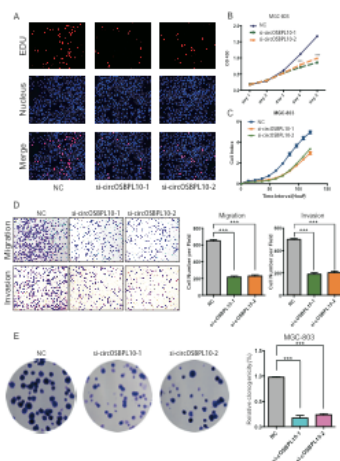

Fig. 5. Silencing of circOSBPL10 inhibits the proliferation of GC cells and suppresses GC cell migration and invasion

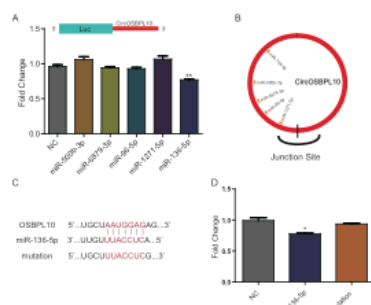### Fig. 5. circOSBPL10 serves as a miRNA sponge for the miR-136-5p.

Fig. 6. circOSBPL10 is an independent prognostic marker of survival in patients with gastric cancer.
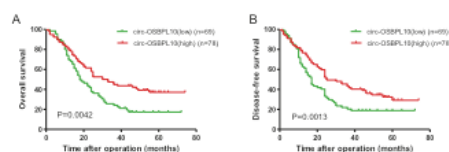
Fig. 1. Identification of circular RNAs by RNA-seq analyses in gastric cancer;Fig. 2. The characteristics of the circular RNA circOSBPL10 in gastric cancer cells;Fig. 3. Silencing of circOSBPL10;Fig. 4 an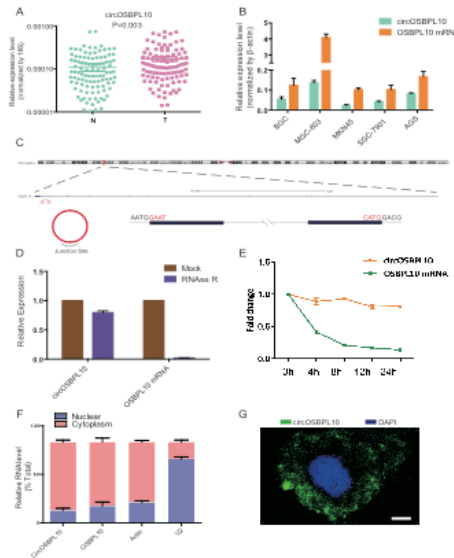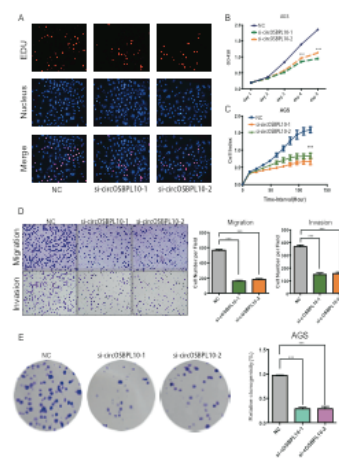d 5. Silencing of circOSBPL10 inhibits the proliferation of GC cells and suppresses GC cell migration and invasion;Fig. 6. circOSBPL10 serves as a miRNA sponge for the miR-136-5p;Fig. 7. circOSBPL10 is an independent prognostic marker of survival in patients with gastric cancer.

Incidence proportions and prognosis of breast cancer patients with bone metastases at initial diagnosis

Yue Gong[1], Jing Zhang[2], Peng Ji[1], Xin Hu[1], Zhi-Ming Shao[1]

[1] *Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China*
[2] *Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, People's Republic of China*

Introduction: Bone is the most frequent site of metastatic lesions among patients with breast cancer. Hence, we conducted this study to analyze the incidence proportions and prognostic factors of patients with breast cancer and bone metastases at the time of cancer diagnosis.

Methods: Women with primary invasive breast cancer and bone metastases at initial diagnosis between 2010 and 2014 were identified using the Surveillance, Epidemiology, and End Results program. Multivariable logistic regression were performed to identify predictors of the presence of bone metastases at diagnosis. Univariate and multivariate analyses were performed to determine the effects of each variable on overall survival (OS) and breast cancer-specific survival.

Results: We included 229,195 patients with primary invasive breast cancer. Of these, 8,295 patients had bone metastases at initial diagnosis, reflecting 3.6% of the entire study population and 65.1% of the subset with metastatic disease to any distant site. Among entire cohort, multivariable logistic regression identified age, sex, race, histology, grade, molecular subtype, extraosseous metastatic sites, marital status, insurance, income and education as predictors of the presence of bone metastases at diagnosis. Median OS for the patients with bone metastases was 30.0 months. Patients with HR-positive HER2-positive subtype had the longest median OS (41.0 months) and patients with triple negative subtype showed the shortest median OS (11.0 months). On multivariable Cox regression for overall mortality, patients with older age, lobular histology, higher grade, triple negative subtype and more extraosseous metastatic sites had significantly shorter OS.

Conclusion: The findings of this study provides population-based estimates of the incidence and prognosis for patients with bone metastases at initial diagnosis of breast cancer.

Keywords:
Breast cancer; Bone metastasis; Incidence; Prognosis

References:
1. Piccioli A, et al. J Orthop Traumatol 2015; 16: 81-86.
2. Torre LA, et al. CA Cancer J Clin 2015; 65: 87-108

P2.4

Clinicopathologic features and prognostic factors in Alpha-Fetoprotein-Producing colorectal cancer: analysis of 78 cases

Qingguo Li, Yang Feng, Sanjun Cai

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Alpha-fetoprotein-producing colorectal cancer (AFPP-CRC) is very rare. This study aimed to elucidate the clinicopathologic characteristics and prognostic factors of AFPP-CRC.
Methods: Among 5,051 colorectal cancer patients receiving surgery in the Fudan University Shanghai Cancer Center from 2006 to 2016, we identified 78 patients with elevated serum level of AFP (>10 μg/L) preoperatively. Due to marked disparities in baseline characteristics and cohort size, a propensity score matching (PSM) analysis was performed which matched 75 AFPP-CRC patients to the same number of AFP-negative colorectal cancer (AFPN-CRC) patients. Kaplan-Meier curves were compared using the log-rank test and multivariable analysis was performed to evaluate the effect of AFP-positivity while adjusting confounding factors. Functional assays were used to characterize the tumorigenicity of AFP.
Results: Patients with AFPP-CRC had a significantly higher incidence of advanced TNM stage and liver metastasis. Overall survival was significantly different between two groups before and after PSM, and AFP-positivity was one of the strongest predictors of overall survival in the multivariable model (HR 4.11, CI 95%: 1.43-11.76, p = 0.009) after PSM. We further investigated prognostic factors affecting prognosis in AFPP-CRC and found that the presence of liver metastasis was the only independent prognostic factor (HR 4.95, CI 95%: 1.48-16.48, p = 0.009). Transwell invasion assay revealed significantly increased cell motility with AFP overexpression.
Conclusion: AFP-positivity is a significant negative predictor of overall survival in patients with colorectal cancer.
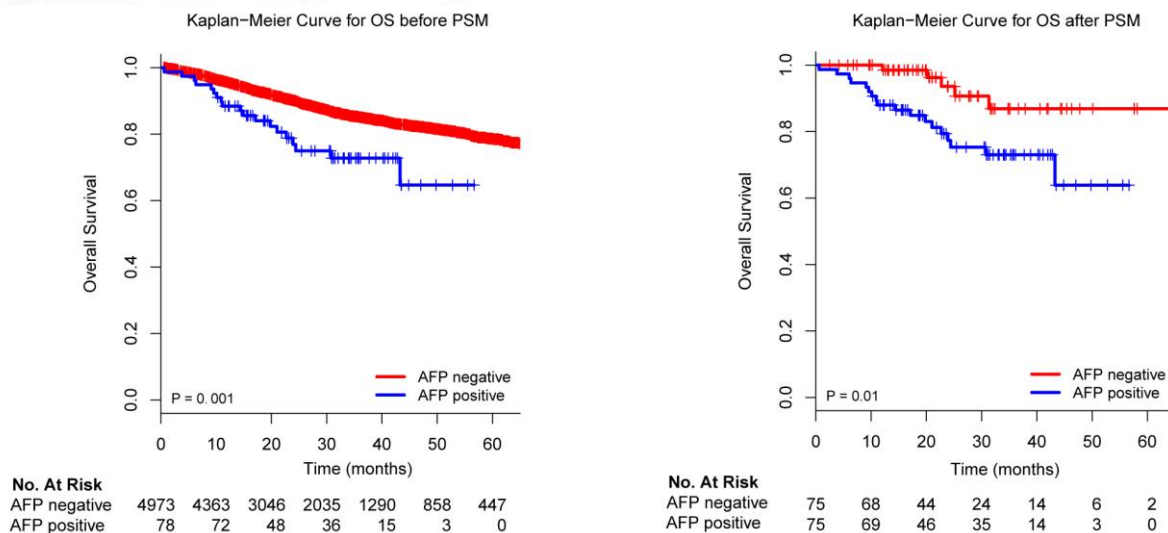
Fig. 1. Overall survival (OS) related to AFP-producing colorectal cancer before and after propensity score matching (PSM).

A nomogram to predict synchronous colorectal peritoneal metastases: study of its utilities using decision curve analysis

Shaobo Mo, Guoxiang Cai

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: The objective of this study was to summarize the clinicopathological and molecular features of synchronous colorectal peritoneal metastases (CPM) compared to non-peritoneal metastases. We then combined clinical and pathological variables associated with synchronous CPM into a nomogram and confirmed its utilities using decision curve analysis.

Methods: Synchronous metastasis colorectal cancer (mCRC) patients who received primary tumor resection and underwent KRAS, NRAS, and BRAF gene mutation detection at the Fudan University Shanghai Cancer Center from January 2014 to September 2015 were included in this retrospective study. An analysis was performed to investigate the clinicopathological and molecular features for independent risk factors of synchronous CPM and to subsequently develop a nomogram for synchronous CPM based on multivariate logistic regression. Model performance was quantified in terms of calibration and discrimination. We studied the utility of the nomogram using decision curve analysis.

Results: In total, 226 patients were diagnosed with synchronous mCRC, of whom 50 patients (22.1%) presented with CPM. After uni- and multivariate analysis, a nomogram was built based on tumor site, histological type, age, and T4 status. The model had good discrimination with an area under the curve (AUC) at 0.777 (95% CI 0.703-0.850) and adequate calibration. By decision curve analysis, the model was shown to be relevant between thresholds of 0.22 and 0.66.

Conclusion: This is the first nomogram to predict synchronous CPM. To ensure generalizability, this model needs to be externally validated.

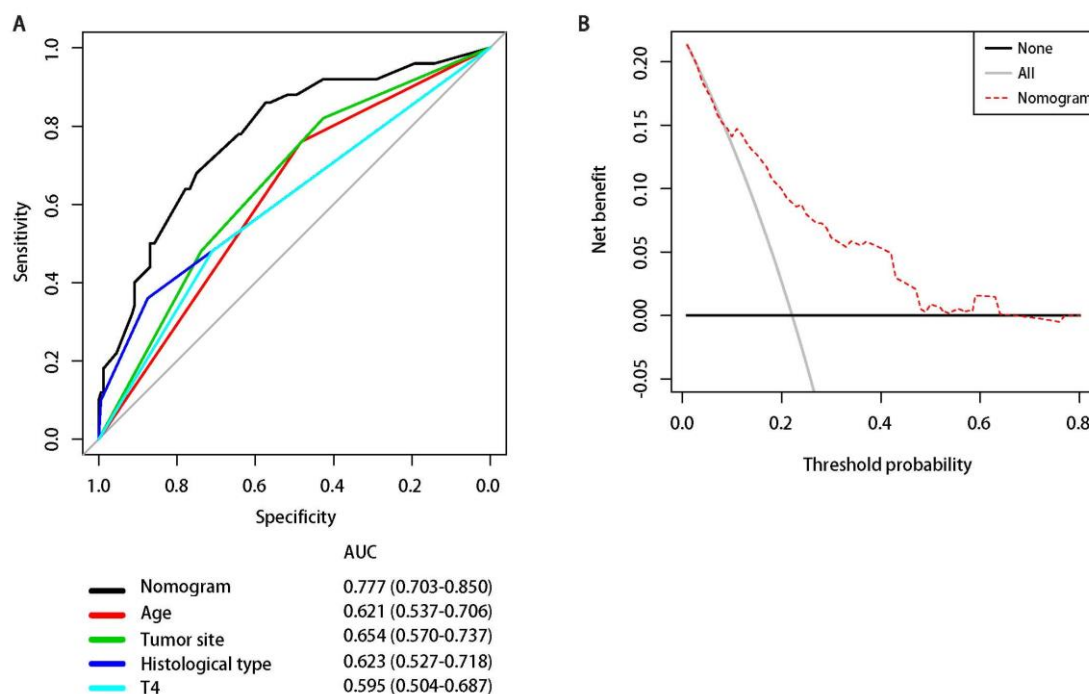|	AUC
Nomogram	0.777 (0.703-0.850)
Age	0.621 (0.537-0.706)
Tumor site	0.654 (0.570-0.737)
Histological type	0.623 (0.527-0.718)
T4	0.595 (0.504-0.687)

Fig. 1. The model had good discrimination with an area under the curve (AUC) at 0.777 (95% CI 0.703-0.850) (A). By decision curve analysis, the model was shown to be relevant between thresholds of 0.22 and 0.66 (B).

Prognostic and Predictive Value of an Autophagy-related Signature for Early Relapse in Stage I-III Colon Cancer

Shaobo Mo, Guoxiang Cai

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Early relapse of colon cancer has been proved to be associated with poor survival. Nevertheless, current staging methods can not accurately evaluate the risk of disease recurrence and predict patients' prognosis. Therefore, we postulated that expression differences of autophagy-related genes are instrumental in stratifying the risk of early relapse after surgery and evaluating the prognosis of patients with stage I-III colon cancer.

Methods: Information of 232 autophagy genes was obtained from the Human Autophagy Database and three independent microarray datasets of stage I-III colon cancer samples were extracted from Gene Expression Omnibus database. Propensity score matching analysis was performed between patients in early relapse group and long-term survival group from GSE39582 test series and internal validation series. The differentially expressed autophagy genes were identified by Linear Models for Microarray data method. Then a nine-autophagy-related signature was built using Cox regression model, and we subsequently validated its prognostic value in both internal validation set and two external independent groups in GSE14333 and GSE33113 datasets.

Results: Using Cox regression model, a nine-autophagy-related signature (CAPN2, ATG16L2, TP63, SIRT1, RPS6KB1, PEX3, ATG5, UVRAG, NAF1) was established to classify patients into those at high risk of early relapse (high-risk group), and those at low risk of early relapse (low-risk group). Relapse free survival (RFS) was significantly different between the two groups in test (HR: 2.019, 95% CI: 1.362–2.992, p<0.001), internal validation (HR: 2.464, 95% CI: 1.196–5.079, p<0.001) and another two external validation series (GSE14333 HR: 2.250, 95% CI: 1.227–4.126, p=0.007; GSE33113 HR: 5.552, 95% CI: 2.098–14.693, p<0.001). Based on RFS, we developed a nomogram, integrating the nine-autophagy-related classifier and four clinicopathological risk factors to evaluate prognosis of stage I-III colon cancer patients.

Conclusions: We identified and built a nine-autophagy-related signature, a credible approach to early relapse prediction in stage I-III colon cancer patients, which may assist physicians in devising more efficient therapeutic strategies.

Keywords:
Autophagy, Early Relapse, Prognosis, Colon Cancer
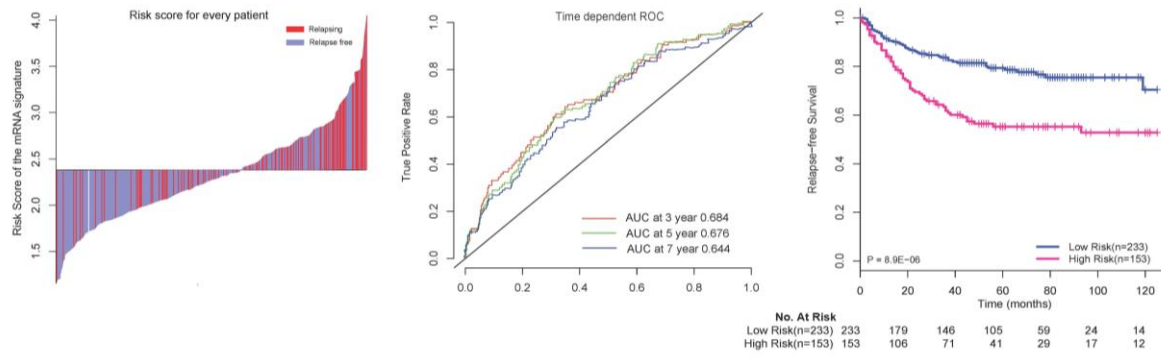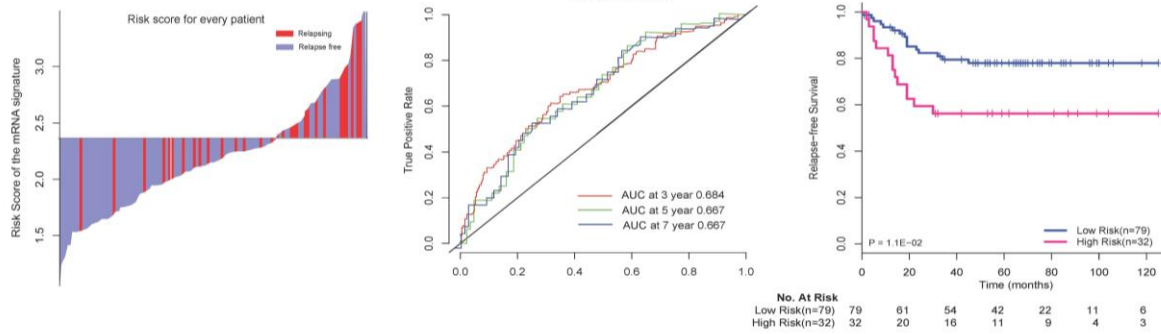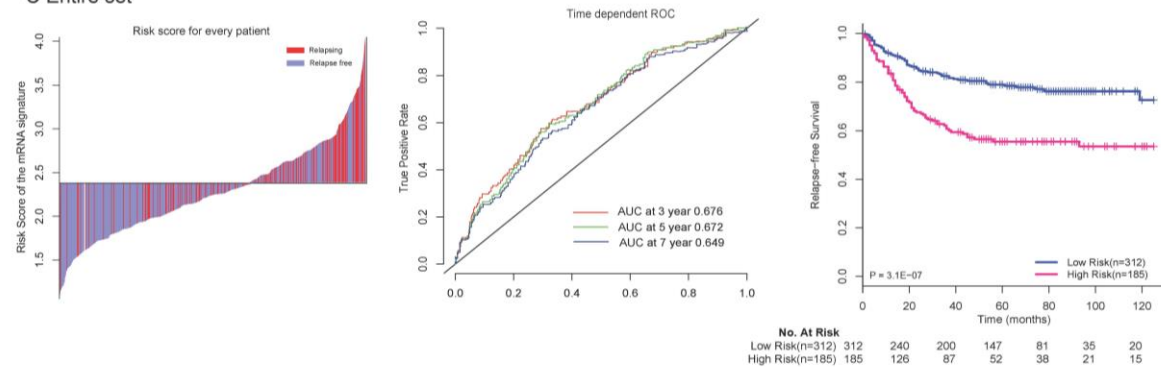
Fig. 1. Distribution of risk score, time dependent ROC curves at 3, 5 and 7 years and Kaplan-Meier survival analysis between patients at low and high risk of relapse in test set (A), internal validation set (B), and entire dataset (C).

Transcriptome profiling reveals an integrated mRNA-lncRNA signature with predictive value of early relapse in colon cancer

Weixing Dai, Guoxiang Cai

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: As one of the most common malignant disease worldwide, colon cancer is extremely diverse in different patients with variable outcomes. The aim of our study was to develop an RNA signature to identify a group of patients who are at high risk for early relapse in stage II-III colon cancer.

Methods: Public transcriptome microarray data of colon cancer were extracted from Gene Expression Omnibus database and comprehensively analyzed. Firstly, propensity score matching was conducted between patients in early relapse group and long-term survival group from GSE39582 training series (N=359) and patients were 1:1 matched. Global transcriptome analysis was then performed between the paired groups to identify tumor specific mRNAs and lncRNAs. Finally, using LASSO Cox regression model, we built a multigene early relapse classifier incorporating mRNAs and lncRNAs. The prognostic and predictive accuracy of the signatures were internally validated in 102 colon cancer patients and externally validated in other 241 patients.

Results: In the training set, patients with high risk score were more likely to suffer from relapse than patients with low risk score (HR: 2.67, 95% CI: 2.07–3.46, P<0.001). The results were validated in the internal validation set(HR: 2.23, 95% CI: 1.23–3.78, P=0.003) and external validation (HR 1.88, 95% CI 1.42–2.48; p<0.001) set. Time-dependent receiver operating curve at 1 year showed that the integrated mRNA–lncRNA signature (AUC=0.742) had better prognostic accuracy than AJCC TNM stage (AUC=0.615) in the entire 702 patients. In addition, survival decision curve analyses revealed the better clinical usefulness of the integrated mRNA–lncRNA classifier.

