50P Transcriptomic analysis of host immune response for precision drug prediction for SARS-CoV-2 infected patients: An evidence-based approach

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Background: SARS-CoV-2 Virus can trigger severe pneumonia and lead to acute respiratory distress syndrome. Data from clinical, in vitro, and in vivo suggest that virus-induced cytokine dysregulation is a contributory factor to the pathogenesis. Drugs targeting the same are being tried.

Methods: To obtain a better understanding of the molecular events, we studied the transcriptome of infected macrophages and obtained a list of incriminated pathways using Gene Set Enrichment Analysis. Co-expression gene analysis was further used to predict drug targets.

Results: Immune system, hemostasis, RNA metabolism, cellular response to external stimuli, vesicle- mediated transport, cell cycle mechanisms, DNA replication, and repair are upregulated. Interferon alpha, beta, and gamma are upregulated. IL-1,6,10, 13, TNF NF-KB are upregulated. Signaling by non-receptor tyrosine kinase, NOTCH, Sonic Hedgehog, lLeptin, MAP kinase-6, 4, and estrogen-mediated signaling are increased. Olfactory sensation, transmission across chemical synapses, and sperm motility appear to be downregulated. Disease host signature has resemblance with cystic fibrosis, thrombophilia, leishmania, influenza, CMV, HIV and SARS infection, and diseases of programmed cell death like neurodegenerative diseases. We have predicted 24 precise drugs which can be explored in clinical trials of SARS-CoV 2 treatment.

Conclusions: Our study provides important mechanistic insights into the understanding of SARS-CoV-2 viral pathogenesis and the multi-faceted host immune responses.

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51P A novel crosstalk between pyridoxal 5′-phosphate (PLP)-dependent enzymes, CBS and CSE, modulated by MALAT-1/STAT-3 axis

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Background: Aberrant expression of the pyridoxal 5′-phosphate (PLP)-dependent enzymes: cystathionine- β-synthase (CBS) and cystathionine-γ-lyase (CSE), is generally observed in oncological contexts. Our group has recently recognized MALAT-1 as a pioneer lncRNA that modulates STAT-3-regulated hydrogen sulfide (H_2S) production via CSE in breast cancer (BC), thereby nominating MALAT-1/STAT-3/CSE as a novel pathway that regulates H_2S levels. Additionally, we elucidated the importance of dual suppression of MALAT-1 and CSE in BC to by-pass the compensatory feedback loop employed by CSE to restore H_2S levels. The tightly regulated, and highly resistant protective mechanism of CSE has prompted us to bioinformatically explore potential non-coding RNAs (ncRNAs) that can directly and effectively target both H_2S synthesizing enzymes.

Methods: Twenty-five BC patients were recruited. MDA-MB-231 cells were cultured and transiently transfected with CBS siRNAs and miR-30-5p oligonucleotides. Total RNA was extracted, reverse transcribed and quantified by qRT-PCR.

Results: CBS was upregulated in BC tissues compared to its normal counterparts. In MDA-MB-231 cells, knockdown of CBS resulted in a marked repression of MALAT-1 and STAT-3 levels as opposed to CSE which was still noticeably elevated. To overcome the protective mechanism of CSE, in-silico analysis was performed to identify a ncRNA that can successfully bind to and repress both CBS and CSE. MiR-30-5p was found to simultaneously target both enzymes; this was validated as the forced ectopic expression of miR-30-5p led to a distinct reduction in both CBS and CSE transcript levels in TNBC cells.

Conclusions: This study validates the compensatory mechanism applied by CSE and showcases its resilience against repression attempts to consistently maintain H_2S levels in the cells. Moreover, it categorizes miR-30-5p as an efficient dual repressor of H_2S-synthesizing enzymes, CBS and CSE, thereby paving the way towards promising therapeutic approaches in aggressive BC phenotypes.

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52P MiRNA profile associated with the invasiveness in non-functioning pituitary adenomas

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Background: Non-functioning pituitary adenomas (NFPAs) account for nearly 35% of all pituitary tumours but since no hormonal hypersecretion has been observed, the diagnosis of NFPAs remains difficult. Although the NFPAs are considered benign, in a proportion of patients, tumour recurrence and invasion have been observed. So far, no biomarkers for NFPAs monitoring are available. Differentially expressed microRNAs (miRs) have been shown biomarker potential in many solid tumours. In the present study, miRs expression profiles were analysed in patients with invasive (with or without recurrence) and non-invasive NFPAs.

Methods: MiRs profiles were analysed in 12 patients with non-invasive and 8 patients with invasive NFPAs using miRCURY LNA miRNA PCR® (Qiagen). ROC curve analysis evaluated the diagnostic ability of the selected miRNAs. $P<0.05$ was assumed for statistically significant and was calculated using un-paired, 2-tailed Student's t-test.

Results: Four miRs were found differentially expressed related to invasion and recurrence of NFPAs. MiR-106a, miR-17, miR-20 were up-regulated in patients with recurrent invasive NFPAs, with an AUR$>$ 0.9 (95% CI = 0.839-1), while miR-210 was down-regulated in invasive NFPAs compared to patients with non-invasive NFPAs.