Conclusions: We successfully developed an integrated mRNA–lncRNA signature based on sixteen mRNAs and two lncRNAs. This multigene signature may help to identify high-risk colon cancer patients who deserve an enhanced follow-up and therapeutic program.
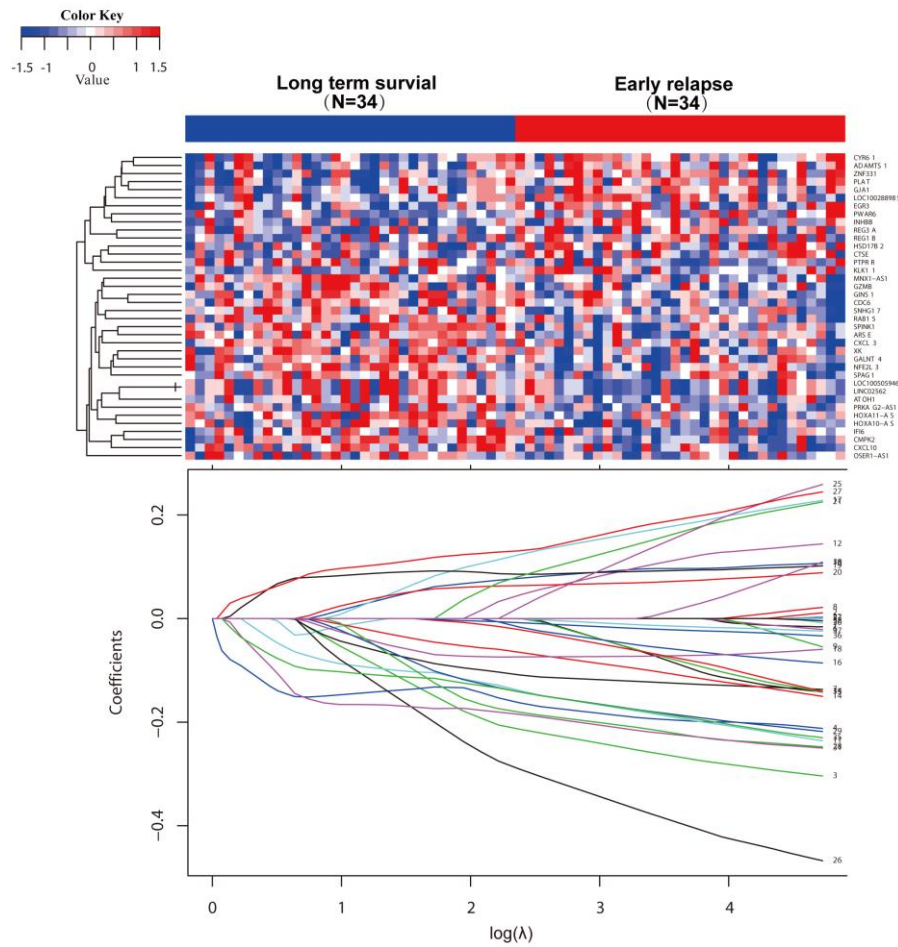
Figure 1

Fig. 1. (A) Heat map showed eighteen differentially expressed mRNAs in colon cancer between early relapse and long-term survival group both in discovery set. (B) LASSO coefficient profiles of the early relapse associated mRNAs. A vertical line is drawn at the value chosen by 10-fold cross-validation.

P2.8

Monitoring serum VEGF in neoadjuvant chemotherapy for TNBC patient: a new strategy for earily prediction of treatment response and survival

Ruo-Xi Wang, Sheng Chen, Liang Huang, Zhi-Ming Shao

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: This study aimed to investigate the clinical utility of serum biomarker changes during neoadjuvant chemotherapy (NAC) for triple-negative breast cancer (TNBC).

Methods: A total of 303 TNBC patients from an NAC clinical trial cohort were enrolled in this study. All patients were treated according to the same protocol, with weekly paclitaxel and carboplatin. Serum samples were taken at three time points during NAC: baseline, prior to the 3rd cycle, and prior to surgery. Samples from 37 patients were assayed using the Luminex multibiomarker panel for 29 serum biomarkers (IL-10, IL-12P40, IL-12P70, IL-13, IL-15, IL-17A, IL-1RA, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IFN-γ, IFN-α2, eotaxin, MCP-1, EGF, TNF-α, TGF-β, VEGF,IP-10, GM-CSF, G-CSF,MIP-1α, and MIP-1β) to detect their correlation with NAC response. The predictive and prognostic value of each selected biomarker was then validated in the whole study population.

Results: Vascular endothelial growth factor (VEGF) was the only biomarker that correlated with treatment response, with a decreasing trend in pathological complete response (pCR) patients relative to non-pCR patients (p<0.001). Univariate and multivariate analyses revealed that the relative reduction in VEGF after 2 cycles of NAC had a remarkable predictive value for both pCR and pathological non-response (pNR), with high sensitivity and specificity (AUC=0.788 and 0.862, respectively) compared to Ki-67 expression and clinical response based on MRI (see ROC curves in figure 1). Patients in the VEGF-reduced group would have a trebled possibility of achieving pCR (67.0% vs 19.8% of VEGF-stable and 15.7% of VEGF-elevated). The VEGF level prior to surgery was also independently correlated with disease-free survival (DFS) in non-pCR responders (HR=1.008, 95% CI: 1.003-1.014; P=0.004).

Conclusions: Our findings indicate that monitoring serum VEGF could help identify patients with different responses at an early time point of NAC and at varying risk of disease relapse. Serum VEGF may also serve as an alternative to traditional response-evaluating methodologies in tailoring and modifying the NAC strategy for both operable and advanced TNBCs.

Keywords:
Triple-negative breast cancer, VEGF, neoadjuvant chemotherapy, biomarker

Fig 2
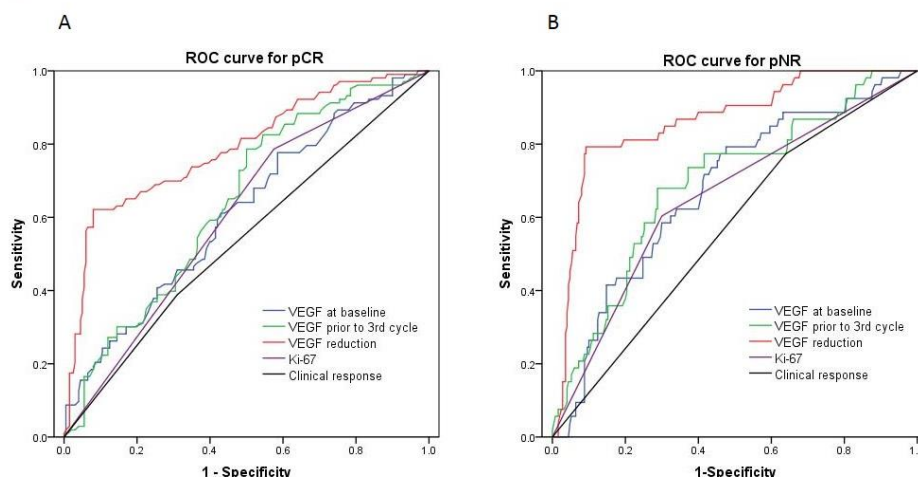
ROC curves for predictors of pCR and pNR. (A)The AUC of VEGF change (red line) was 0.788 (95% CI: 0.732-0.845, P<0.001) ; (B)The AUC of VEGF change (red line) was 0.862 (95% CI: 0.806-0.918, P<0.001)

The size and depth of lesions measured by gastroscopy at diagnosis are prognostic factors of primary gastric diffuse large B-cell lymphoma

Yi-Zhen Liu[1], Kai Xue[2], Bo-Shi Wang[3], Chun-Yan Li[2], Fang-Fang Lv[2], Jia Jin[2], Qun-Ling Zhang[2], Zu-Guang Xia[2], Dong-Mei Ji[2], Hui Sun[2], Jia-Chen Wang[2], Jun-Ning Cao[2], Xiao-Nan Hong[2]

[1] *Fudan University Shanghai Cancer Center, Shanghai, China, People's Republic of China*
[2] *Department of Medical Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, People's Republic of China*
[3] *State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, People's Republic of China*

Objective: Diffuse large B cell lymphoma (DLBCL) is one of the predominant histological subtypes of primary gastric lymphomas (PGLs). Factors that contribute to precise stratification and guide the treatment of this disease are still not well understood. This study aims to identify clinical features and prognostic factors of PG-DLBCL patients.

Methods: A total of 73 patients were included in this retrospective study who were diagnosed as PG-DLBCL and treated in Shanghai Cancer Center, China from January 2007 to July 2017. The demographic details, clinico-pathologic characteristics of the patients were summarized. Kaplan-Meier survival curves were constructed for survival analyses. The prognostic factors were determined by univariate and multivariate Cox analyses.

Results: 34 (46.6%) men and 39 (53.4%) women were enrolled in this study, and median age was 50 years old. Univariate Cox regression analysis showed that PG-DLBCL patients characterized by late stage (Stage III/IV) (P =0.00015), multiple localization (sites ≥ 1) (P =0.034), B symptoms (P =0.001), lower serum albumin (ALB) level (≤ 35g/L) (P =0.00005) and elevated LDH level (>250U/L) (P =0.001) had a shorter overall survival (OS). Multivariate Cox regression analysis demonstrated that ALB ≤ 35g/L (HR=0.154, 95% CI 0.041-0.584, P=0.006), staging III/IV (HR=4.893, 95% CI 1.182-20.253, P=0.028), and multiple sites localization (HR=1.849, 95% CI 1.148-2.976, P=0.011) were independent adverse prognostic factors. Significantly, in thirty-five patients who received endoscopy at diagnosis, Kaplan-Meier analyses indicated that patients with large (≥3cm) and deep lesions (≥11mm) had an inferior OS compared to those with small, superficial lesions (P = 0.01, = 0.039).

Conclusions: Our work revealed that ALB ≤ 35g/L, staging III/IV and multiple sites localization were independent adverse prognostic factors of PG-DLBCL patients. The PG-DLBCL patients with large (≥3cm) and deep (≥11mm) lesions under endoscopic ultrasound were indicated to have a worse OS.

P2.10

Mass spectrometry imaging and LC-MS/MS studies of mouse malignant brain tumor in situ

Chiungyin Huang, Kuo Chen Wei

Chang Gung University, Taoyuan, Taiwan

Lipids perform multitude of functions in the biological system and reflect the physiological status of cells and tissues. We investigated the changes in phospholipids (PLs) and sphingolipids (SLs) in the rodent model of fast-growing in situ malignant brain tumorusing matrix-assisted laser desorption-ionization mass spectrometry imaging (MALDI-MSI) and liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques to characterize the qualitative and quantitative lipidomic features of this rapid growing neoplasm. The MALDI-MSI demonstrated the significant down- and up-regulation of palmitoyl-phosphatidylcholines (PCs) species, and the heterogeneity of such changes in these and other tumoral PC and lyso-PC species. The distribution of certain PCs also signified the peripheral shell and the deep core regions of the tumor, while others served as the earmarks of the cortical or striatal tumor parenchyma. The MSI of SLs showed the overall changes of sphingomyelin (SM) and the heterogeneous, ceramide-enriched tissue patch embedded deep in the tumor tissue. The LC-MS/MS analysis of tumor tissue lipids supported and complemented the MALDI-MSI observation, and provided a more comprehensive phospholipid (PL) overview.

When consulting the gene expression profile in the malignant brain tumor, we found that the altered expression of certain lipid-metabolism machineries like phospholipase, fatty acyl transferases, and fatty acid binding protein were among the affected genes that supported the mechanistic insights to the lipidomic observation presented herein. Putting together, this study integrated observed lipidomic changes with the regulation of upstream gene candidates of metabolic machineries to cast a more holistic and dogmatic view of phospholipidome in the malignant brain tumors.

Reg4 expression as a subtype indicator of lung adenocarcinoma with mutated of KRAS and negative TTF1

Hui Yu[1], Si Sun[2], Jialei Wang[2], Xingjiang Hu[3]

[1] Fudan university;Cancer hospital; Shanghai, People's Republic of China
[2] Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China
[3] First Affiliated Hospital, Zhejiang University, Hangzhou, People's Republic of China

Background: Mutations in KRAS are presented in up to 30% of lung cancer cases. No effective therapies specifically targeting mutant KRAS have been developed. In recent studies as well as TCGA database, the KRAS mutated patients were classified as three subtypes according to concurrent molecular alterations: KL subtype (LKB1/STK11 mutation), KP subtype (TP53 mutation), KC type (TTF-1 negative), which accounts for about 44%, 34% and 22%, respectively. In KP subtype, patients were sensitive to checkpoint inhibitor treatment, however, for those with KP or KC subtypes, the biological character and mechanism were still unclear.

Methods & Results: We analyzed the publicly available E-GEOD-31210 microarray and TCGA database, and found REG4, an important regulator of GI carcinogenesis, was highly expressed in KRAS mutated lung adenocarcinoma with low expression of TTF-1 (KC subtype). We validated these results using quantitative real-time PCR in an independent 55 clinical samples from Fudan University Shanghai Cancer Center (FUSCC); the results showed that REG4 expression was much higher in KC subtype than KL and KP subtypes (P<0.01). We selected two cell lines DV90 and NCI-H727, which have KRAS mutation and high REG4 expression, to conduct the in vitro experiments. Results showed that cell proliferation was significantly reduced when silencing the REG4 gene (P<0.001). Immunochemistry study showed that REG4 expression was limited to the TTF1 negative patients. RNA sequencing revealed that REG4 downregulation resulted in an induction of p16, which negatively regulates E2F pathway and blocks the G1/S transition.

Conclusions: The results of this study highlighted an insight into REG4 was high expression in KRAS mutation with TTF1 negative lung adenocarcinoma. We had discovered REG4 downregulation resulted in an induction of P16, which negatively regulates E2F pathway and blocks the G1/S transition. These work provided evidence that REG4 may play an important role in tumorgenesis. Additional studies are warranted to evaluate the clinical value of KRAS inhibitor treatment in KC subtypes lung adenocarcinoma.

Identification and Validation of a 23-gene Expression Signature for Medulloblastoma Molecular Classification

Qinghua Xu[1], Qifeng Wang[2], Jinying Chen[3], Chengshu Chen[3], Yifeng Sun[3], Lei Chen[3], Qing Ye[4], Xiang Du[2]

[1] *Tongji University, Shanghai, People's Republic of China*
[2] *Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China*
[3] *Canhelp Genomics, Hangzhou, People's Republic of China*
[4] *Nanjing Drum Tower Hospital, Nanjing, People's Republic of China*

Background: Medulloblastoma is the most common malignant brain tumor in children accounting for about 10% of all pediatric cancer deaths. According to the 2016 WHO Classification, four molecular subtypes of medulloblastoma including WNT, SHH, Group 3 and Group 4 were characterized by high--throughput gene expression profiling. These molecular subtypes display distinct clinical, demographic and genetic features that are associated with prognostic and therapeutic differences. Because it is currently impractical to perform microarray analysis in clinical settings, distinguishing the four molecular subgroups of medulloblastoma in the daily treatment of patients, as well in the setting of clinical trials, remains an important challenge.

Materials and methods: Three medulloblastoma microarray datasets were curated to perform an integrative analysis. To identify a reliable biomarker, a training--validating approach was adopted in this study. The gene expression profiles of 103 samples were selected as a training set for signature identification. Additional 358 samples were used for signature validation. The Gene Ontology and KEGG pathway analysis were performed to reveal the biological features of candidate genes.

Results: A 23--gene expression signature derived from the training set was strongly associated with the molecular subtypes of medulloblastoma. The 23--gene expression signature was validated in 8 WNT, 61 SHH, 62 Group 3 and 227 Group 4 samples. With the 23-gene expression signature, 8 samples were classified as WNT, 64 as SHH, 71 as Group 3 and 215 as Group 4. The gene expression- based assignments reached a 95.5% overall agreement with the reference diagnoses (342 of 358; 95% CI: 0.928 to 0.974). Sensitivity ranged from 94% to 100%, while specificity ranged from 96% to 100%. The functional enrichment analysis showed that 23 genes were significantly associated with signal transduction and WNT signaling pathway.

Conclusions: A 23--gene expression signature that could accurately discriminate molecular subtypes of medulloblastoma was identified in this study. Our results may prompt further development of this gene expression signature into a molecular assay amenable to routine clinical practice.

Keywords: medulloblastoma, gene expression profile, molecular classification

Table 1. Performance characteristics of the 23-gene signature in the Test Set

a.

Prediction	Reference				
	Group 3	Group 4	SHH	WNT	Total
Group 3	60	11	0	0	71
Group 4	2	213	0	0	215
SHH	0	3	61	0	64
WNT	0	0	0	8	8
Total	62	227	61	8	358

b.

	Sensitivity	Specificity	PPV	NPV
Group 3	97%	96%	85%	99%
Group 4	94%	98%	99%	90%
SHH	100%	99%	95%	100%
WNT	100%	100%	100%	100%

*PPV: Positive Predictive Values NPV: Negative Predictive Values

Table 1. Performance characteristics of the 23-gene signature in the test set

P2.13

Immune profile of BRAF mutated metastatic colorectal tumors with good prognosis

Tae Won Kim[1], Jeong Eun Kim[1], Ji-Hun Kim[1], Yong Sang Hong[1], Sun Young Kim[1], Sang-Yeob Kim[2], Jaekyung Cheon[3]

[1] *Asan Medical Center, Seoul, South Korea*
[2] *Asan Institute for Life Sciences, Seoul, South Korea*
[3] *Ulsan University Hospital, Ulsan, South Korea*

Background: BRAF mutated (mBRAF) metastatic colorectal cancers (mCRC) are known to be related to poor prognosis and low response to current chemotherapy. We investigated immune profile of mBRAF tumors to define other mechanism related to prognosis.

Patients and Methods: A total of 2313 patients (pts) with mCRC were treated and received BRAF testing between Jan 2005 and OCT 2015 at Asan Medical Center. We analyzed clinical outcomes of all pts and investigated immune profile of mBRAF tumors using multiplex immunohistochemistry of immune related markers; cytokeratin (CK), programmed death-ligand 1(PD-L1), Programmed cell death protein 1 (PD-1), cluster of differentiation 8(CD8).

Results: Out of 2313 tumors, 2190 were wild type BRAF (wBRAF) and 123 were mBRAF tumors; 119 had BRAF mutation of V600E; 3 of K6001E; 1 of V600E and K600E. Overall survival (OS) were longer in wBRAF than mBRAF tumors (32.8 vs. 14.6 months, p30 months), intermediate (27 pts, 12-36 months), poor (25 pts, <12 months). In microsatellite stable (MSS) mBRAF tumors with good prognosis, proportion of PD-L1- positive tumor cell was lower, while stromal CD8-positive cell infiltration was higher.

Conclusion: Clinical outcomes of unselected mBRAF mCRC patients treated in real clinical practice were generally similar to previous studies. However, distinct subgroup of mBRAF patients showed comparable survival to wt BRAF. With immune profile analysis, lower PD-L1 expression and higher stromal CD8-positive cell infiltration was shown in mBRAF tumors with good prognosis.

Keywords:
CRC, BRAF, Prognosis

P2.17

Molecular pathway activation – new type of biomarkers for tumor morphology and personalized selection of target drugs

<u>Anton Buzdin</u>[1], Maxim Sorokin[1], Alf Giese[2], Nicolas Borisov[1], Andrew Garazha[3], Ella Kim[4], Amir Samii[2], Xinmin Li[5], Christian Hartmann[6], Elena Poddubskaya[1], Marina Sekacheva[1]

[1] *I.M. Sechenov First Moscow State Medical University, Moscow, Russia*
[2] *International Neuroscience Institute, Hannover, Germany*
[3] *Omicsway Corp., Walnut, CA, United States*
[4] *Johannes Gutenberg University Mainz, Mainz, Germany*
[5] *University of California, Los Angeles, CA, United States*
[6] *Institute of Neuropathology at Hannover Medical School, Hannover, Germany*

Background: Anticancer target drugs (ATDs) specifically bind and inhibit molecular targets that play central roles in intracellular signaling pathways, thus regulating cancer development and progression. Different ATDs have different mechanisms of action and are effective for different individuals. Advanced molecular approaches are needed to personalize selection of ATDs, but now they are used for only a minor fraction of patients.

Materials and Methods: We proposed a new type of biomarkers: degree of molecular pathway activation. Pathway activation value is the function of (i) normalized expression levels for the respective genes, and (ii) of the coefficients describing their molecular roles in a pathway. We compiled data on >3.100 human molecular pathways.

Results: Pathway approach showed strong ability to improve stability of expression data by increasing correlations between the data obtained using different platforms. For 12 major cancer types pathways showed better AUC values than the enclosed individual genes taken one by one. We proposed an approach of using the pathway activation metrics as the response markers for ATDs. For 5 different ATDs used for 7 cancer types, we correlated activities of marker pathways with published clinical trial-based response to treatments with a correlation 0.77, p = 0.023. Cancer applications included finding retrospective biomarkers of cetuximab response for colorectal; vemurafenib for melanoma (n=51); sorafenib for thyroid (n=18), lung (n=50) and renal (n=39); sunitinib for lymphomas (n=15); imatinib for thyroid (n=8); bevacizumab for colorectal (n=28) and kidney (n=182); imatinib for sarcomas (n=30).

In a preliminary prospective trial, the same platform was used to support personalized choice of ATDs prescription to the advanced solid cancer patients (n = 35). The efficacy of ATD treatments was 61% (CR+PR, RECIST), or 79 % (CR+PR+SD). The drugs prescribed were bevacizumab, sorafenib, imatinib, pazopanib, aflibercept, paclitaxel and docetaxel.

Conclusions: Molecular pathway-based analysis of gene expression may be effective in finding new biomarkers for tumor morphology and treatment response. We seek collaborations to study gene expression features in relation to response on anticancer therapies.

HER2 1+/2+ FISH positive breast cancer shows undifferentiated response to neoadjuvant chemotherapy compare with HER2 3+ tumors

Xiaoli Xu, Rao Jia

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Breast cancer with HER2 over expression detected by immunohistochemistry (IHC) and/or HER2 amplification identified by fluorescence in situ hybridization (FISH) is eligible for anti-HER2 therapy. Whether the two groups of tumor have the same response to anti-HER2 neoadjuvant therapy is unclear. We sought to compare the pCR rate in neoadjuvant chemotherapy (NAC) and anti-HER2 therapy between HER2 IHC 3+ and HER2 FISH positive (IHC 1+/2+) breast cancers.

Methods: 110 cases of HER2-positive breast cancer in pre-NAC and anti-HER2 targeted therapy core needle biopsies were retrieved in this study, including 72 that had HER2 IHC (3+) positive tumors and 38 with FISH-positive tumors (IHC 1+/2+). Information regarding ER status and HER2/CEP17 FISH ratios were obtained from the electronic chart. HER2 IHC and FISH results were assessed according to the current American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) recommendations. The response of NAC was assessed using Miller-Payne system. Statistical analysis was performed using two-tailed Fisher's exact test.

Results: Overall, 34 showed pathological complete response in this study, the pCR rate was 30.9%. The pCR was 33.3% (24/72) in HER2 IHC 3+ cases and 26.3% (10/38) in HER2 IHC 1+/2+ FISH positive cases. The difference was not significant (P>0.05). HER2/CEP17 FISH ratios for the IHC 1+/2+ cases ranged from 1.9 to 6.53 (median: 3.59). The pCR was not related with FISH ratio. (P>0.05) Among IHC 3+ tumors, 45.8% (33/72) were ER positive and 40% (10/25) of them were pCR. There was no statistically significant difference in response to NAC in ER+/IHC+ vs. ER-/IHC+ tumors (p>0.05). Among FISH-positive (IHC 1+/2+) tumors, 52.6% (20/38) were ER positive and 33.3% (3/9) of them were pCR. There was no statistically significant difference in response to NAC in ER+/FISH+ versus ER-/FISH+ tumors (p>0.05).

Conclusions: In our study, the response to NAC in HER2 FISH positive (IHC 1+/2+) breast cancers is undifferentiated compared with HER2 IHC 3+ cases. There was no difference in response to NAC regardless of ER status in the HER2 3+ and HER2 1+/2+ FISH positive cases. These findings suggest that the breast cancer patients with HER2 IHC 1+/2+ also benefit from anti-HER2 neoadjuvant therapy.

A model-based prediction of survival outcome for breast cancer patients stratified to be integrative cluster-2 in the METABRIC cohort

Carlos Traynor[1], Michael Chappell[1], Neil Evans[1], Tarjinder Sahota[2], Helen Tomkinso[2]

[1] *University of Warwick, Coventry, United Kingdom*
[2] *Astrazeneca, Cambridge, United Kingdom*

Background: Recently, the study of the METABRIC cohort [1] using integrative clustering confirmed that there are at least 10 subtypes of breast cancer which have different clinical outcomes. Moreover it was established that the integrative cluster-2 (iC-2), currently classified as ER+, has poor prognosis. The aim of this study is two-fold: to compare iC-2 to ER+/HER2- as a covariate selection model, and to describe the genomic predictors associated with survival in iC-2.

Methods: Two datasets were filtered with ER+/HER2- (n=1523) and iC-2 (n = 72) patients. The survival model comprised a Cox grouped Lasso, which penalised the combined genomic and transcriptomic coefficients within a clinico-genomic model, assuming age and NPI to be well-established clinical factors. A Monte Carlo cross-validation was conducted in a test dataset, including iC-2 patients only (not used in the training dataset); and prediction performance was measured via the survival Brier score (BS).

Results: Table 1 summarises the BS for each model. The probability mass of the null model did not overlap the iC-2 model (i.e. practically negative = 1 at ± 0.05); whilst iC-2 and ER+/HER2- models overlapped, with iC-2 being the best model (practically equivalent = 0.442 at ± 0.05). An association of poor prognosis with the oncogenic signature was found for the iC-2 predictors, with Wnt pathway component LEF1 (HR: 1.598, 95% CI 1.080-2.365) and mutant K-ras upregulated genes (HR: 2.055, 95% CI 1.249-3.382).

Conclusion: This study shows that the iC-2 stratification yields superior predictions of clinical outcomes than ER+/HER2- pooling. Our results reveal a gene signature for iC-2 that may support the development of tailored therapies by mapping the Wnt and K-ras signalling pathways. All relevant code can be found at https://github.com/csetraynor/iclust2prog.

References:
[1] Pereira, B. et al. Nat. Commun. 7, 2016.

Model	Lower (5%)	Mean	Upper (95%)	Practically neg	Practically equiv	Practically pos
iC-2	0.062	0.069	0.077			
ER+/HER2-	0.109	0.120	0.133	0.558	0.442	0
Null	0.161	0.178	0.195	1	0	0

Table 1: Posterior distribution of BS for each model summarised by lower quantile 0.05, mean, and upper quantile 0.95. Bayesian variable selection model comparison of ER+/HER2- and Null against the iC-2. Key: "Practically neg", practically negative; "Practically equiv", practically equivalent; "Practically pos", practically positive.

Application of the immunoscore as prognostic biomarkers in patients with high-grade serous ovarian cancer

Yong-Man Kim, Shin-Wha Lee, Young-Jae Lee, Young-Tak Kim

Ulsan University, ASAN Medical Center, Seoul, South Korea

Background: The analysis of single parameters alone may not provide sufficient insights about complex immune system–tumor interactions. This study is to validate the immune contexture as prognostic biomarkers in high-grade serous ovarian cancer (HGS-OC) and to find new era of immunoscore in HGS-OC.

Materials and methods: We collected FFPE samples from 187 patients with HSOC and produced TMA samples. We accomplished the OPAL multiplex IHC assay for the quantitative analysis of immune markers, including CD4, CD8, CD20, FoxP3, PD-L1, and CK. Multiplex Biomarker Imaging and in-Form® Image Analysis Software was used.

Results: FIGO stage III and IV patients were 84.5% (158/187). The optimal debulking surgery was done in 66.8% (125/187). The 3-year disease free survival and 5-year overall survival were 35.1% and 50.0%, respectively. Any single marker was not related to the survival including CD8, FoxP3, and PD-L1. However, high CD8:FoxP3 and CD8:PD-L1 ratios were correlated with the good survival. In cox regression model, the risk factors for HGS-OC survival were FIGO stage (HR 1.784, 95% CI: 1.295-2.457, $p<0.001$) and platinum resistance (HR 4.257, 95% CI: 2.753-6.582, $p<0.001$). Additionally, CD8:PD-L1 ratio was a favorable prognostic factor (HR 0.621, 95% CI: 0.042-0.917, $p=0.017$).

Conclusions: These findings indicate that, although any single immune marker is not related to the survival, CD8:FoxP3 and CD8:PD-L1 ratios provide the positive correlation with the prognosis in HGS-OC. Especially, CD8:PD-L1 ratio is prognostic biomarker which is comparable to clinical biomarkers. The next study for immunoscore is necessary to define immunoscore in ovarian cancer.

Long non-coding MIR31HG outlier expression defines colorectal cancers with inferior outcome independent of consensus molecular subtypes

Peter W. Eide, Ina A. Eilertsen, Anita Sveen, Ragnhild A. Lothe
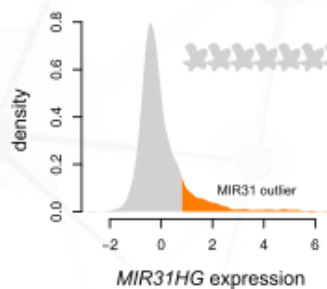
Institute for Cancer Research, Oslo, Norway

Background: MicroRNA-31 has oncogenic properties and clinicopathologcial associations in colorectal cancer (CRC). We hypothesize that miR-31 and the co-transcribed long non coding RNA MIR3HG are prognostic biomarkers for CRC independent of gene expression based consensus molecular subtypes (CMS)

Materials and methods: Totally 1993 pre-clinical and clinical CRC samples were analyzed for expression of the mature miR-31-5p and/or long non-coding MIR31HG in smallRNA sequencing and transcriptome microarrays/RNA sequencing data. Molecular and clinicopathological associations were determined in an aggregated set of three patient cohorts (total=1265). Cell line (n=78) gene set expression analysis was applied to biologically characterize subgroups and associations verified in patient-derived xenografts (PDX, n=51) and primary CRCs (n=1864).

Results: miR-31-5 had a wide expression range of ~500-fold in CRC cell lines, and a strong expression correlation with the MIR31HG. MIR31HG outlier expression was observed in 12% of pCRCs and was associated with depletion of CMS2-canonical subgroup and shorter relapse-free survival (RFS) in multivariable analysis (HR=2.2 [1.6-3.0]). MIR31HG outlier was associated with inferior outcome also within the poor prognostic CMS4-mesenchymal gene expression subtype specifically. Both primary tumors and pre-clinical models with MIR31HG outlier expression were characterized by elevated EMT gene signature and reduced differentiation .

Conclusions: We here present the first evidence that MIR31HG expression provides clinical stratification beyond major gene expression phenotypes, and we propose that increased expression is an indicator of a cellular state conferring intrinsic invasive and/or immuno-evasive capabilities.
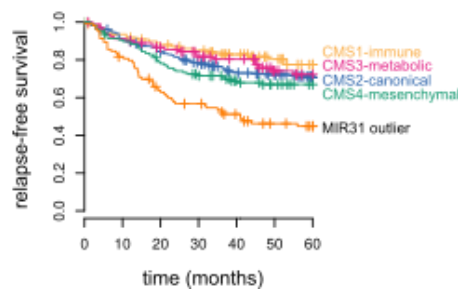
Figure 1: (left) A subset of primary colorectal cancers exhibit MIR31HG outlier expression. (right) MIR31HG outlier status is associated with poor outcome independent of consensus molecular subtypes (CMS).

Exome analysis to reveal genomic markers associated with better efficacy of nivolumab in lung cancer patients

Corentin Richard, Jean-David Fumet, Laurent Arnould, Caroline Truntzer, Romain Boidot, François Ghiringhelli

Centre Georges François Leclerc, Dijon, France

Background: Monoclonal antibodies targeting immune checkpoint inhibitors revolutionized the treatment of lung cancer. However, only one-quarter of non-small cell lung cancer patients (NSCLC) benefit from these new therapies. The assessment of PD-L1 tumor expression by IHC is used to select responder patients and is considered as the gold standard biomarker in many studies; however, this marker does not predict the absence of anti-PD-1 efficacy. Recent studies suggest that high tumor mutational burden (TMB) is associated with better efficacy of Nivolumab compared with chemotherapy. In addition, transcriptomic and exome analyses have revealed potential biomarkers needing further confirmation. It is thus of major importance to define biomarkers that efficiently predict Nivolumab efficacy.

Methods: We performed somatic and constitutional exome analyses for 77 patients with NSCLC treated with Nivolumab. We studied: 1-tumor-related characteristics: aneuploidy, tumor copy number alteration clonality, mutational signatures, TMB, mutations in WNT, AKT, MAPK and DNA repair pathways, and 2-immunological characteristics: number of intratumoral TCR clones, HLA type, number of neoantigens, and Type I IFN mutation pathway; and 6 clinical parameters.

Results: A high TMB per Mb, a high number of neoantigens, mutational signatures 1A and 1B, mutations in DNA repair pathways and a low number of TCR clones are associated with greater PFS. Using the least absolute shrinkage and selection operator method, we established an exome-based model with 9 parameters that could discriminate patients with good or poor PFS ($p < 0.0001$) and OS ($p = 0.002$) independently from clinical data (Figures BC). Moreover, this model had improved ability to predict outcomes compared with a PD-L1 clinical model with or without TMB (Area Under Curve: Clinical = 0.80, TMB = 0.81, exome-derived = 0.93) (Figure A). The model was externally validated on another cohort of 34 patients treated with Pembrolizumab (Rizvi et al., 2015) (Figure 4).

Conclusions: Altogether, these data provide a validated biomarker that predicts the efficacy of Nivolumab or Pembrolizumab in lung cancer patients. Our biomarker appears to be superior to PD-L1 labelling and TMB models.
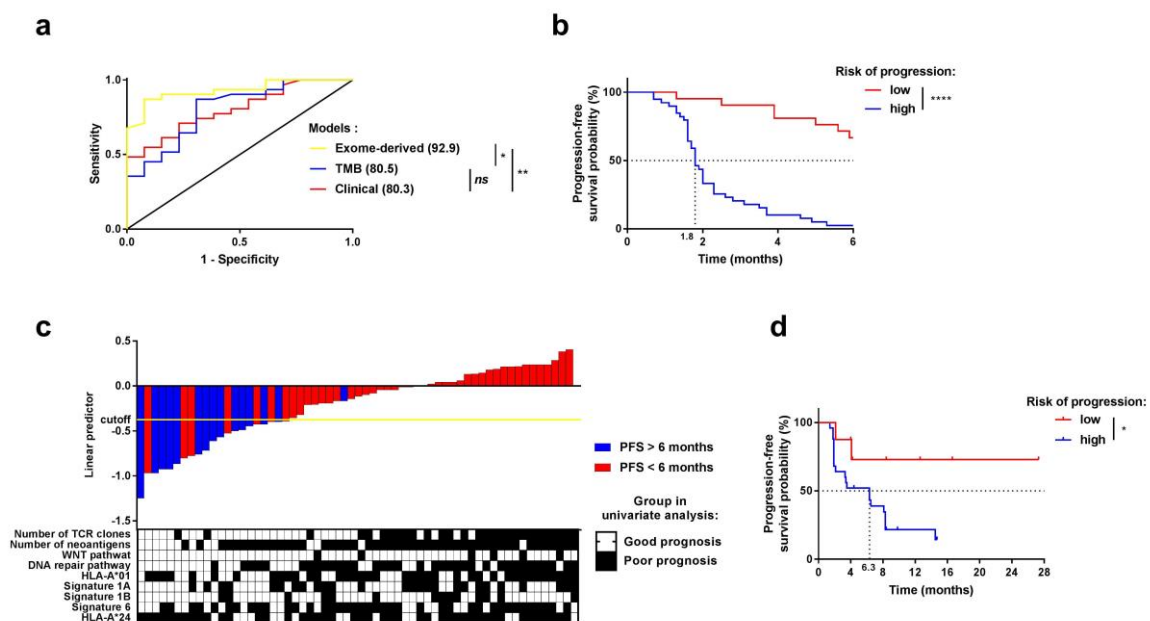
Figure: Prognostic role of the composite exome biomarker. (A) ROC curves estimated using the linear predictor of the clinical and PD-L1 Cox model (Clinical; in red), the clinical and tumor mutational burden model (TMB; in blue), and the exome-derived model (in yellow). For each model, the area under the curve is given in brackets. (B) Kaplan-Meier estimates for progression-free survival (PFS); patients were stratified according to the value of the linear predictor estimated from the exome-derived Cox model: high risk (in blue) or low risk (in red). (C) Top: Barplot showing the values of the linear predictor estimated from the exome-derived model for each patient: patients with PFS greater than 6 months in blue and lower than 6 months in red. Bottom: Heatmap showing how patients are stratified given the threshold defined in the univariate analysis of each variable selected in the exome-derived model: good prognosis (white) and poor prognosis (black). (D) Kaplan-Meier estimates for PFS; patients from Rizvi et al. (2015) cohort were stratified according to the value of the linear predictor estimated from the exome-derived Cox model: high risk (in blue) or low risk (in red). All the cutoffs were defined with the Cutoff Finder method. *: p < 0.05; **: p < 0.01; ***: p < 0.001; ****: p < 0.0001; ns: not significant.

Factors that contributed to racial/ethnic differences in the outcomes of patients with metastatic breast cancer

Jin-Xiao Ren, Yue Gong, Hong Ling, Xin Hu, Zhi-Ming Shao

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Population-based estimates of racial disparities in metastatic breast cancer are lacking. In this study, we quantified the contributions of demographic, socioeconomic, tumor and metastatic characteristics to racial differences in metastatic breast cancer and characterized the most disproportional subgroup.

Patients and methods: Patients diagnosed with stage IV breast cancer between 2010 and 2014 were identified using the Surveillance, Epidemiology, and End Results database. A multivariable Cox proportional hazards model was used to adjust each set of variables. The excess relative risk of cancer-specific and all-cause death in non-Hispanic black (NHB) versus non-Hispanic white (NHW) women with metastatic breast cancer was expressed as a percentage and was stratified by the age at diagnosis.

Results: We identified 12,263 female patients. NHB women exhibited substantially higher morbidity and mortality than women of other races/ethnicities. The greatest excess mortality risk for NHB women was observed in the young-onset group (18-49 years; hazard ratio: 1.70), followed by the middle-age group (50-64 years; hazard ratio: 1.48); the trend was not significant among the elderly group. Socioeconomic factors stably explained one-third of the excess risk, whereas the contribution of tumor characteristics obviously decreased with age (18-49 years, 40.1%; 50-64 years, 30.0%), and the metastatic pattern accounted for less than one-fifth of the excess risk. Additionally, the disproportional death burden of NHB women persisted in less aggressive subgroups, underscoring the importance of implementing more intensive strategies for detecting and treating metastases in non-elderly NHB women with lower grade (I/II) tumors at T2 stage with the ductal histological type, human epidermal growth factor receptor-2+/ hormone receptor- subtype and a lack of existing organ-specific metastases (bone/brain/liver/lung).

Conclusions: Socioeconomic factors and tumor characteristics are the two dominant contributors to white-black survival differences in metastatic breast cancer, and the latter further explained the strengthened disparities among the younger group. We urge targeted preventions that can be undertaken instantly in the public health, policy, and clinical arenas.

Personalized & Precision Medicine (PPM) as a model of healthcare services of the newest generation

Sergey Suchkov

Sechenov University, Moscow, Russia

A new systems approach to diseased states and wellness result in a new branch in the healthcare services, namely, personalized and precision medicine (PM). To implement of PPM into the daily practicey, it is necessary to create a new strategy based upon the recognition of biomarkers of hidden abnormalities long before the disease clinically manifests itself.

Each decision-maker values the impact of the decision to use PPM on their own budget and well-being, which may not necessarily be optimal for the society. It would be extremely useful to integrate data harvesting from different databanks for applications such as prediction and personalization of further treatment to thus provide more tailored measures for the patients and persons-at-risk resulting in improved outcomess. One of the most advanced areas in cardiology is atherosclerosis, cardiovascular and coronary disorders as well as in myocarditis. A lack of medical guidelines has been identified by the majority of responders as the predominant barrier for adoption, indicating a need for the development of best practices and guidelines to support the imple-mentation of PPM into the daily practice of cardiologists!

Implementation of PPM requires a lot before the current model "physician-patient" could be gradually displaced by a new model "medical advisor-healthy person-at-risk". This is the reason for developing global scientific, clinical, social, and educational pro-jects in the area of PM to elicit the content of The new branch.

P4.1

Phase I oncology studies: evidence that in the era of immunotherapies, patients on lower doses do not fare worse

Vivek Subbiah, Shiraj Sen, David Hong, Aung Naing, Funda Meric-Bernstam, Kenneth Hess

The University of Texas MD Anderson Cancer Center, Houston, TX, United States

Background: PK and PD studies demonstrate drug exposure and target saturation of immune checkpoint inhibitors (ICI) at doses below MTD. MTD remains a primary endpoint in ICI phase 1 trials and the optimal dosing to minimize toxicity and optimize response remains unclear.

Methods: We analyzed clinical data from pts treated in phase 1 ICI dose escalation trials at MD Anderson Center for Targeted Therapy. Patients were stratified into a low-dose [LDG] (< 33% MTD), medium dose [MDG] (34-66% MTD), high dose [HDG] (67-100% MTD), or very high dose [VHDG] (> 100% MTD) group. Groups were compared for irAE, PFS, OS, ORR (CR + PR), and DCR (CR + PR + SD > 6 months).

Results: Among 90 pts treated with escalating doses of ICI (57 CTLA4- and 33 PD1-based) between April 2013 and December 2015, median age was 59 years (range: 20-86 years) and 37 (41%) were females. The most common tumor types treated included renal cell carcinoma (n = 23; 25%), melanoma (n = 16; 18%), sarcoma (n = 10; 11%), and GIST (n = 10; 11%). PFS in the LDG (n = 16) was 2.76 months (mo) (95% CI 1.48-NA), MDG (n = 21) was 2.76 mo (95% CI 1.48-NA), HDG (n = 36) was 2.46 mo (95% CI 1.84-3.29), and VHDG (n = 17) was 3.68 mo (95% CI 2.76-NA). Log rank p = 0.22. OS in LDG was 6.18 mo (95% CI 3.45-NA), MDG was 17.05 mo (95% CI 3.94-NA), HDG was 5.16 mo (95% CI 4.24-7.62), and VHDG was 7.49 mo (95% CI 5.59-NA). Log rank p = 0.0070. In all evaluable patients, ORR in LDG, MDG, HDG, and VHDG was 0%, 6%, 6%, and 12% (p = 0.47) and DCR was 62%, 71%, 41%, and 81% (p = .027), respectively. irAE rates in LDG, MDG, HDG, and VHDG were 6%, 10%, 17%, and 29% (p = .045).

Conclusions: Despite a dose-dependent increase in irAE, we identify no improvement in PFS, OS, or DCR with escalating doses of ICI administered in phase I trials but do detect an improvement in ORR. Prospective dose-/exposure-response relationships and biomarker-driven RP2D are warranted on all ICI dose-escalation phase 1 trials. Lower doses may reduce toxicity and cost without compromising disease control or survival.

Dosing	PFS, months (95% CI)	OS, months (95% CI)	ORR (%)	DCR (%)	irAE (%)
LDG	2.76 (1.48-NA)	6.18 (3.45-NR)	0	62	6
MDG	2.76 (1.84-NA)	17.05 (3.94-NR)	6	71	10
HDG	2.46 (1.84-3.29)	5.16 (4.24-7.62)	6	41	17
VHDG	3.68 (2.76-NA)	7.49 (5.59-NR)	12	81	29

Impact of immune checkpoint inhibitor dose on toxicity, response rate, and survival: A pooled analysis of dose escalation phase 1 trials.

P5.2

Clinical Evaluation of a gene expression-based assay in triple-negative breast cancer and its diagnostic utility

Qifeng Wang[1], Qinghua Xu[2], Jinying Chen[2], Chengshu Chen[2], Chenhui Qian[2], Yizuo Chen[3], Midie Xu[1], Xiang Du[1], Wentao Yang[1]

[1] Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China
[2] Canhelp Genomics, Hangzhou, People's Republic of China
[3] The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China

Background: Triple-negative breast cancer (TNBC) accounts for 12%-20% of all breast cancers. Most breast cancer can be diagnosed based on morphological assessment and immunohistochemistry. However, the diagnosis of TNBC is sometimes challenging, particularly in the metastatic setting when the prior history of breast cancer is not available. Besides, the expression of markers for breast origin, such as GATA3, GCDFP15 and mammaglobin is relatively low in TNBC compared with other subtypes of breast cancer. Hence, efforts have been made to establish new diagnostic tools for TNBC. Molecular profiling is a promising diagnostic approach, which has the potential to provide an objective classification of metastatic tumors with an unknown tissue of origin.

Methods: In this study, the performance of a novel 90-gene expression signature-based assay for the determination of the site of tumor origin was evaluated in 115 TNBC samples. For each specimen, the expression profile of the 90 tumor-specific genes was analyzed, and similarity scores were obtained for each of the 21 tumor types on the test panel. The predicted tumor type was compared with the reference diagnosis to calculate the accuracy. Furthermore, the Rank Product Analysis was performed to identify genes that were differentially expressed between TNBC and other types of tumor.

Results: The 90-gene expression signature resulted in a 97.4% (112/115, 95%CI: 0.92-0.99) agreement with the reference diagnosis. Among all specimens, the signature correctly classified 97.6% of TNBC from the primary site (41/42) and lymph node metastasis (41/42) and 96.8% of distant metastatic tumors (30/31). Furthermore, a list of genes including KRT14, KRT15, KRT19 and SFRP1 were identified differentially expressed between TNBC and other types of tumor, suggesting their potential use as discriminative markers.

Conclusions: The overall accuracy of 97.4% demonstrated the excellent performance of the 90-gene expression signature-based assay for the identification of the tumor origin in a cohort of both primary and metastatic TNBC samples. These findings show promise for the use of the 90-gene assay to aid in the differential diagnosis of TNBC, especially in the metastatic setting.

Keywords:
Triple-negative breast cancer, gene expression profiles

Table 1. The Performance of 90-gene Signature in Triple-Negative Breast Cancer

Tumor Type	n	Agreement	Accuracy
Primary Tumor	42	41	97.6%
Lymph Node Metastasis	42	41	97.6%
Distant Organ Metastasis	31	30	96.8%
Total Accuracy = **97.4%**			

Table 1. The Performance of 90-gene Signature in Triple-Negative Breast Cancer

P5.3

Clinical evaluation of a gene expression-based assay for tissue origin diagnosis of brain metastases

<u>Yulong Zheng</u>[1], Yongfeng Ding[1], Cheng Xiao[1], Weixiang Zhong[1], Xiaodong Teng[1], Qiqi Gao[1], Qifeng Wang[2], Yifeng Sun[3], Chengshu Chen[3], Lei Chen[3], Jiajun Zhu[3], Qinghua Xu[3], Nong Xu[3]

[1] *The First Affiliated Hospital, Zhejiang University, Hangzhou, People's Republic of China*
[2] *Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China*
[3] *Canhelp Genomics, Hangzhou, People's Republic of China*

Background: Brain metastases (BM) are the most common intracranial tumors affecting about 8-10% of all cancer patients. Morphology and immunohistochemical staining are two common approaches used to identify the primary sites of BM samples, but morphology fails to identify poorly differentiated tumors and IHC markers usually lack specificity. About 2-14% of BM patients still present with unknown primary sites. A 90-gene assay proposed in our previous study is an RNA-based gene expression test to identify the origin sites of tumors. This study aims to evaluate the performance of the 90-gene assay in BM samples.

Methods: The sequence-based gene expression profiles of 708 primary brain tumors (PBT) collected from TCGA database were performed by a 90-gene expression signature, with a similarity score for each of 21 common tumor types. We used Optimal Binning algorithm to generate a threshold for separating PBT from BM. Eighteen PBT samples from Fudan University Shanghai Cancer Center were analyzed to substantiate the reliability of the threshold. In addition, the performance of the assay for identifying the tissue of origin was validated in a cohort of 48 BM samples with known origin from The First Affiliated Hospital, Zhejiang University. For each BM sample, the tumor type with the highest similarity score was considered tissue of origin. When a sample was diagnosed as PBT but the similarity score below the threshold, the second prediction was considered as the primary site.

Results: A threshold of the similarity score, 70, was identified to discriminate PBT from BM (PBT: ≥70, BM: <70) with an accuracy of 99% (703/708, 95%CI: 0.98-1.00). Eighteen PBT and 44 BM were performed by the 90-gene assay. The results of 18 PBT samples matched reference diagnosis with a concordance rate of 100% and all similarity scores were above 70. Of 44 BM samples, the 90-gene assay accurately predicted primary sites in 89% (39/44, 95%CI: 0.75-0.96) of the cases.

Conclusions: The 90-gene assay showed promising discriminatory ability to separate PBT from BM and identify the primary site of BM. Our findings demonstrated the potential that 90-gene assay can serve as a powerful tool for accurately identifying the tissue of origin for BM samples.

Keywords:
Brain metastases, gene expression profiles

Table 1 The Performance of 90-gene Signature in Brain Tumors

Reference Diagnosis	No. of Specimens	Agreement	Accuracy
Primary Brain Tumors			
Meningiomas	10	10	100%
Gliomas	7	7	100%
PNET	1	1	100%
Total	18	18	100%
Origin of Brain Metastasis			
Lung	26	21	81%
Colorectal	6	6	100%
Breast	6	6	100%
Neuroendocrine	4	4	100%
Cervix	1	1	100%
Liver	1	1	100%
Total	44	39	89%

Abbreviation: PNET, Primitive neuroectodermal tumor

Table 1. The Performance of 90-gene Signature in Brain Tumors

Development and Validation of a 90-Gene Real-Time PCR Assay for Tumor Origin Identification

Qing Ye[1], Qifeng Wang[2], Peng Qi[2], Jinying Chen[3], Wanli Ren[3], Midie Xu[2], Gang Ji[2], Jun Yang[1], Ling Nie[1], Yifeng Sun[3], Chengshu Chen[3], Qinghua Xu[3], Xiaoyan Zhou[2]

[1] Nanjing Drum Tower Hospital, Nanjing, People's Republic of China
[2] Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China
[3] Canhelp Genomics, Hangzhou, People's Republic of China

Introduction: Accurate identification of tumor origin in patients with metastatic cancer is critical for effective treatment selection, but remains a challenge. The primary goal of this research is to develop and validate a gene expression assay for tumor classification and integrate it with histopathology to identify origin site for cancer of uncertain primary.

Materials and Methods: We established a 90-gene classifier of tumor types based on high-throughput gene expression profiling in a training set of 5800 samples of 21 tumor types. Then, we transferred the 90-gene signature from microarray platform to a real-time PCR assay which would allow broader access in the clinical setting. A total of 385 formalin-fixed paraffin-embedded specimens archived from 2012 to 2017 were retrospectively collected for the clinical validation. The cohort consisted of 308 primary tumors, 71 metastatic tumors and 6 well-characterized cases of uncertain primary. We applied the developed real-time PCR assay to predict tumor type. The accuracy of predictions from the 90-gene assay was validated using comprehensive imaging/histological tests, as well as follow-up for subsequent clinical detection of primary sites.

Results: The tumor type classifier based on the 90-gene expression profiles showed an overall 99.5% specificity, 88.6% sensitivity, 91.2% positive predictive value, and 99.5% negative predictive value in the validation set of 385 tumors. More specifically, the classification accuracy reached 90.2% (278/308, 95% CI: 86.5%-93.2%) for primary tumors, and 87.3% (62/71, 95% CI: 76.8%-93.7%) for metastatic tumors. There was no significant difference in terms of the assay accuracy between primary and metastatic tumors (P=0.605). For the 6 cases of uncertain primary, the assay predictions were further confirmed by latent primary site or assay-directed pathological/imaging tests.

Conclusions: We show that the development of a 90-gene real-time PCR assay can classify a broad spectrum of tumor types with high level of accuracy. This assay could be a useful tool to unmask the origin of cancer of uncertain primary and a step towards the improvement of the clinical management of these patients.

Keywords:
Tumor classification, cancer of uncertain primary, gene expression profiles, real-time PCR assay

Table 1: Performance characteristics of the 90-gene real-time PCR assay in cases of primary tumor, metastatic tumor and cancer of uncertain primary.

a.

Tumor Type	n	Primary Tumor Sensitivity (%)	Primary Tumor Specificity (%)	n	Metastatic Tumor Sensitivity (%)	Metastatic Tumor Specificity (%)
Adrenal	11	100	100	1	100	100
Brain	18	100	99.3	0	/	100
Breast	25	100	99.6	14	100	93
Cervix	13	84.6	99.3	3	100	100
Colonrectum	12	100	98.3	8	100	100
Endometrium	17	70.6	99.7	0	/	100
Gastroesophagus	24	91.7	98.9	2	100	100
Germ Cell	10	70	99.7	0	/	98.6
Head and Neck	10	70	100	4	50	100
Kidney	15	100	100	6	83.3	100
Liver/ cholangiocarcinoma	14	92.9	99.7	1	100	100
Lung	13	100	99.3	2	100	100
Melanoma	10	100	100	6	33.3	98.5
Mesothelioma	11	72.7	100	0	/	100
Neuroendocrine	22	81.8	99.3	2	100	98.6
Ovary	15	86.7	98	12	91.7	98.3
Pancreas	13	84.6	99.7	1	100	100
Prostate	11	100	99.7	2	100	100
Sarcoma	19	89.5	99.7	1	100	98.6
Thyroid	13	100	99.7	2	100	100
Urinary	12	91.7	100	4	75	100

b.

Cancer of Uncertain Primary						
Cases	Age/Sex	Biopsy site	Work-up	Diagnosis at presentation	90-Gene RT-PCR Assay prediction	Follow up
1	50/F	Left clavicle	PET-; IHC inconclusive	Poorly differentiated carcinoma	Breast	16 months later, the core needle biopsy of left breast showed invasive carcinoma
2	30/M	Left clavicle	PET-	Adenocarcinoma	Germ cell	IHC (SALL4+, CD30+), pelvic MRI and postoperative pathology confirmed embryonal carcinoma
3	48/M	Left chest and back	36 days, biopsy twice, 33 IHC tests (Arg-1+, Hepa-1+)	Liver cancer	Liver/ cholangiocarcinoma	/
4	47/F	Left neck	ER-, PR-, Her2 0	Squamous cell carcinoma	Breast	Ultrasound of breast: BI-RADS subcategory 4c
5	63/M	Neck	TTF-1-, CgA-, Syn-, CD56-, CK7-, Napsin-, CDX-2-, CK19-, P504S+, PSA-, STATB-, Villin-, Ki67+20%	Metastatic kidney cancer?	Kidney	Inter-institutional pathology consultation: kidney cancer
6	52/F	Right axillary fossa	IHC inconclusive (Hepatocyte+)	Poorly differentiated adenocarcinoma, metastasis of hepatocellular carcinoma?	Gastroesophagus	Chemotherapy regimen for gastric cancer, CT showed a decrease in tumor size after 4 cycles

Table 1. Performance characteristics of the 90-gene real-time PCR assay in cases of primary tumor, metastatic tumor and cancer of uncertain primary

Identification of Extracellular RNA panel for Detection of Malignant Pleural Mesothelioma

Hany Lotfy

Armed Forces College Of Medicine (AFCM), Cairo, Egypt

Background: Malignant pleural mesothelioma (MPM) is a highly aggressive cancer mainly caused by chronic exposure of asbestos. In this pilot study, we aimed to investigate the expression of an extracellular RNA (exRNA)-based biomarker panel assessing the clinical utility of exRNAs as diagnostic & prognostic MPM biomarkers.

Methods: Using in silico data analysis, we chose a MPM-specific RNA-based biomarker panel based on the integration of differential DNA Damage Regulated Autophagy Modulator 1 (DRAM1) & arylsulfatase A (ARSA) gene expression with their selected epigenetic regulators microRNA (miR-2053) & long non-coding RNA (lncRNA-RP1-86D1.3). This was followed by quantitative real-time PCR (qPCR) validation in sera of 60 MPM patients, 20 chronic asbestos exposure patients and 20 healthy volunteers. Furthermore, the prognostic power of the selected panel was explored.

Results: The positivity rates of DRAM1 mRNA, ARSA mRNA, hsa-miR-2053 & lncRNA-RP1-86D1.3 were 78.3%, 90%, 85% and 83.3% in sera samples of MPM patients, respectively. The chosen exRNAs were able to discriminate between cases and controls with high accuracy and their combined sensitivity reached 100% for the diagnosis of MPM. The predictive power of exRNAs was validated (R square=0.91, p<0.01). Cox regression analysis including gender, age and smoking; showed lncRNA-RP1-86D1.3 to be an independent prognostic factor of MPM (95% CI 0.002-0.96; HR 0.04).

Conclusion: These preliminary findings imply that the proposed integrative approach identified exRNAs that play crucial roles in driving MPM development suggesting their potential as a diagnostic panel. Further larger as well as in vitro studies are strongly recommended.

Keywords:
Mesothelioma, MicroRNA, lncRNA Diagnosis, Bioinformatics.

References:
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Role of Midkine in predicting malignancy in patient with solitary thyroid nodule

Ahmed Hamam

Aremd Forces College of Medicine, Cairo, Egypt

Background: Solitary thyroid nodules are a common clinical problem. Unfortunately none of sonographic features alone is sufficient to discard or detect malignancy efficiently (Russ et al. 2017). Midkine is a novel heparin-binding growth factor, plays critical roles in carcinogenesis. There was release of midkine from cancer tissue into the blood (Kuzu et al. 2016). In this study, we aimed to evaluate serum midkine levels in patients with solitary thyroid nodules to predict malignancy.
Methods: A total of 100 patients (68 women and 32 men) with thyroid solitary thyroid nodules were enrolled. The level of serum midkine was measured. Fine needle aspiration cytology was done to all nodules (25 malignant/suspicious and 75 benign).
Results: Serum midkine was found to be higher in nodules with irregular border (P = 0.013) and calcification (p = 0.003). Serum midkine was direct correlated with age and TSH (r=0.327, P = 0.02 and r= 0.285, P = 0.045 respectively). The most important predictors of midkine was calcification (B = -0.260, β = -0.453, t = -3.519, P = 0.01) followed by gender (B = -0.394, β = -0.358, t = -2.985, P = 0.004) followed by nodular border (B =0.300, β = 0.268, t = 2.181, P = 0.034). The area under curve at receiver operating characteristic analysis was 0.860 (95% CI). The sensitivity of serum midkine for prediction of solitary thyroid nodule malignancy was 76% and specificity was 82% at cut off value of 0.680 ng/ml.
Conclusion: Serum midkine can predict malignancy in solitary thyroid nodule and also well correlated with sonographic features of thyroid nodules. We suggest that midkine levels may serve as a novel biochemarker in association with sonographic features in evaluation of solitary thyroid nodules.

Keywords:
Midkine and solitary thyroid nodule

Kuzu, Fatih et al. 2016. "Midkine : A Novel Biomarker to Predict Malignancy in Patients with Nodular Thyroid Disease." 2016.

Russ, Gilles, J. Bonnema, Murat Faik, and Cosimo Durante. 2017. "European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults : The EU-TIRADS." 13:225–37.
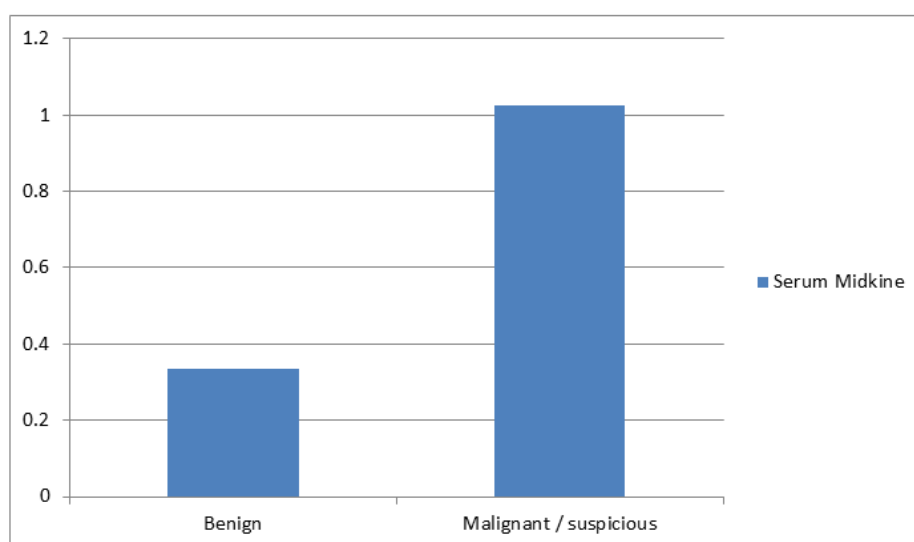
Bar chart shows higher level of serum midkine in malignant / suspicious than benign solitary thyroid nodule (P < 0.001)

POSTER SESSION 6: Drug design and discovery

P6.2

[Experimental] PARP1-hyperactivation driven necrosis by DDS-assisted Zn(2+) and its evidence of global compatibility with I/O drugs

Jinhyuk Fred Chung[1], Zhisheng Her[2], Qingfeng Chen[2]

[1]XYLONIX PTE LTD, Singapore, Singapore
[2]AStar, Singapore, Singapore

Background: Zn-γPGA (XNX-C003/C004) is a proprietary DDS-assisted Zn(2+) agent that is designed to exclusively induce PARP1-hyperactivation driven necrosis against cancer. The PARP1-hyperactivation driven necrosis is mediated by the ATP and NAD+ depletion arising from its DNA repair activities. This unique mechanism by Zn-γPGA stipulates two positive ways of its global compatibility with immuno-oncology drugs (I/O drugs). (1) Its immunogenic tumoricidal mechanism and subsequent immunofocusing effect, and (2) its enhanced activity against cancer types with genetic instabilities (the proven conditions of the site-independent approval to pembrolizumab and nivolumab) that may uniquely enable novel site-independent clinical trial design approaches. Hence, experimental validation of the stipulated effects were commenced.

Materials and Methods: GMP-grade Zn-γPGA injectable solution (Sterile, 1 mg Zn/mL as zinc sulfate, pH 7-adjusted by Tris) was produced by consignment to Novell Pharmaceutical Laboratories PTE (Jakarta, Indonesia). 50 cell line screening was consigned to Crown Biosciences Inc. (Santa Clara, US/Beijing, China). HCC PDX-HuMice studies and TIL analysis were performed by Humanized Mouse Unit of IMBC, AStar (Singapore).

Results: In vitro, the Zn-γPGA IC50 value distribution in the 50 cell line screening was narrower and more consistent (median-normalized Z distribution between 0.15-3.77, with 49/50 cell lines under Z=2) when compared to cisplatin (median-normalzied Z distribution between 0.12-8.50, with 37/50 cell lines under Z=2). Statistical analyses on the IC50 value distribution in respect to the genetic instability gene mutations (GIMs: MLH1, MSH2, PMS2, PARP1, BRCA1/2, TP53) revealed statistically significant pattern of IC50 reduction in those with more GIMs.

In vivo, daily intravenous injection of Zn-γPGA to HCC PDX-HuMice led to significant inhibition of tumor growth rate, which was coincided with significantly increased uptake of various T cells into the tumor.

Conclusions: DDS-assisted Zn2+ agent Zn-γPGA presents positive global compatibility with I/O drugs by (1) immunofocusing of various T cells into tumor space, and (2) demonstrating enhanced cytotoxicity against cancer types carrying genetic instability gene mutations.
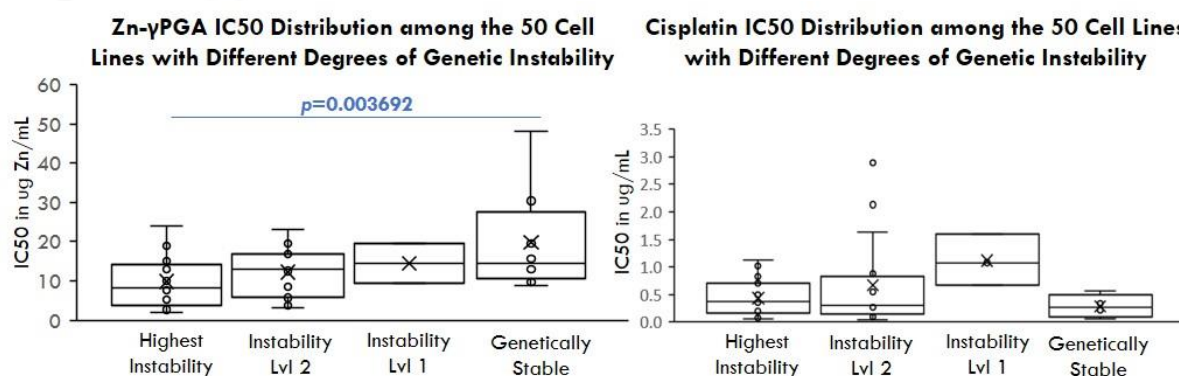
The effect of accumulating genetic instability gene mutations toward in vitro efficacy of Zn-γPGA

P7.1

CapG renders breast cancer resistance to paclitaxel by activating PI3K/Akt signaling through transcriptionally upregulating p50

Yayun Chi

Breast Cancer Institute, People's Republic of China

Chemotherapy resistance in breast cancer is a major problem in breast cancer treatment and a leading cause of mortality in breast cancer patients. Various molecular signaling events including increased PI3K activation have been shown to contribute to the chemotherapy resistance, while the underlying mechanisms are not completely understood. Here we show that the expression of actin-binding protein CapG significantly associated with paclitaxel resistance in breast cancer cells. High CapG level correlated with short relapse-free survival as well as hyper-activation of PI3K/Akt signaling in breast cancer patients. Mechanistically, CapG enhanced PIK3R1/p50 transcription which led to increased PI3K/Akt activation. Unexpectedly, CapG was found to bind to the variant-specific promoter of PIK3R1/p50, where it associated with and enhanced the recruitment of p300/CBP to this promoter region, thereby increasing PIK3R1/p50 transcription by promoting histone H3K27 acetylation. Consistently, inhibiting p300/CBP with C646 substantially decreased CapG-dependent upregulation of PIK3R1/p50 and subsequent PI3K/Akt activation, resulting in increased sensitivity to paclitaxel treatment in breast cancer cells. Altogether, our data indicate that CapG may serve as a novel predictive marker for paclitaxel response in breast cancer patients, as well as a potential therapeutic target for mitigating resistance to chemotherapy.
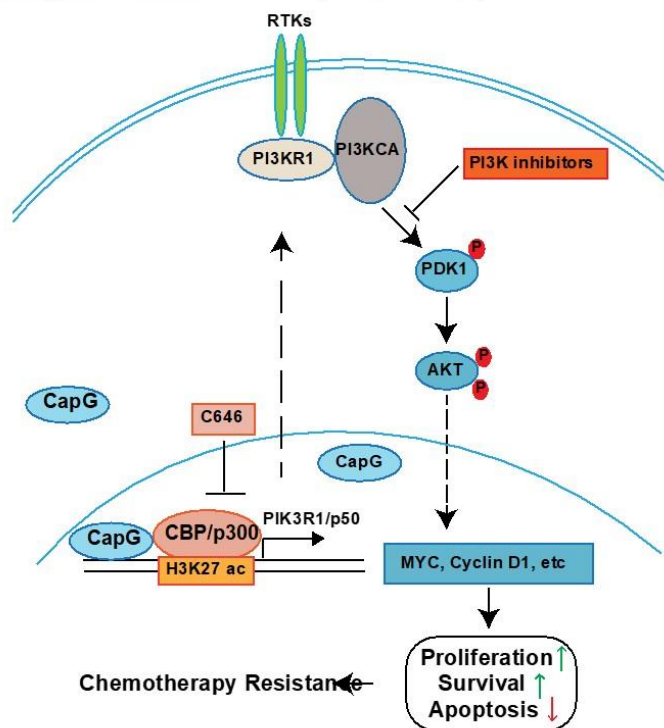
A model depicting the potential role of CapG in modulating breast cancer cell response to chemotherapy.

P7.2

Dynamic monitoring HER2 amplification of circulating DNA in metastatic colorectal cancer patients treated with cetuximab

Rujiao Liu, Zhiyu Chen

Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Addition of cetuximab has shown clinical benefit in RAS and BRAF wild-type (wt) patients with metastatic colorectal cancer (mCRC). Human epidermal growth factor receptor 2 (HER2) amplification was reported to be one of the resistance mechanisms to cetuximab. We investigated whether monitoring of HER2 amplification in circulating tumor DNA (ctDNA) allows early detection of progression and informs about resistance to cetuximab.

Methods: We analyzed HER2 amplification in ctDNA by 8 week-interval using droplet digital polymerase chain reaction (ddPCR) from 36 RAS and BRAF wt patients, who progressed after failure of cetuximab contained regimens between Jul 2015 and Jan 2018.

Results: 13.8% (5/36) of patients exhibited dynamic fluctuations of HER2 amplification in plasma, one of whom were found to be positive for HER2 amplification in matched tumor specimens using FISH. Two patients had HER2 and c-MET co-amplification. All 5 primary sites were left side, 4 rectums and 1 descending colon. 3 patients received cetuximab as first line therapy, whereas 2 patients in the 2nd line setting. Among these 5 patients, changes in ctDNA levels showed good agreement with changes in tumor volume. Furthermore, quantifications of HER2 amplification showed obvious increase with an average lead time of 2 months compared with CT documented progress. But interestingly, there was no difference in progression-free survival (PFS) between these 5 patients and the others without HER2 amplification (HR=0.89, 95%CI: 0.34-2.37, p=0.820).

Conclusions: Plasma HER2 amplification detected by ddPCR was showed dynamic changing over time and would predict resistance to cetuximab-containing treatment. Average 2 months lead time was observed and need to validate in the further study.

Case No.	Gender	Age	Primary site	Baseline HER2 expression of FFPE (IHC)	Baseline HER2 expression of FFPE (FISH)	Baseline HER2 copy number of plasma	Treatment status	Regimen combined with cetuximab	PFS(Days)
Case 1	M	43	Descending colon	-	Negative	2.38	1st line	FOLFIRI	293
Case 2	M	59	Rectum	3+	Positive	13.63	3rd line	IRI	118
Case 3	M	62	Descending colon	-	Negative	6.57	2nd line	IRI	429
Case 4	M	59	Rectum	Partly +	Negative	1.87	1st line	FOLFOX	212
Case 5	F	52	Rectum	3+	Positive	2.27	1st line	FOLFOX	325

Table 1 Clinical characteristics of 5 HER2 amplification patients received cetuximab

STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in KRAS-Mutant Lung Adenocarcinoma

Ferdinandos Skoulidis[1], Michael Goldberg[2], Danielle Greenawalt[3], Matthew Hellmann[4], Mark Awad[5], Justin Gainor[6], J.Jack Lee[7], Jianjun Zhang[7], Vassiliki Papadimitrakopoulou[7], Ignacio Wistuba[7], Vincent Miller[8], Gareth Frampton[8], Jedd Wolchok[4], Alice Shaw[6], Pasi Jänne[5], Philip Stephens[2], Charles Rudin[4], William Geese[3], Lee Albacker[2], John Heymach[1]

[1] *The University of Texas MD Anderson Cancer Center, Houston, TX, United States*
[2] *Foundation Medicine Inc, Cambridge, MA, United States*
[3] *Bristol-Myers Squibb, Princeton, NJ, United States*
[4] *Memorial Sloan Kettering Cancer Center, New York, NY, United States*
[5] *Dana Farber Cancer Institute, Boston, MA, United States*
[6] *Massachusetts General Hospital, Boston, MA United States*
[7] *The University of Texas MD Anderson Cancer Center Houston TX United States*
[8] *Foundation Medicine Inc., Cambridge, MA, United States*

KRAS is the most common oncogenic driver in lung adenocarcinoma (LUAC). The genomic determinants of response to immunotherapy in these patients are largely unknown. We previously reported that STK11/LKB1 (KL) or TP53 (KP) co-mutations define distinct subgroups of KRAS-mutant LUAC . Here, we report the impact of KRAS co-mutations on the efficacy of PD-1 inhibitors. In a cohort of 174 patients with KRAS-mutant LUAC (SU2C cohort) objective response rates to PD-1 blockade differed significantly between KL (7.4%), KP (35.7%) and K-only (28.6%) subgroups (P<0.001). Similar results were obtained in patients with KRAS-mutant non-squamous NSCLC that were treated with nivolumab in the CheckMate-057 randomized phase 3 trial (0% vs 57.1% vs 18.2%, P=0.047). In the SU2C cohort, KL LUAC exhibited shorter progression-free survival (P<0.001) and overall survival (P=0.0015) compared to KRASMUT;STK11/LKB1WT LUAC. In an independent large cohort of 924 LUAC with comprehensive NGS-based genomic profiling (FM cohort), STK11/LKB1 alterations were the only genomic marker significantly associated with PD-L1 negativity in TMBIntermediate/High LUAC. The impact of STK11/LKB1 alterations on clinical outcomes with PD-1/PD-L1 inhibitors extended to PD-L1-positive NSCLC. In Kras-mutant murine LUAC models, Stk11/Lkb1 loss fostered establishment of a non-T-cell inflamed tumor immune micro-environment and directly promoted PD-1/PD-L1 inhibitor resistance, suggesting a causal role. These results identify STK11/LKB1 alterations as a major genomic driver of primary resistance to PD-1 axis blockade in KRAS-mutant LUAC and suggest that development of precision immunotherapy approaches should be tailored to the co-mutation status of individual tumors.

POSTER SESSION 9: Experimental therapies – clinical

P9.1

Mirtazapine as a secondary prophylactic for delayed vomiting induced by highly emetogenic chemotherapy

Jun Cao[1], Xichun Hu[1], Biyun Wang[1], Zhonghua Wang[1], Jian Zhang1, Leiping Wang[1], Quchang Ouyang[2], Shusen Wang[3]

[1] *Fudan University Shanghai Cancer Center, Shanghai, People's Republic Of China*
[2] *Hu Nan Tumor Hospital, Changsha, People's Republic of China*
[3] *Zhongshan Medical, Guangzhou, People's Republic of China*

Background: We examined the efficacy of mirtazapine for the prevention of delayed nausea and vomiting in patients who had experienced delayed nausea and vomiting resulting from highly emetogenic chemotherapy (HEC).

Methods*:* Patients with breast cancer who had experienced delayed emesis after receiving EC or cisplatin-containing regimens with no use of aprepitant as antiemesis, and would subsequently accept at least 3 more cycles of the same chemotherapy were randomly assigned to a mirtazapine group (15 mg daily on days 2 to 4) or control group, both with aprepitant, a 5-HT3 receptor antagonist (RA) and dexamethasone (7.5 mg on days 2 to 4). Primary end point was complete response (CR) to vomiting (no emesis and no rescue treatments) in the delayed phase (25 to 120 h) during cycle 1. Impact on quality of life (QOL) was assessed by modified Functional Living Index–Emesis (FLIE) questionnaire on days 2 and 6 of each cycle.

Results: Of 95 enrolled patients, 46 were assigned to the mirtazapine group and 49 to the control group. Compared with the control group, delayed CR rates were significantly higher with mirtazapine group in cycle 1 (78.3% versus 49.0%, respectively, P=0.003, OR 3.75, 95% CI: 1.529-9.196), in cycle 3 (88.2% versus 55.0%, respectively, P=0.010, OR 7.50, 95% CI: 1.421-39.590), as well as in the whole study (P=0.0043, OR 3.30, 95% CI: 1.414-6.452). Adverse events were mild to moderate. The mirtazapine group had increased somnolence and weight gain. Mean total FLIE scores were similar between the two arms during cycle 1 both on day 2 and 6.

Conclusions*:* Compared to standard aprepitant-containing triplet regimen with CR rate of about 50% for HEC-induced delayed vomiting, mirtazapine could further increase the rate to 75% , and had no impact on QOL in patients with breast cancer who had experienced delayed emesis with the same prior chemotherapy.

Figure 2A. Percent of Patients with a Complete Response in Cycle 1

MIR, mirtazapine; 5-HT3 RA, 5-HT3 receptor antagonist; DEX, dexamethasone; APR, aprepitant
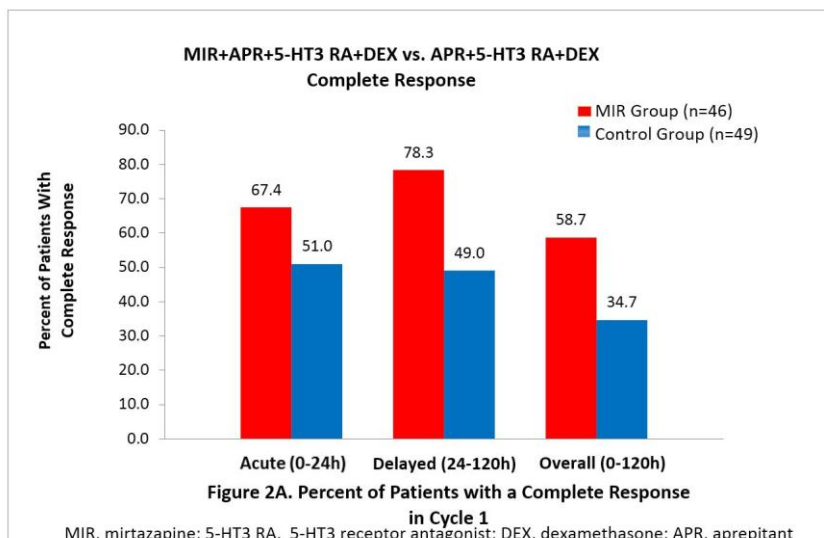
Percent of patients with a complete response in cycle 1

P9.2

Pegylated IL-10 (AM0010, Pegilodecakin) with immune checkpoint blockade in NSCLC and RCC

<u>Aung Naing</u>[1], Deborah Wong[2], Jeff Infante[3], Raid Aljumaily[4], Kyriakos Papadopoulos[5], W. Michael Korn[6], Jeff Schneider[7], Manish Patel[8], Karen Autio[9], Gerald Falchook[10], Nashat Gabrail[11], Annie Hung[12], Peter VanVlasselaer[12], Joseph Leveque[12], Eddie B Garon[2], Nizar Tannir[13], Martin Oft [12]

[1] MD Anderson, Houston, TX, United States
[2] UCLA, Los Angeles, CA, United States
[3] SCRI, Nashville, TN, United States
[4] OUHSC, Oklahoma City, OK, United States
[5] START Center for Cancer Care, San Antonio, TX, United States
[6] UCSF, San Francisco, CA, United States
[7] Winthrop University Hospital, Mineola, NY, United States
[8] Sarah Cannon Research Institute/Florida Cancer Specialists, Sarasota, FL, United States
[9] Memorial Sloan-Kettering Cancer Center, New York, NY, United States
[10] Sarah Cannon Research Institute at HealthONE, Denver, CO, United States
[11] Gabrail Cancer Center Canton, OH, United States
[12] ARMO BioSciences Redwood City, CA, United States
[13] MDACC, Houston, TX, United States

Pegilodecakin stimulates the cytotoxicity, survival and proliferation of intratumoral antigen activated CD8+ T cells in pre-clinical cancer models. Antigen stimulation leads to upregulation of immune checkpoints and the IL-10R on CD8 T cells. This suggests that a combination of AM0010 with PD-1 inhibitors provides therapeutic benefit. Tolerability and anti-tumor activity of pegilodecakin alone and in combination with anti-PD1 immune checkpoint inhibitors was explored in a Phase 1 trial. Previously we reported partial responses (PRs) in 4 of 16 RCC pts treated with pegilodecakin alone (25%ORR).
A total of 34 NSCLC pts. were enrolled on pegilodecakin (10-20ug/kg QD, SC) and pembrolizumab (2mg/kg, q3wk IV; n=5) or nivolumab (3mg/kg, q2wk IV; n=29). Pts. had a median of 2 prior therapies (range 0-5). 37 RCC patients were enrolled on AM0010 and pembrolizumab (2mg/kg, q3wk IV; n=8) or nivolumab (3mg/kg, q2wk IV; n=29), with a median of 1 prior therapy (0-5) and at least one anti-angiogenic therapy. Tumor responses were assessed by irRC. Tumor mutational burden (TMB), IFNg signature (GEP), serum cytokines, peripheral T cell activation and clonality were analyzed.
Pegilodecakin + anti-PD-1 was well tolerated. TrAEs included anemia, thrombocytopenia and fatigue, and were reversible and transient. The incidence of clinically relevant immune related TrAEs was less than 50% as expected from historical controls. As of May 1 2018, PRs were observed in 11 of 26 evaluable NSCLC pts (41%), including 4 (of 12) with PD-L1+ <1% and 4 (of 5) with PD-L1+ >50% cancers. 5 of 8 patients with NSCLC with liver metastasis had a PR. Patients with low TMB or GEP had an equal propensity for PR.
PRs were observed in 14 of 34 evaluable RCC pts (41%). An additional 15 RCC pts had stable disease (44%), 7 of those had a tumor reduction > 30%. The mPFS and mOS has not been reached, the mFU is 15.2 m (range 0.5-30.6).
Updated data will be presented.
Induction of immune cytokines (IL-18) in the serum, invigoration of CD8+ T cells and the increase of newly expanding T cell clones correlated with tumor response to pegilodecakin alone.
Conclusion: The encouraging efficacy with the robust CD8 T cell activation is promising and phase 2 studies of Pegilodecakin (at 10ug/kg) in combination with nivolumab and pembrolizumab are in progress.

P9.3

Targeting oncogenic gene fusions in a phase 1 clinical trials program: a personalized therapy approach

Vivek Subbiah, Roman Groisberg, Kenneth Hess, David Hong, Funda Meric-Bernstam

The University of Texas MD Anderson Cancer Center, Houston, TX, United States

Background: Oncogenic fusions occur when two genes are connected by a chromosomal rearrangement or deletion, translocation, inversion, duplication, or when two genes are read in a single transcription event. Fusions are identified in a growing number of hematologic and solid tumor malignancies and may lead to constitutive activation of a driving tyrosine kinase resulting in cell growth. Tyrosine kinases inhibitors can target these fusions. We reviewed the effect of targeted therapy on patients with gene fusions in a large phase 1 program.

Methods: Charts of patients participating in clinical trials with targeted therapies in our phase 1 clinic were reviewed. Patients were treated between 2009 and 2017. We defined targeted therapy as directed either toward the gene itself or in a downstream pathway. Demographic information including diagnosis, age, sex, date of first dose on trial, date of progression, best response by RECIST, and if applicable date of death. Information on fusions was obtained from next generation sequencing or FISH performed as part of oncologic management.

Results: Patient demographics including number of patients treated, diseases included, fusions identified, and best responses are presented in table 1. Majority of drugs were targeted to the gene fusion (RET, NTRK) with the exception of sarcomas which targeted downstream pathways (c-MET, IGF1R, lurbinectedin). Median progression free survival was 7.1 months, 95% CI [4.8,15.4] and overall survival was 19.6 months, 95% CI [10.8, 29.4].

Conclusion: Gene fusion driven cancers are actionable with multi-kinase or selective kinase inhibitors. Patients with fusions have improved response rates to historical molecularly matched targeted therapy data in our phase 1 group trials, and have longer overall survival. Further study is needed for molecular mechanisms of response and resistance to fusion targeted therapies.

Table 1	# Pts	%
Total	85	
Treated with targeted therapy	59	69.41%
Not treated with target therapy	26	30.59%
Male	46	54.12%
Female	39	45.88%
Tumor Types		
Sarcomas	49	57.65%
NSCLC	17	20.00%
Cholangiocarcinoma	7	8.24%
Salivary gland	4	4.71%
Thyroid	3	3.53%
HG-NET	2	2.35%
Glioblastoma	2	2.35%
Colon	1	1.18%
Lymphoma	1	1.18%
Best Response with Targeted Therapy		
Complete Response	3	5.08%
Partial Response	12	20.34%
Stable Disease	21	35.59%
Clinical Benefit	36	61.02%
Fusions identified		
ALK-MEMO	FGFR2-FOXP1	
ASPL-TFE3 t(X;17)	FGFR2-LPXN	
CCDC6-RET	FGFR3-TACC3	
DCTN1-ALK	KIAA1549-BRAF	
EWS-ATF1	KIF5B - RET	
EWSR1- FL1	NCOA4-RET	
EWSR1/CREB1	NPM1-ALK	
FGFR2-AFF4	NTRK1	
FGFR2-AHCYL1	NTRK3	
FGFR2-BICC1	ROS1	
FGFR2-DZIP1		

Demographics of patients with Gene fusions enrolled in Phase 1 trials

MOST - A Phase II evaluating the benefit of maintenance treatment targeting molecular alterations in advanced solid tumors: sorafenib cohort

Christelle DeLafouchardière[1], Christophe Le Tourneau[2], Antoine Italiano[3], Maud Toulmonde[3], Laure Motané[1], François Bertucci[4], Anthony Goncalvès[4], Delphine Loirat[2], Sylvestre Le Moulec[3], Isabelle Ray-Coquard[1], Sophie Cousin[3], Carlos-Alberto Gomez-Roca[5], Olivier Tredan[1], Philippe Casssier[1], Gwenaëlle Gravis-Mescam[4], Véronique Corset[1], Vincent Agrapart[1], Andrea Dargenio[1], David Perol[1]

[1] *Centre Léon Bérard, Lyon, France*
[2] *Institut Curie, Paris, France*
[3] *Institut Bergonié, Bordeaux, France*
[4] *Institut Paoli Calmettes, Marseille, France*
[5] *Institut Claudius Régaud Oncopole, Toulouse, France*

Background: MOST (My Own Specific Therapy) is a multicenter, randomized, open-label, 2-period, adaptive Phase II conducted in patients (pts) with advanced progressive solid tumors (any histological type). This trial aims to evaluate the clinical benefit of different molecular targeted therapies (MTT) when actionable molecular alterations were identified.

Methods: Data from the maintenance period for the sorafenib cohort of the MOST trial are reported here. Based on the presence of oncogenic variants in the RAS pathway, pts were treated with sorafenib. Pts with tumors for which sorafenib is approved were not eligible. After 12 weeks of treatment (induction period), pts with objective response were proposed to continue the MTT, while pts with stable disease (SD) were randomly assigned (1:1, maintenance period) to continuation or interruption of MTT. Sequential statistical analyses were carried out every 10 randomized pts using a Bayesian approach with pre-defined stopping rule. Primary endpoint of the maintenance period was the proportion of pts who were progression-free at 16 weeks after randomization according to RECIST1.1 (PFR16W). Secondary endpoints included PFS, OS and toxicity.

Results: From Feb. 2014 to Mar. 2018, 147 pts were enrolled and treated in the sorafenib cohort (400mg BID, PO). Following the 12-week induction treatment, 33 pts with SD were randomized: 17 and 16 pts in the maintenance arm and the interruption arm, respectively. The PFR16w after randomization were 63.2% versus 26.7%, respectively. Baseline characteristics, median PFS and OS are presented in Table 1.

Conclusion: The probability that the estimated PFR16W in the maintenance arm is higher than the interruption arm is 0.99. According to the pre-defined stopping rule, the sorafenib cohort was closed to enrolment.

Clinical trial information:
NCT02029001.

Keywords:
Genomic-driven clinical trial, sorafenib.

Table 1

	MAINTENANCE (N=17)	INTERRUPTION (N=16)
Median age at inclusion, years [min-max]	61.0 [51-76]	65.0 [48-78]
Sex, n		
M	8	6
F	9	10
Primary tumor location, n		
Colorectal	4	2
Lung	4	8
Pancreas	0	2
Ovary/endometrium	6	4
Thyroid	1	0
Retroperitoneal region	2	0
Number of prior lines, median [min-max]	2.0 [1-7]	3.0 [1-6]
Metastatic sites, n		
≤ 2	13	9
> 2	3	7
Locally advanced disease	1	0
Molecular alteration matching the MTT : sorafenib [§] Gene Name and type of alteration, n		
KRAS		
○ mutation	14	15
○ amplification	2	
RET		0
○ mutation	1	0
Patients progression-free at 16 weeks after randomization	11 / 17	3 / 13*
PFR_{16w} after randomization**, % [95% CI]	63.2% [41.0-82.7]	26.7% [8.4-50.8]
PFS, events median, months [95% CI]	15 events 5.6 months [1.97-8.57]	13 events 2.1 months [1.61 – 3.91]
OS, events median, months [95% CI]	9 deaths 16.2 months [8.87-38.73]	13 deaths 7.2 months [46.3-89.8]

*: 3 patients in the interruption arm were randomized despite PD after the induction period and are considered non evaluable.
** Bayesian estimation [95% credibility interval], §: one patient was enrolled and randomized without any documented molecular alteration matching the MTT sorafenib.

Table 1 - Baseline characteristics and efficacy endpoints

Development of allogeneic gene-edited CAR T-cells: From preclinic to clinical proof of concept

Agnès Gouble[1], Roman Galetto[1], Julianne Smith[2], Philippe Duchateau[1], Laurent Poirot[1], Stéphane Depil[1]

[1] *Cellctis, Paris, France*
[2] *Cellectis Inc, New York, NY, United States*

Adoptive immunotherapy using engineered T-cells has emerged as a powerful approach to treat cancer. The potential of this approach relies on the ability to redirect the specificity of T cells through ex vivo genetic engineering and transfer of chimeric antigen receptors (CARs) or engineered TCRs. The transduction of patients' blood cells with an anti CD19 CAR for the treatment of Acute Lymphoblastic Leukemia (ALL) have led to complete responses in the large majority of treated patients and early approval of two products. These autologous treatments require a complex manufacturing process and are dependent on the existence of a healthy T-cell population despite previous heavy chemotherapy lines of treatment. The use of allogenic cells (derived from healthy donors rather than the patients themselves) allows preparation of cells ahead of a patient's need for treatment, in depth characterization of starting material, production of multiple treatment doses from one run and more affordable access to treatment.

Using our proprietary nuclease-based gene editing technologies, we showed our capability to efficiently edit any gene in primary T-cells with very high precision. Here, we describe how the TALEN gene-editing technology allows to create CAR T-cells that can be used in allogenic setting but also empower them with additional safety and efficacy attributes. These new features include, among other possibilities, control properties, resistance to standard oncology treatments, and prevent fratricide killing of engineered CAR T-cells.

We were able to develop GMP-compliant manufacturing of TALEN® edited CAR T-cells for clinical use. Two allogenic CAR T-cell products are currently in the clinic. Preliminary data show expansion of those allogeneic cells associated with antitumor activity, providing first clinical proof of concept of the allogeneic approach. This technology offers therefore unparalleled possibilities to design next generation cell immunotherapies in hematological malignancies as well as in solid tumors.

P10.1

Repurposing endogenous immune pathways to improve chimeric antigen receptor T-cells potency

Mohit Sachdeva[1], Julien Valton[1], Philippe Duchateau[2], Stéphane Depil[2], Laurent Poirot[2]

[1] *Cellectis Inc, New York, NY, United States*
[2] *Cellectis, Paris, France*

CAR T-cell therapies hold great promise for treating a range of liquid malignancies but are however challenged to access and eradicate solid tumors. To overcome this hurdle CAR T-cell were engineered to secrete different cytokines known to improve T-cell antitumor activity, prevent T-cell anergy and reduce activation induced cell death. While cytokine-expressing CAR T-cell were shown to be highly active against solid tumor in in vivo models, they have also led to toxicity associated with the systemic release of cytokine. Therefore, new engineering strategies enabling the fine tuning of cytokine secretion by CAR T-cell are warranted.
We sought to explore one these engineering strategies by integrating an IL-12 chimeric heterodimer expression cassette under the control of the endogenous promoters regulating PD1 or CD25. Because both genes are known to be activated upon tumor engagement by CAR T-cells, they could be repurposed to secrete cytokine only in the vicinity of a given tumor. This approach would reduce the potential side effects induced by their systemic secretion while maintaining their capacity to improve antitumor activity.
By combining TALEN® technology with AAV6 repair vectors delivering the CAR to the TRAC locus and the IL-12 to the CD25 or PD1 loci, we have engineered CAR and IL-12 expressions under the respective control of TCR and CD25 or PD1 regulatory elements. This double targeted insertion led to the disruption of PD1 and TRAC genes, to the expression of an anti-CD22 tool CAR and to the conditional secretion of IL-12 in the media. Such secretion was found to be transient, dependent on tumor engagement and to follow the regulation patterns of CD25 or PD1 genes, commonly observed upon T-cell activation. In addition, it was also found to enhance the antitumor activity and the proliferative capacities of CAR T-cells. Similar results were obtained when IL-15 was substituted for IL-12.
This proof of concept paves the way for seamless multi-repurposing of immune pathways to generate smarter CAR T-cells able to sense and react to their environment in a highly regulated and specific manner.

Corosolic acid inhibits hepatic cancer via dowregulation of JAK2/STAT3 signaling and induction of apoptosis

Sarita Saraswati[1], Abdulqader A Alhaider[2], Abdelgalil Abdelgadir[3]

[1] CNIC, Madrid, Spain
[2] King Saud University, Riyadh, Saudi Arabia
[3] College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, Riyadh, Saudi Arabia

Corosolic acid (CA) possess numerous biological properties, including antidiabetic, anti-inflammatory, antiproliferative, and protein kinase C inhibition activity, but its effect of hepatic cancer is not expolored yet. We investigated the anti-cancer effect of CA on an experimental carcinogenesis model of liver cancer by studying the anti-oxidant, anti-inflammatory, anti-proliferation, pro-apoptotic activities of CA in vivo. We evaluated the effects of CA on N-nitrosodiethylamine (DENA)-induced hepatocarcinogenesis in rats. We initiated hepatocarcinogenesis by intraperitoneal injection of diethylnitrosamine (DENA) followed by promotion with phenobarbital. Administration of CA at doses of 15, 30, and 50 mg/kg/day was started 4 weeks prior to the DENA injection and was continued for 22 weeks. CA decreased the incidence, total number, multiplicity, size and volume of preneoplastic hepatic nodules in a dose-dependent manner. Furthermore, CA counteracted DEN-induced oxidative stress in rats as determined by restoration of superoxide dismutase, catalase, and glutathione-S-transferase levels and diminishing of myeloperoxidase activity, malondialdehyde and protein carbonyl formation in liver. CA resulted in normal serum levels of IL-2, IFN-γ, AFP and AFU. We found that JAK2/STAT3 signaling was significantly up-regulated in DEN-treated group compared to that in control group. Western blot analysis showed that CA inhibited phosphorylation of STAT3 and its principal upstream kinase, Jak2. Further, it also decreased expression of cyclin D1 and Bcl-2 with activation of caspase-3 and increased expression of Bax. Immunohistochemical demonstrated the decreased expression of the PCNA, VEGF cyclooxygenase 2, iNOS, nuclear factor-kappa B p-65. This study suggests that CA exerts a significant chemopreventive effect against liver cancer via inhibition of cell proliferation and induction of apoptosis mediated by JAK2/STAT3 signaling pathway. This study also demonstrated that CA protects rat liver from cancer via modulating oxidative damage and suppressing inflammatory response.

P10.3

Chrysin attenuates cell viability of colorectal cancer cells through autophagy different from 5-fluorouracil/oxaliplatin combination

Yun-Ju Chen[1], Yueh-Ming Lin[2], Chih-I Chen[3], Yi-Ping Hsiang[3], Yung-Chia Hsu[3], Pei-Hsuan Chien[3], Chien-Chang Lu[2]

[1]I-SHOU University, Kaohsiung, Taiwan
[2]Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan
[3]E-Da Cancer Hospital, Kaohsiung, Taiwan

Chemotherapeutic 5-fluorouracil (5-FU) combined with oxaliplatin is often used as standard treatment for colorectal cancer (CRC). Due to disturbing side effects and drug resistance, commonly observed in chemotherapy, it urges us to develop alternative optimal therapeutic options for CRC treatment. Chrysin, a natural and biologically active flavonoid abundant in propolis, is reported to have anti-tumor effect on a few of CRC. However, whether and how chrysin achieves similar effectiveness as to 5-FU combination is not clearly clarified. In this study, MTT, western blot, fluorescence microscopy, reactive oxygen species (ROS) production were assayed. We found that chrysin exhibited similar viability inhibition as to 5-FU combination in a panel of human CRC cells. Furthermore, the results showed that chrysin significantly increased LC3-II amount, an autophagy-related marker, in CRC cells, which was not observed in 5-FU combination. More importantly, blockage of autophagy induction restored chrysin-attenuated CRC cell viability. Further mechanistic analysis revealed that chrysin, not 5-FU combination, induced ROS generation and in turn phosphorylations of Akt and mammalian target of rapamycin (mTOR) were inhibited. Collectively, these results imply that chrysin may be in the future as a potential replacement of 5-FU and oxaliplatin combination to achieve anti-tumor activity through autophagy for CRC treatment.

Matrix stiffness enhances angiogenesis in hepatocellular carcinoma by upregulate the expression of VEGFR2 in endothelial cells

Yaohui Qang[1], Weimin Wang[2], Wentao L[1]I, Jiefeng Cui[2]

[1]Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China
[2]Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China

Matrix stiffness as a novel regulation factor involves in modulating the pathogenesis of hepatocellular carcinoma (HCC) invasion or metastasis. However, the mechanism by which matrix stiffness modulates HCC angiogenesis remains unknown. Here, using buffalo rat HCC models with different liver matrix stiffness backgrounds and an in vitro cell culture system of mechanically tunable Collagen1 (COL1)-coated polyacrylamide gel, we investigated the effects of different matrix stiffness levels on vascular endothelial growth factor receptor 2 (VEGFR2) expression in human umbilical vein endothelial cells (HUVECs) and explored its regulatory mechanism for controlling HCC angiogenesis. Tissue microarray analysis showed that the expression levels of VEGFR2 was gradually upregulated in tumor tissues with increasing COL1 and lysyloxidase (LOX) expression, indicating a positive correlation between tumor angiogenesis and matrix rigidity. Differential translation factors (TFs) in HUVECs with different matrix stiffness were screened and identified with Protein/DNA assay. Over 102 TFs were found in HUVECs that have associated with the matrix stiffness. The expression of VEGFR2 and the SP1 were all upregulated in HUVECs on high-stiffness gel than on low-stiffness gel. Meanwhile, the expression of PI3K/Akt was found to be the most distinctive, implying that it might mediate the response of HUVECs to matrix stiffness simulation. After PI3K/Akt was blocked in HUVECs, the expression of VEGFR2 and the SP1 were accordingly suppressed and downregulated in the treatment group as compared with those in the control group. All data suggested that the extracellular matrix stiffness upregulated VEGFR2 expression by activated the PI3K/Akt/Sp1 pathway. This study unveils a new paradigm in which matrix stiffness as initiators to modulate HCC angiogenesis.

Mechanism-based approach to identify apoptosis in live tumor cell by triple fluorescence following anti-cancer drugs

Pradip De, Jennifer Carlson, Nandini Dey, Brian Leyland-Jones

Avera Cancer Institute, Sioux Falls, SD, United States

Introduction: Here we present a protocol for live cancer cell imaging by triple fluorescent staining to test three crucial mechanisms of apoptosis; the enzymatic activity of the executioner caspase3, caspase-dependent phosphatidylserine presentation on the cell surface and mitochondrial function.

Methods: We standardized a protocol to co-stain live tumor cells with the fluorescent markers NucView488 Casp3 substrate, CF594 AnnexinV, and MitoView Blue in ovarian and breast cancer models. We used chemotherapeutic agents (paclitaxel and carboplatin) and targeted drugs (olaparib, BKM120, AZD2014, and AZD5363). Fluorescent imaging of cells using simultaneous live/dead cell markers (Calcein AM green/EthD-1 red) was used to validate this protocol. We used quantitative confluence (Essen), AnnexinV-PE staining, expression of apoptotic signals (cl-caspase3, cl-PARP; WB) and mitochondrial potential (TMRE-A) as validation criteria for the triple co-fluorescence staining in A2780 & OVK18 cells following paclitaxel and BKM120 treatment.

Results: Paclitaxel alone or in combination with BKM120 decreased proliferation, induced early apoptosis, increased apoptotic signaling, and increased mitochondrial depolarization, as compared to control. Dual treatment blocked MitoViewBlue staining while increasing both nuclear NucView488 Casp3 substrate staining and red membranous CF594 AnnexinV staining. Similarly, characteristic features of apoptosis were identified in carboplatin, olaparib, and AZD5363 or AZD2014 treated MDA-MB468 and SUM149 BC cells. Merged images of triple fluorescence showed 100% mutual exclusivity between Mitoview Blue and caspase 3 or AnnexinV stains in both control and treated cells as determined by overlap and colocalization coefficients. However, caspase 3 and AnnexinV staining in the treated cells could be found both individually as well as overlapping (yellow fluorescence), indicating the sequence of apoptotic events in an individual cell in response to drug treatment.

Conclusion: This staining protocol will help in deciphering the mechanistic involvement of different stages of apoptosis in live tumor cells following treatment with different anti-cancer drugs in real time.

Fasudil suppress lung cancer endothelial cell viability and migration via inhibiting RhoA/ROCK signaling

Zhe Zhang, Wen-Wu Cheng, Li Nong, Zhenyu Li

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

The aim of this study was to investigate the effect of fasudil on lung carcinoma-conditioned endothelial cells (LCc-ECs). To obtain LCc-ECs, human umbilical vein endothelial cells (HUVECs) were treated with conditioned cell culture media from human A549 lung adenocarcinoma cells. The effect of fasudil on the viability of LCc-ECs was assessed using the MTT assay, in vitro invasive ability was evaluated using the transwell chamber assay and cytoskeletal changes were detected using fluorescein-labelled phalloidin immuno- cytochemistry. RhoA mRNA and p-MLC protein expression were measured using RT-PCR and western blotting. Fasudil significantly and dose-dependently inhibited LCc-EC prolif- eration and in vitro invasive ability. Fasudil also led to stress fibre breakage and fracture in LCc-ECs, indicating that fasudil impacts polymerisation of the cytoskeletal actin filament network. Expression of RhoA mRNA and protein expression of the ROCK substrate p-MLC were reduced by fasudil, suggesting that fasudil can inhibit RhoA/ROCK signalling and attenuate angiogenesis in LCc-ECs. This study indicates that fasudil is an anti-angiogenic agent with potential application for the treatment of cancer, especially lung adenocarcinoma.

Table 1

	MAINTENANCE (N=17)	INTERRUPTION (N=16)
Median age at inclusion, years [min-max]	61.0 [51-76]	65.0 [48-78]
Sex, n		
M	8	6
F	9	10
Primary tumor location, n		
Colorectal	4	2
Lung	4	8
Pancreas	0	2
Ovary/endometrium	6	4
Thyroid	1	0
Retroperitoneal region	2	0
Number of prior lines, median [min-max]	2.0 [1-7]	3.0 [1-6]
Metastatic sites, n		
≤ 2	13	9
> 2	3	7
Locally advanced disease	1	0
Molecular alteration matching the MTT : sorafenib § Gene Name and type of alteration, n		
KRAS		
○ mutation	14	15
○ amplification	2	
RET		0
○ mutation	1	0
Patients progression-free at 16 weeks after randomization	11 / 17	3 / 13*
PFR16w after randomization**, % [95% CI]	63.2% [41.0-82.7]	26.7% [8.4-50.8]
PFS, events median, months [95% CI]	15 events 5.6 months [1.97-8.57]	13 events 2.1 months [1.61 – 3.91]
OS, events median, months [95% CI]	9 deaths 16.2 months [8.87-38.73]	13 deaths 7.2 months [46.3-89.8]

*: 3 patients in the interruption arm were randomized despite PD after the induction period and are considered non evaluable.
** Bayesian estimation [95% credibility interval], §: one patient was enrolled and randomized without any documented molecular alteration matching the MTT sorafenib.

Fasudil treatment disrupts cytoskeletal actin filament integrity in LCc-ECs.

## BRAF and CRAF mutations: actionable targets in lung cancer

Amir Noeparast, Philippe Giron, Carolien Eggermont, Gil Verschelden, Ulrike De Ridder, Lore Decoster, Rajendra Bahadur Shahi, Sylvia De Brakeleer, Erik Teugels, Jacques De Grève

Vrije Universiteit Brussel, Brussels, Belgium

In three sequential studies, we pre-clinically investigated several previously unexplored lung cancer-derived BRAF and CRAF mutations and their response to clinically available targeted therapeutics. During the FIELT I clinical study at UZ-Brussel (De Grève et al. PLoS ONE, 2016) in which 229 NSCLC patients were prospectively investigated at the genomic level, twelve patients (5.2%) were identified to harbor a BRAF mutation in their tumor. As opposed to melanoma, 75% of these NSCLC-derived BRAF mutations were non-V600. RAF inhibitors have only been clinically developed against BRAF V600 mutations because of concerns of paradoxical effect in non-V600 mutant cancers. The status of non-V600 mutations with regard to BRAF inhibition effects was unknown. We functionally analyzed 13 such tumor-derived BRAF non-V600 mutations and we demonstrated in three preclinical models (A) that all types of BRAF mutations cause pathway activation and are sensitive to clinically relevant doses of a combination of type I RAF-inhibitor (Dabrafenib) and that paradoxical pathway activation is abrogated by MEK-inhibition (Trametinib). This entails that dual inhibition of non-V600 mutations is effective and safe. Further, we investigated the comparative efficacy of two modes of RAF inhibition (type I vs. type II) in suppressing mutant BRAF-induced ERK signaling (B). Our preclinical findings in non-V600 BRAF expressing cellular models suggest that the type II RAF-inhibition (AZ628) has superior potential than type I RAF-inhibition (Dabrafenib) both as single agent and combined with MEK inhibition in suppressing the ERK pathway independent of BRAF mutation type. We also explored two somatic CRAF mutations not identified before in lung cancer (C). In the HEK293T and BEAS-2B cellular models, we demonstrate that one of these mutations, CRAF P261A, is ERK pathway-activating and predicts sensitivity to type II but not type I RAF inhibition. Our findings support the clinical investigation of BRAF non-V600 or CRAF mutated lung or other cancers with dual RAF and MEK inhibition.

Graphical summary of methods
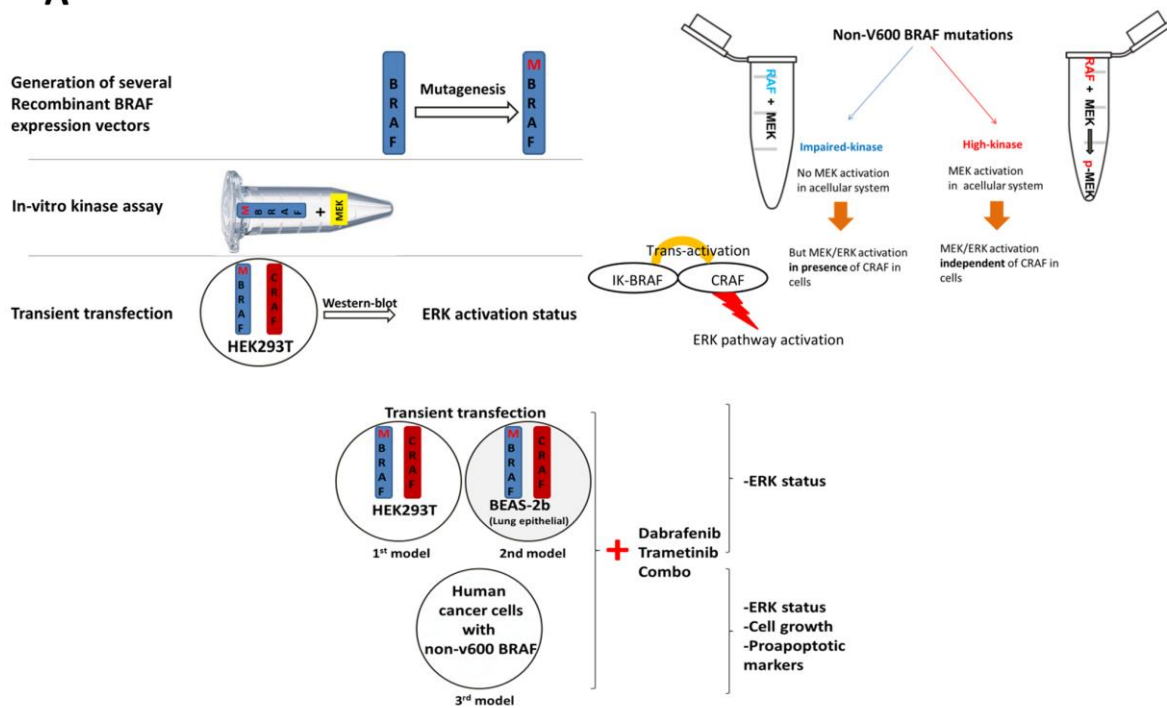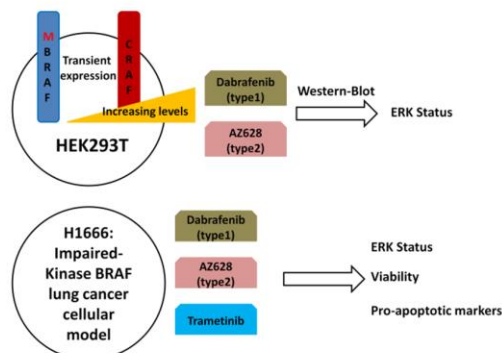
Graphical summary of methods

Anti cancer and immune stimulating activities of a novel oncolytic virus -- VG161 in colorectal cancer mice model

Ronghua Zhao[1], Jun Ding[1], Dmitry Choujenko[1], Yanal Murad[1], Erica Lee[1], Guoyu Liu[1,2], Luke Bu[1,2], William Jia[1,2]

[1] *Virogin Biotech, Vancouver, BC, Canada*
[2] *University of British Columbia, Vancouver, BC, Canada*
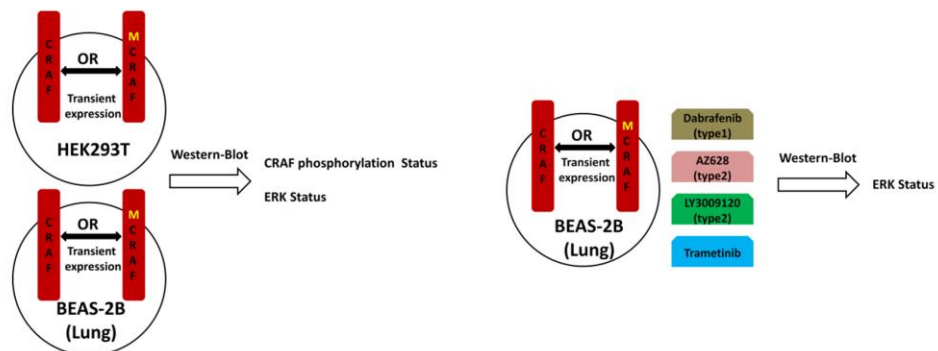
Background: Oncolytic viruses (OVs) can not only cause cancer cell lysis but more importantly, their replication in tumor cells induces anti-tumor immune response from the host, resulting in lasting anti-tumor immunity. Also, synergistic stimulation of anti-tumor immune response requires multiple immune regulatory factors. Therefore, OVs armed with multiple immune modulating factors are among the most powerful approaches in cancer immunotherapy. The large genomic capacity of Herpes Simplex Virus type-1 (HSV-1) renders the virus to carry multiple exogenous genes.
This study aimed to look at the anti cancer and immune stimulating activities of a novel HSV-1 oncolytic viral vector (VG161) which was constructed to simultaneously express IL12, IL15 with its receptor alpha unit and a PDL-1 blocking peptide.|
Method: Anti-tumor activity of VG161 was tested in both immune competent mice for murine (CT26) and nude mice for human (LS174T) colorectal cancer models. Since CT26 is poorly permissive for HSV-1 replication, the mouse tumor model was able to demonstrate the anti-tumor immune response induced by VG161 while oncolytic activity of VG161 was demonstrated in LS174T model since the immune system is compromised in the model. The specific anti-cancer immunity was determined by T cell subpopulation analysis, ELISPOT, and re-challenge test as well.
Results: VG161 caused complete tumor eradication in all the models tested and the animals survived tumor-free for many months till sacrificed. The virus induced tumor oncolysis caused complete tumor destruction in LS174T tumor. In the CT26 model, no tumor could be found after re-challenging with the same tumor cells. Number of CD8+ tumor infiltrated T cells was significantly increased, and systemic specific anti cancer immunity was confirmed by ELISPOT assay on T cells isolated from spleen.
Conclusions: VG161 is a novel oncolytic virus that are of both strong in stimulating anti-tumor immunity and oncolytic activity. Intratumorally expressing multiple immune regulatory factors by an oncolytic virus may significantly change the tumor immune microenvironment and stimulating anti cancer immunity, to enhance the efficacy of the oncolytic virus.

P11.1

SNPs in PSEN1 and MAML2 predict overall survival in Chinese patients with epithelial ovarian cancer

Mengyun Wang, Yuan Xu, Lei Cheng, Ruoxin Zhang

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: To identify genetic variants in Notch signaling pathway genes that may predict overall survival of Han Chinese patients with epithelial ovarian cancer (EOC), we performed an analysis using a subset of available genotyping data from a published EOC genome-wide association study (GWAS).
Methods: The present study included genotyping data for a total of 1,273 single nucleotide polymorphisms (SNPs) within 75 Notch genes in 480 EOC patients. The death-risk associated SNPs were identified by multivariate Cox proportional hazards regression analysis, with the correction of false-positive report probability (FPRP) <0.2.
Results: Overall, we found that two SNPs (i.e., PSEN1 rs165934 and MAML2 rs76032516) were associated with survival of EOC patients. Specifically, the PSEN1 rs165934 AA genotype was associated with a poor survival (adjusted hazards ratio [HR]=1.41, 95% CI=1.07-1.84, and P=0.014), compared with the CC+CA genotype, while MAML2 rs76032516 AA+AC genotypes were associated with a poor overall survival (adjusted HR=1.58, 95% CI=1.16-2.14, P=0.004), compared with the CC genotype; when these risk genotypes were combined, the death risk increased as the number of risk genotypes increased in a dose-dependent manner (Ptrend < 0.001), which also significantly improved the time-dependent receiver operating characteristic analysis with an increased concordance index (P<0.001 for both additive and dominant models). Finally, an expression quantitative trait loci (eQTL) analysis found that the mRNA expression levels of PSEN1 increased with a borderline significant trend for the rs165932 (in high LD with rs165934; r2=0.946) allele change of A to C(P=0.060), but this was not observed for MAML2.
Conclusions: The associations of PSEN1rs165934 and MAML2 rs76032516 of the Notch signaling pathway genes with overall survival in Chinese Han EOC patients are novel findings but need to be validated in other large and independent studies.

P11.2

A multi-omic approach as powerful tool for the identification of actionable targets

Alexandra Samsen, Silvia von der Heyde, Sandra Schneider, Wolfgang Saeger, Susanne Arbogast, Eike von Leitner, Hartmut Juhl

IndivuTest GmbH, Hamburg, Germany

Background and aim: Molecular profiling of patients tumor samples for the identification of targeted treatment options is often limited to genetic testing. The number of tumor-agnostic and approved targeted agents is increasing and hundreds are in clinical development. The stratification of patients for the most promising targeted therapy option is a challenge. In this study tumor tissues of patients with different tumor types were analyzed in a multi-omic approach. The aim was to evaluate if genetic testing as stand-alone method is sufficient to implement precision medicine.

Materials and methods: Tumor tissue of 83 patients was collected under highly standardized conditions and snap frozen within seconds following tissue collection to preserve the (phospho-) protein pattern to generate reliable data. In addition to targeted sequencing, alterations were also detected on protein expression- and pathway activity levels of cancer relevant signaling pathways.

Results: For 29% of the patients' actionable alterations (drug targets or drug exclusion targets) were detected by sequencing, mainly in the genes KRAS (57%), PIK3CA (24%), EGFR (5%), ERBB2 (5%), FLT3 (5%), KIT (5%), BRAF (5%) and IDH2 (5%). Increased pathway activity could be identified for 81% of the patients, specifically ERK1/2 (62%), MEK1/2 (79%) and Akt (55%). Overexpression of receptor proteins and nuclear proliferation marker was found for 91% of the patients, specifically EGFR (71%), HER-2 (24%), c-MET (87%), IGF-1R (84%), PD-L1 (36%), VEGFR-2 (0%), EML4-ALK (9%), Ki-67 (94%) and Topoisomerase-II-alpha (84%). The integrative analysis of all methods together tripled the number of identified drug targets in comparison to consider sequencing results only.

Conclusions: Using this multi-omic approach offers valuable insights in the individual tumor biology and identifies additional druggable alterations. Through the limitation to genomic alterations important potential drug targets would remain unexploited.

Keywords:
Multi-omics, Pathway activity

Identification of long non-coding RNAs (lncRNAs) involved in chromosomal instability in a prostate cancer model.

Rogelio Montiel Manríquez, Cristian Arriaga Canon, Luis A. Herrera

Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas, Instituto Nacional de Cancerología, Mexico City, Ciudad de México, Mexico

In this study, we aimed to identify new lncRNAs important for prostate carcinogenesis using RNA-seq data, and to analyze whether those transcripts could be associated with chromosomal instability (CIN).
Using RNA-seq data from two prostate cell lines, neoplastic (LNCaP) and non-neoplastic (PrEC), we have identified a new lncRNA adjacent to CEP55 (centrosomal protein 55), a gene associated with CIN. This lncRNA, which we have named lncRNA-CEP55, is 1.6 kb long and it is downregulated in the neoplastic cell line. Downregulation was also seen in CEP55, which expression decreased in the neoplastic line suggesting a possible role of the loss of this transcript in carcinogenesis.
lncRNA-RFC4 was a second lncRNA, with differential expression in both cell lines. lncRNA-RFC4 is an annotated gene in the human genome GRCh38/hg38, but it has not been studied in cancer. This lncRNA is highly expressed in LNCaP whereas its expression is very low in PrEC, which suggests a possible role of this lncRNA in cancer development. lncRNA-RFC4 is 4.3 kb away from RFC4 (Replication Factor C Subunit 4), a protein coding gene related to mismatch repair and DNA Double-Strand Break Repair, abnormalities in this coding gene have been associated with CIN in other cancers. lncRNA-RFC4 could be playing an important role in the cis regulation of RFC4 gene.
lncRNA-CEP55 and lncRNA-RFC4 are important to study because of their differential expression in both cell lines, as well as for their possible association with the regulation of adjacent genes related to CIN, an enabling characteristic that facilitates the acquisition of the hallmarks of cancer. The role of lncRNAs as drivers of CIN in CaP is a promising field to understand CaP progression and are promising biomarkers in this disease.

P11.4

TP53 mutation-associated immunomodulation and prognostic impact is specific to CMS1-immune colorectal cancers

Anita Sveen[1], Jørgen Smeby[2], Christian H. Bergsland[1], Ina A. Eilertsen[1], Peter W. Eide[1], Merete Hektoen[1], Marianne G. Guren[2], Arild Nesbakken[2], Jarle Bruun[1], Ragnhild A. Lothe[1]

[1] Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
[2] Oslo University Hospital, Oslo, Norway

Background: Emerging data indicate that TP53 has a role in tumor immune responses, but the clinical significance of mutations of this well-known tumor suppressor in colorectal cancer (CRC) remains unknown.
Materials and methods: We performed mutation analysis of the full coding region and splice sites of TP53 in a single-hospital series of 401 stage I-IV CRCs. Transcriptional consequences and the consensus molecular subtypes (CMS) were analyzed by gene expression profiling, and quantification of tumor immune cell infiltration was validated by multiplex, fluorescence-based immunohistochemistry. For prognostic analyses according to CMS, the patient series was pooled with a publicly available French multi-center cohort.
Results: TP53 mutations were found in 60% of the CRCs and were weakly enriched in the CMS2-epithelial/canonical subtype (79%). However, gene set enrichment analyses showed that the mutations had strongest transcriptional effects in CMS1-immune and CSM4-mesenchymal CRCs. This subtype-specificity was most strongly seen as upregulation of gene sets reflecting cell cycle progression in CMS4, and downregulation of T cell activity in CMS1. The latter was accompanied by significant depletion of several immune cell populations in mutated compared to wild-type tumors in CMS1, including cytotoxic lymphocytes (adjusted P-value in CMS1 = 0.002 and CMS2-4 > 0.9; gene expression-level, MCP-counter algorithm). CMS1-specific immune cell depletion was validated by immunohistochemistry-based quantification of CD8+ cells. Furthermore, the immunomodulatory effect was strongest among microsatellite stable (MSS) tumors, and in the pooled cohort of 635 patients, this translated into a negative prognostic association for TP53 mutations specifically in the CMS1/MSS subtype (HR = 5.52 (1.21-25.3) for overall survival, P = 0.028; test for interaction of TP53 mutation and microsatellite instability status in CMS1, P = 0.049).
Conclusions: Integration of TP53 mutation status with the CMS framework revealed subtype-dependent immunomodulation and a subsequent negative prognostic association, indicating clinical relevance of TP53 in primary CRC, potentially also with immunotherapeutic implications.

Machine learning for prognostic stratification and driver gene identification using copy number variations in anaplastic oligodendroglioma

Shai Rosenberg[1], Francois Ducray[2,3,4], Agusti Alentron[5,6,7], Caroline Dehais[7], Nabila Elarouci[8], Aurelie Kamoun[8], Yannick Marie[6], Marie-Laure Tanguy[7], Aurélien De Reynies[8], Karima Mokhtari[5,7], Dominique Figarella-Branger[9,10], Jean-Yves Delattre[5,7], Ahmed Idbaih[5,7]

[1] Hadassah - Hebrew University Medical Center, Jerusalem, Israel
[2] Hospices Civils de Lyon, Hôpital Neurologique, Lyon, France
[3] Cancer Research Centre of Lyon, Lyon, France
[4] Université Claude Bernard Lyon, Lyon, France
[5] Sorbonne Universités, Paris, France
[6] Institut du Cerveau et de la Moelle épinière, Paris, France
[7] AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière- Charles Foix, Paris, France
[8] Ligue Nationale Contre Le Cancer, Paris, France
[9] Université de la Méditerranée, Aix-Marseille, Marseille, France
[10] Hôpital de la Timone, Marseille, France

Background: Anaplastic oligodendrogliomas have variable clinical behavior. We have recently shown that the common 9p21.3 allelic loss is an independent prognostic factor in this tumor type. The aim of this study is to identify less frequent genomic Copy Number Variations (CNV) with clinical importance that may shed light on molecular oncogenesis of this tumor type.

Materials and Methods: A cohort of 197, anaplastic oligodendroglioma was collected as part of the French POLA network. Clinical, pathological and molecular information were recorded. CNV analysis was performed using SNP arrays. Computational biology and feature selection based on a random forests method were used to identify CNV events associated with overall survival and other clinical-pathological variables

Results: Recurrent chromosomal events were identified in chromosomes 4, 9, and 11. 46 focal amplification events and 22 focal deletion events were identified. 24 focal CNV areas were associated with survival and five of them were significantly associated with survival after multivariable analysis. 9/24 CNV events, were validated using an external cohort of The Cancer Genome Atlas. Five of the validated events contain a cancer related gene or MIR: CDKN2A deletion, SS18L1 amplification, RHOA/MIR191 copy-neutral loss-of-heterozygosity, FGFR3 amplification, and ARNT amplification. Using multivariable model the CNV profile contributes to better survival prediction compared to clinical based risk assessment.

Conclusion: Several recurrent CNV events, detected in anaplastic oligodendroglioma, enable better survival prediction. More important, they help in identifying potential genes for understanding oncogenesis and for personalized therapy.

Study of the regulation by the lncrna terra in the telomere healing process of a myeloid cell model

Diego Oliva Rico, Rodrigo Cáceres Gutiérrez, Eunice Fabián Morales, Julieta Dominguez Ortiz, Fernanda Cisneros Soberanis, Cristian Arriaga Canon, Luis Alonso Herrera

Unidad de Investigación Biomédica en Cáncer, Instituto Nacional de Cancerología-Instituto de Investigaciones Biomédicas, UNAM, Ciudad de México, Mexico

Telomeres and sub-telomeric regions are enriched in histone marks associated to constitutive heterochromatin. The marks are essential for the maintenance of telomere integrity and therefore for genomic stability, but there is another kind of regulation that depends on ncRNAs. This is the case of the lncRNA Telomere repeat-containing RNA (TERRA), an element required for telomeric integrity. Telomeric instability leads to chromosomal aberrations that could produce a malignant cell lineage, however, in order for this event to take place, a telomere healing process must occur so that the ribonucleoproteic complex of the telomere can be stabilized once more. Studies have been carried out to relate changes in TERRA expression with progression, aggressiveness and prognosis of certain types of cancer, and in spite of the evidence discovered so far, a constant association has not been determined yet between this RNA's expression and cancer development. For this reason it is important to study the role that the lncRNA TERRA plays in telomere stabilization and how its malfunctioning can set on changes in cancer prognosis.

We worked with two cell lines of the same cell lineage K562 & SC, in order to have a leukemic and a non-transformed cell model. In order to enrich the amount of TERRA after RNA extraction, we synthesized cDNA using both dT primers and telomere repeat-containing oligonucleotides. The cDNA was used to evaluate the total amount of TERRA in both cell lines using the expression of the ncRNAs U6 as an amplification reference. We analyzed the sub-telomeric region of the chromosome arms 5, 10, 11 and X in search of a potential TERRA transcription site. We looked for elements associated to promoter regions such as euchromatin-associated histone modification marks and potential transcription start sites (TSS). We later evaluated the enrichment of chromatin marks on the chosen loci and also quantified the molecules transcribed from them for a set of population doubling events.

We observed that the TERRA total concentration not only varies between different cell types, but also according with the senescence of the culture. This phenomenon was more evident in the control cell line, that doesn't express Telomerase (Saos2). TERRA expression varies according to the analyzed locus, which allowed classifying this molecules according with the changes in their expression. Chromatin marks associated with transcriptional repression (H3K9me3, H3K27me3, H4K20me3) showed an increase in the loci where expression dropped or remained unchanged. Whereas transcription associated marks (H3K4me3, CTCF) increased in loci where TERRA expression went up as the culture aged. While a relation has been found in the cell lines used, these study still needs to be carried out in lymphocytes of CML blood patients at different stages of their disease and treatment, in order to strengthen the association discovered.

P11.7

Phynotype feature of achromotopsia in Saudi Arabia

Nuha Alsalameh

Ksu, Riyadh, Saudi Arabia

Achromatopsia is an autosomal recessive visual defect that is marked by color blindness in which the colors of the spectrum are seen as tones of white, gray, and black, poor visual acuity, and extreme sensitivity to bright light.The known causes of the congenital forms of achromatopsia are all due to malfunction of the retinal phototransduction pathway. Specifically, this form of ACHM seems to result from the inability of cone cells to properly respond to light input by hyperpolarizing. Known genetic causes of this are mutations in the cone cell cyclic nucleotide-gated ion channels the most prevalent is mutation in CNGB3 (ACHM2) followed by CNGA3 (ACHM3) ,while mutations in PDE6C and GNAT2 genes considered less common.

The electroretinogram (ERG) is a diagnostic test that measures the electrical activity generated by neural and non-neuronal cells in the retina in response to a light stimulus. The ERG can provide important diagnostic information on a variety of retinal disorders including, but not limited to congenital stationary night blindness, Leber congenital amaurosis, and cancer-associated retinopathy. Moreover, an ERG can also be used to monitor disease progression or evaluating for retinal toxicity with various drugs or from a retained intraocular foreign body. In achromatopsia ERG will shows an absence of conventional cone responses, with normal rod ERG. Dark adaptometry shows no cone plateau, and no cone-rod break. There are two on going clinicl trail gene therapy, one on AAV Gene Therapy(rAAV2tYF-PR1.7-hCNGB3) administered to one eye by subretinal injection in Patients With CNGB3 Achromatopsia , measuring their safety and efficacy,with Estimated outcome Date on 2022.

other clinical trail on a Single Subretinal Injection of(rAAV.hCNGA3) in Patients With CNGA3 Achromatopsia,measuring their safty and efficacy ,with estimated outcome date on 2017

POSTER SESSION 12: New combinations (agents and modalities)

P12.1

Combining targeted therapies to chemosensitize prostate cancer cells

Annamaria Bevilacqua[1], Angela Calastretti[1], Giuliana Gatti[1], Gianfranco Canti[2]

[1] Università degli Studi di Milano, Milan, Italy
[2] School of Medicine University of Milano, Milan, Italy

The specific aim of our research project is to overcome therapeutic resistance and restore the sensitivity to anti-cancer drugs in prostate cancer cells combining selective inhibitors of the PI3K/AKT/mTOR cascade. We previously demonstrated that down-regulating AKT and/or Bcl-2 levels enhances the sensitization to cell death, induced by association of starvation plus taxanes [1]. Here prostate cancer cells have been treated with different inhibitors of the PI3K/AKT/mTOR pathway as single agents or in combination with siRNAs, starvation and taxanes (Figure 1). Cell viability of prostate cancer cells, reflecting a range of cancer progression and androgen sensitivity, was differently affected by PI3K/AKT/mTOR pathway inhibitors. The percentage of growth inhibition significantly increased in combined treatments with starvation and taxanes. In particular, prostate cancer cells treated with siRNAs directed towards mTOR in association with starvation and taxanes displayed a dramatic reduction of viable cell number. A correlation between combined targeted therapies and PTEN status could be hypothesized.

Overall, we demonstrated that it is possible to induce prostate cancer cell death through selective inhibition of specific components of PI3K/AKT/mTOR cascade, combining treatments with taxanes and metabolic starvation.
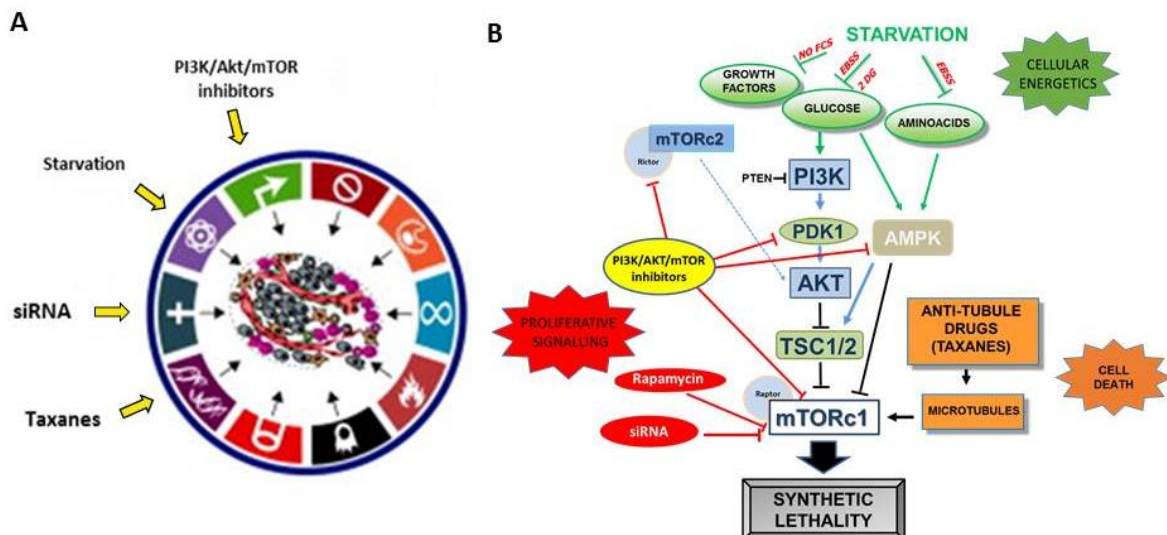
Figure 1 – Model depicting the combination of targets to induce cell death in prostate cancer cells. A) Targeting of different cancer's capabilities, modified from [2]. B) Synergic role of taxanes, metabolic inhibition and down-regulation of PI3k/AKT/mTOR.

P13.1

Concurrent neoadjuvant chemotherapy and estrogen deprivation in patients with ER-positive, HER 2-negative breast cancer (CBCSG-036 trial)

Keda Yu

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Background: Limited studies existed to demonstrate clinical benefit from co-administration of neoadjuvant chemotherapy (NCT) and endocrine therapy by estrogen deprivation. Therefore, we conducted a clinical trial to investigate the efficacy of concurrent neoadjuvant chemotherapy (NCT) and estrogen deprivation in patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer.

Methods: In this open-label, multicenter, randomized controlled trial, eligible patients with stage IIB–IIIC, ER-positive, HER2-negative breast cancer (having indications for chemotherapy) were enrolled and randomly assigned to receive NCT with or without estrogen deprivation (an aromatase inhibitor with or without GnRH-a). The primary endpoint was the objective response rate (ORR). The secondary endpoints included Ki67 proliferation marker changes, progression-free survival (PFS), and safety.

Results: 249 patients were randomly assigned to two groups, with 125 patients in the neoadjuvant chemo-endocrine therapy (NCET) and 124 patients in the NCT group. In the intention-to-treat analysis, the ORR was significantly higher in the NCET group than that in the NCT group (84.8% vs 72.6%, P=0.019; odds ratio=2.11, 95% confidence interval 1.13–3.95, P=0.020). The efficacy of concurrent estrogen deprivation was more prominent in tumors with higher Ki67 (Ki67 >20%)(P=0.001). Although there is no significant difference of PFS between the two groups (P=0.188), patients with higher Ki67 at baseline might get more PFS benefit from concurrent NCT and estrogen deprivation (2-year PFS: 91.5% in NCET group vs 76.5% in NCT group, P=0.058). Adding endocrine agents to NCT was well tolerated and did not result in significant differences in adverse events (grade 3 or 4 toxicity) between two groups.

Conclusions: Addition of estrogen deprivation to NCT improves the clinical response in patients with ER-positive, HER2-negative breast cancer, especially for those with higher Ki67. Although early survival analysis indicates that patients with higher Ki67 might get more PFS benefit from concurrent NCT and estrogen deprivation, longer follow-up will be necessary to fully evaluate survival benefit.

Targeting casein kinase II (CK2) pro-oncogene to restore ikaros tumor suppressor activity: Targeted therapy for high risk pediatric leukemia

Chandrika Gowda, Chunhua Song[2], Sinisa Dovat[2]

[1] *Penn State University Medical Center, Hershey, PA, United States*
[2] *Penn State College of Medicine, Hershey, PA, United States*

Recent genome-wide studies of leukemic blasts have detected genetic lesions such as deletions or mutations in IKZF1, a gene that encodes the lymphoid transcription factor Ikaros which is a master regulator of lymphopoiesis and a tumor suppressor. Phosphorylation of Ikaros by CK2 impairs Ikaros functions. We recently reported that targeted inhibition of CK2 restores Ikaros tumor suppressor function in high-risk B-ALL and exhibits an anti-leukemic effect in primary xenograft models of high-risk B-ALL.

Objective: Global genome-wide binding studies using chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq) demonstrate that the Ikaros binds to the promoter of Bcl-xL gene. Bcl-xL (B cell lymphoma extra-large) is an anti-apoptotic gene that is overexpressed in leukemia.

Methods and Results: Overexpression of Ikaros in B-ALL cells results in reduced expression of Bcl-xL at both the mRNA and protein levels. Ikaros silencing with shRNA results in increased transcription of Bcl-xL. These data suggest that Ikaros represses transcription of Bcl-xL. Previous studies suggested that CK2 impairs the ability of Ikaros to regulate transcription of its target genes in leukemia. Since CK2 is overexpressed in B-ALL, we tested whether CK2 can regulate expression of Bcl-xL in this leukemia. Results showed that molecular inhibition of CK2 with shRNA, as well as pharmacological inhibition with a specific CK2 inhibitor, CX-4945, results in reduced transcription of Bcl-xL in B-ALL cell lines and in primary B-ALL cells. This was associated with increased binding of Ikaros to the Bcl-xL promoter, as evidenced by qChIP. Ikaros knock-down with shRNA abolished the ability of CK2 inhibitors to repress Bcl-xL transcription in B-ALL. These data demonstrate that Ikaros is a regulator of Bcl-xL transcription in B-ALL, and that CK2 inhibitors repress transcription of Bcl-xL by increasing Ikaros binding to the Bcl-xL promoter in B-ALL. Several currently used anti apoptotic agents show synergistic activity with CK2 inhibitors both in vitro and inpatient derived xenograft leukemia models. In conclusion, targeting pro oncogene CK2 in order to restore function of tumor suppressor Ikaros is a novel therapeutic strategy and potentially effective in treating high risk leukemia.

The AA protein-based model for cancer genesis: a new concept for new therapeutic avenues

Adouda Adjiri

Sétif-1University, Sétif, Algeria

Background: Cancer is a defiant disease which cure is still far from being attained besides the colossal efforts and financial means deployed towards that end. The continuing setbacks encountered with today's arsenal of anti-cancer drugs invites inevitably for new concepts and ideas. Therefore and after having challenged the role of DNA mutations in cancer initiation; the question asked next is: If DNA mutations are not the initiating events in cancer, how then to explain malignancy? A thorough analysis of cancer hallmarks provides us with initial answers.

Results: This radical approach brings to light the following points: (i) Cancer with its plethora of genetic and cellular symptoms could originate from one major event switching a cell from normalcy-to-malignancy; (ii) This switching event is postulated to involve a pathological breakup of a non-mutated protein, called here AA protein, resulting in the acquisition of new cellular functions present only in cancer cells; (iii) Following this event, DNA mutations begin to accumulate as secondary events to ensure perpetuity of cancer; (iv) The AA protein-based model reconciles together the clonal-and-stem cell theories; (v) Cancer hallmarks are but adaptive traits, earned as a result of the switch from normalcy-to-malignancy.

Conclusions: Adaptation of cancer cells to their microenvironment and to different anti-cancer drugs is deemed here as the ultimate cancer hallmark, that needs to be understood and controlled. Today's targeted therapies may give positive and lasting effects if associated with inhibitors capable of blocking the adaptive power of cancer cells. This unprecedented analysis while demystifying cancer; puts the finger on the core problem of malignancy, offering thus ideas for its control with the ultimate goal of leading to its cure.

Keywords:
Cancer hallmarks; the switch from normalcy-to-malignancy; the AA protein-based model for cancer genesis; cancer therapy.

P13.4

Pharmacogenomically driven therapy for HER2-negative breast cancer in the neoadjuvant setting

Rachel Elsey[1], Kai Zheng[2,3], Tobias Meissner[1], Bing Xu[1], Casey Williams[1]

[1] Avera Cancer Institute, Sioux Falls, SD, United States
[2] Avera McKennan Hospital, Sioux Falls, SD, United States
[3] University Health Center, Sioux Falls, SD, United States

Background: The Avera Cancer Institute's Precision Oncology service has utilized patient's somatic tumor mutation information to guide cancer treatment choice since 2014. Pharmacogenomic testing offers additional options to patients who are at high risk for poor response to standard neoadjuvant treatment, but additional therapy could also increase risk of adverse effects. This study aims to indicate whether breast cancer patients who received pharmcogenomic testing and possibly targeted neoadjuvant therapy differed in therapeutic outcome when compared to standard of care.

Materials and Methods: This is a retrospective case-control study. Inclusion criteria were: HER-2 negative breast cancer patients who received neoadjuvant treatment between June 2014 until January 2017. Exclusion criteria were: male, pregnant, received radiation therapy, stage 0, and metastatic disease. Patients were considered to be in case group if they received somatic tumor mutation testing in the neoadjuvant setting. Control patients were, if possible, matched by age at diagnosis, ER/PR status, clinical stage, and tumor grade (1:1). The primary outcome of this study is the immediate response from surgical pathology report (pCR) and the secondary outcomes are disease-free survival (DFS) and tolerability.

Results: Twenty patients were included in both the case and control groups. Baseline characteristics were similar between the groups, but, numerically there were more triple negative and stage III patients in the study group. Of the 20 patients in the study group, 18 were found to have actionable mutations, and 11 were able to recieve targeted therapy prior to surgery. The study group had a pCR rate of 25% compared to 35% in the control group (p=0.731). At one year after surgery, 95% of the study group and 85% of the control group were disease-free. Tolerability was similar in the two groups with 55% of the study group and 35% of the control group experiencing grade 3 or 4 adverse events. Fifteen patients in the study group and fourteen patients in the control group required treatment delays.

Conclusions: Sequencing somatic tumor mutations in the neoadjuvant setting offers a unique opportunity to tailor therapy for patients, however, it is still unattainable for many patients for many reasons.

P13.5

Trastuzumab in combination with chemotherapy for treatment of HER2-positive advanced gastric cancer: the experience of a single center

Isabel Domingues, Edgar Pratas, João Carvalho, Sara Pinheiro, Susana Amaral, Mariela Marques, Judy Paulo, Nuno Bonito, João Ribeiro, Ana Pais, Paula Jacinto, Gabriela Sousa

Portuguese Institute of Oncology of Coimbra, Coimbra, Portugal

Background: Chemotherapy associated with trastuzumab (trastuzumab/ChT) for treatment of advanced gastric cancer with overexpression of HER2 increased overall survival.

Methods: We performed a retrospective analysis of patients with HER2-positive gastric or gastro-oesophageal junction cancer treated with trastuzumab/ChT, in our oncology department.

Results: We included 13 patients, treated between May/2010 and December/2018. Their median age were 59 years (48 to 75) and 76.9% (n=10) were male. All patients presented histologically confirmed gastric cancer, intestinal type in 92.3% (12) and diffuse type in 7.7% (1). Previous ChT treatment was performed in 92.3% (12) patients and 7.7% (1) had anthracycline therapy. Previous radiotherapy was made in 15.4% (2) and gastrectomy in 30.8% (4) patients. Tumors were tested for HER2 status with immunohistochemistry (score 3+) in 76.9% (10) and with fluorescence in-situ hybridization (FISH positive) in 23.1% (3).

In the beginning of trastuzumab/ChT, 84.6% (11) patients presented metastatic disease, with at least one, two or three metastatic sites, respectively in 46.2%, 23.1% or 30.8%. Liver, lymph node and lung metastasis were the most common sites. The median number of trastuzumab cycles until the best disease response was 6.2 (3 to 10). The best disease response was partial response in 38.5% (n=5), stable disease in 23.1% (3), complete response in 7.7% (1) and progressive disease in 7.7% patients. The median number of trastuzumab cycles was 8.5 (3 to 16). Trastuzumab/ChT was stopped due to progressive disease in 69.2% (9) patients, and intolerable toxicity in 7.7% (cisplatin neurotoxicity). Median time to progression was 6.1 months (4.7 to 19.8). Second-line therapy was given to all patients.

Eastern Cooperative Oncology Group/Performance status was 0-1 in the beginning and end of treatment. Grade 3-4 toxicities were observed in 69.2% patients, with neutropenia in 53.8% (7).

Median overall survival was 16.8 months (11.1 to 22.5; 95% CI) and median progression free survival was 6.0 months (5.2 to 6.7, 95% CI).

Conclusion: Despite the low number of patients, our analysis didn't show significant differences compared to the literature.

POSTER SESSION 14: New treatment strategies

P14.1

Investigation of Profile-Related Evidence Determining Individualized Cancer Therapy (I-PREDICT): Combinatorial Precision Cancer Therapies

Jason Sicklick[1], Shumei Kato[1], Maria Schwaederle[1], Ryosuke Okamura[1], Michael Hahn[1], Casey Williams[2], Pradip De[2], Amy Krie[2], David Piccioni[1], Heidi McKean[2], Vincent Miller[3], Jeff Ross[3], Adam Benson[3], Jennifer Webster[3], Phillip Stephens[3], J. Jack Lee[4], Paul Fanta[1], Brian Leyland-Jones[2], Razelle Kurzrock[1]

[1] University of California San Diego, La Jolla, CA, United States
[2] Avera Cancer Institute Sioux Falls, SD, United States
[3] Foundation Medicine, Inc., Cambridge, MA, United States
[4] MD Anderson Cancer Center, Houston, TX, United States

Background: With the increasing availability of large gene panels and cognate agents, we hypothesized that offering individualized combination therapies to patients with refractory cancers would improve outcomes.
Methods: I-PREDICT (NCT02534675) is a prospective navigation trial (two centers: UC San Diego and Avera Cancer Institute). Comprehensive genomic profiling (CGP, Foundation Medicine; 315 genes), and, if possible, PD-(L)1 immunohistochemistry, tumor mutational burden and circulating tumor DNA were performed. A molecular tumor board discussed results immediately upon receipt, and emphasized use of customized, matched targeted/ immunotherapy combinations. Final decisions were the treating physician's choice.
Results: Overall, 149 patients with advanced cancers were enrolled: 83 (55.7%) have been treated [73 matched (49.0%); 10 unmatched (16.7%)]. Diverse malignancies including gastrointestinal, hepatobiliary, gynecologic, and brain were represented. Median number of prior therapies = 2; median (range) of genomic alterations per patient = 4 (1-20). Each tumor had a unique genomic profile. The median (IQR) number of agents given was 2 (1-5), dosed individually on an N-of-1 basis. There were no drug-related deaths. We classified the patients based upon their treatment regimens' molecular Matching Score [number of genomic alterations matched/total number of characterized DNA alterations (>50%: "high," n=28; ≤50%: "low," n=55 (includes unmatched (score = 0))]. High vs low Matching Scores independently correlated with better outcomes: SD> 6 months/PR/CR, 50% versus 22% (P = 0.03); median PFS (6.5 vs 3.1 months; P=0.0004); median OS (not reached vs 10.2 months; P = 0.04) (all multivariate) (Figure).
Conclusion: The use of customized, multi-drug combinations targeting >50% of identified genomic alterations recommended by timely molecular tumor boards and based on CGP was safe and associated with improvements in all outcome parameters. These findings underscore the feasibility and the importance of designing precision oncology trials that emphasize tailored combinations rather than monotherapies.
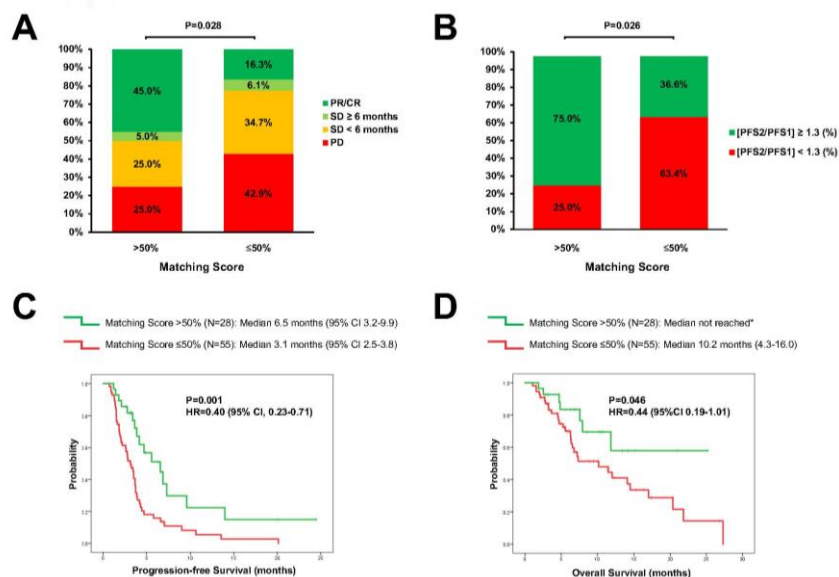
Outcome comparison according to the Matching Score.

POSTER SESSION 15: Patient reported outcomes

P15.1

Analysis of factors leading to non-participation of patients with cancer in immunotherapy clinical trials during the "Washout Period"

Ishwaria Subbiah[1], David Hong[2]

[1] *MD Anderson Cancer Center, Houston, TX, United States*
[2] *The University of Texas MD Anderson Cancer Center, Houston, TX, United States*

Background: Patients with advanced cancers are referred to early phase clinical trials after progression on standard-of-care regimens. However early phase I/II trials tend to have stringent eligibility criteria not only regarding performance status and organ function but also on wash-out periods from prior therapies. In this study, we sought to better characterize the clinical circumstances and outcomes of patients who signed consent to participate in a phase I trial but ultimately did not receive any study drug/therapy on that trial.
Methods: We identified 650 consecutive pts w advanced cancer who consented to participate on a immunotherapy-based phase I trials between 12/2015-12/2017, and collected patient and disease characteristics as well as clinical outcomes, in particular if they in fact did proceed to receive therapy.
Results: Among the 650 pts who initially consented to participate on immunotherapy based phase I trials, 178 (27%) were never treated. Reasons for non-participation in trial were included: 33 (19%) pursued alternate therapies (of whom 13 elected to receive therapy at home), 28 (16%) had an unfavorable result on a pre-trial screening test, 22 (12%) had systemic disease progression, 9 (5%) developed new brain mets. 18 (10%) pts died prior to start of trial, 16 (9%) developed a new lab abnormality (hepatic, renal dysfunction), 14 (8%) had a decline in performance status, 12 (7%) withdrew consent for reasons not provided, 12 (7%) did not have enough biopsy or archival tissue for correlative studies as directed by the protocol, 8 (4%) had no reason documented, 7 (4%) reported other reasons [prohibited concomitant medications, no measureable disease on imaging,]. Six (3%) pts were denied by insurance, to participate on trial & 2 (1%) did not start because that protocol was on an administrative hold.
Conclusions: Almost a third of patients who signed consent on a trial were unable to start on study, for the reasons above. In-depth analysis into these reasons can identify potentially modifiable factors that can streamline the pretrial period to ensure this vulnerable population has maximum access to initiate the study.

Analysis of participation among adolescent and young adults (AYA) with advanced cancers on early phase clinical trials

Ishwaria Subbiah[1], David Hong[2]

[1] *MD Anderson Cancer Center, Houston, TX, United States*
[2] *The University of Texas MD Anderson Cancer Center, Houston, TX, United States*

Background: Patients with cancer aged 15-39 years, the Adolescent and Young Adults (AYA), are significantly underrepresented on late phase clinical trials overall particularly in comparison to their pediatric (age < 15y) and middle age (40-64y) counterparts. With the rapid proliferation of novel anticancer therapies brought to early phase trials, we sought to characterize the participation of AYAs on phase I trials with investigational agents to better understand this unique and vulnerable with advanced cancer.

Methods: We retrospectively identified pts w advanced cancers who received therapy on a phase I trials bw Dec 2004-July 2013. We subdivided pts by age as AYA (15-39y) or middle age (40-64y) and obtained their demographics and clinical characteristics, time to treatment failure (TTF), and RECIST response to therapy per imaging (complete response CR, partial response PR, stable disease SD).

Results: Overall we identified 1489 pts, of whom 991 (67%) were middle age and 220 (15%) were AYAs. The median age in AYA group was 32.6y, with 63% being female, 33% male, and having a median of 3 prior therapies. Most common primary malignancy in the AYA subgroup was gyn (41, 19%), sarcoma (39, 18%), GI (35, 16%), thoracic/H/N (31, 14%), melanoma (27, 12%), & breast (20, 9%) cancers. Both mid age and AYA pts had a similar TTF (3.5m v 3.3m, respectively). Regarding disease response to therapy per imaging, AYAs had 93 (42%) SD inc 42 (19%) prolonged SD (median duration 9.7m), 34 (15%) PR (median duration 9.5m), and 2 pts with a CR lasting 36.2m and 50.1m. To date, none of these trials have reported on patient reported symptoms and outcomes or quality of life metrics.

Conclusions: Adolescent and young adults remain underrepresented on phase I clinical trials with the trials providing little to no information on the needs unique to the AYA population such as psychosocial, family, educational and economic needs. Thorough characterization of barriers and addressing these barriers in the clinical trial design will be crucial to improving trial participation in this vulnerable group.

P16.1

Application of 3D-Tumor spheroids in drug discovery

Mariami Kaviladze

Sechenov Medical University, Moscow, Russia

At present, various models are used in experimental oncology, including spontaneous, transplanted, and induced tumors of animals, human tumors transplanted to animals, various cultures of human and animal tumor cells, as well as molecular genetic models. In these latter days special importance is played to in vitro models based on cell cultures, including multicellular tumor spheroids (MTS) because of the tightening of the requirements for animal experiments. Three-dimensional cultures of tumor cells overcome the limitations associated with such basic characteristics as volume gradients, growth factors, and metabolites and the presence of necrotic, hypoxic, resting, and proliferating cells. The aim was to prove the advantage of the 3D model over the 2D model in order to further integrate the in vitro model of tumor spheroids into the design of anticancer drugs and to use primary tumor cells in drug screening studies for the implementation of personalized cancer treatment.

In the study, multicellular spheroids generated from a suspension of isolated cells of the immortalized adenocarcinoma cell line MCF-7 of human mammary gland were obtained in the serum. Microcapsules with MTS were incubated in 24-well plates with Methotrexate for 48 hours. The control group was presented by the monolayer MCF-7 culture (100,000 cells per well). Quantitative evaluation of the surviving cells was carried out with trypan blue dye in a Fuchs-Rosenthal counting chamber.

The survival rate of viable cells in the control group was 2 times less than in MTS with a Methotrexate concentration of 100 nM. Evaluation of the cytotoxic effect of Methotrexate, based on the size of MTS was also made. When Methotrexate concentration of 100 nM, the number of living cells was 65 and 88% for spheroids with size of 150 and 300 μm, respectively, while in the control group this value was only 35%.

Compared to monolayer cultures, cancer cells in three-dimensional spheroid cultures demonstrate greater resistance to cytotoxic drugs, with the cytotoxic effect of Methotrexate decreasing while MTS size increasing. In this regard, three-dimensional tumor models are a valuable "tool" for cancer research in the context of drug discovery.

Three dimensional organotypic ex vivo culture of resected tumor tissues from patients with lung cancers

Nandini Dey[1], Raed Sulaiman[1], Jennifer Carlson[1], Xiaoqian Lin[1], Megan Quast[1], Yuliang Sun[1], Casey Williams[1], Benjamin Solomon[1], Paul Meyer[1], Molly McAfee[1], Vladimir Lazar[2], Pradip De[1], Brian Leyland-Jones[1]

[1] Avera Cancer Institute,, Sioux Falls, SD, United States
[2] WIN Consortium, Paris, France

Background: Human carcinomas from the same organ-site with similar genomic alterations in different patients may determine their tumorigenic history and their diverse paths of tumorigenic evolution which cause them respond differently to the same anti-tumor drugs and treatment modalities.

Purpose: The uniqueness of genomic profile in tumor and path of tumorigenic evolution in an individual patient makes ex vivo culture an ideal model to test effects of drugs on the individual patient.

Methods: Informed consent was obtained from patients (NCT02470715). The investigation was approved by Avera IRB & the Western IRB. Surgically resected tissues were obtained from patients with lung tumors. Tumor and normal cells from paired specimens at D0 (noncultured) were compared to a cultured specimen of D1-D3 using H&E stain & Ki-67, pS6RP, cl-caspase 3, and CD31 IHC-stains based on criteria for individual protein(s). Anatomic pathological evaluation for the diagnosis of the disease in cultured tissue was carried out based on morphological features of the tumor cells and tumor-adjacent normal tissues.

Results: We report data from 177 independent ex vivo cultures from both tumor (96) and tumor-adjacent normal tissues (81) from 8 patients. The viability of cells was 100% in 96 independent ex vivo cultures over a period of 6-72 hours from tumor-adjacent normal lung tissues, and the viability of tumor cells was 81% out of total 66 independent ex vivo cultures. Ki67, pS6RP, and cl-caspase3 positivity were observed in tumor cells at different time points of the culture with different culture media tested. Ki67 staining was quantified according to standard pathological evaluation criteria. CD31 stains identified tumor endothelial cells in both tumor-adjacent normal tissues and tumor tissues in all ex vivo cultures. The extent of the IHC staining was dependent on the amount of tumor tissue present in the cultured specimen.

Conclusion: We show that tumor-derived tissues survive in 3D Matrigel culture starting up to 72 hours. Our model provides a unique opportunity to evaluate drug response to tumor tissues in comparison with tumor-adjacent normal tissues in an individual patient.

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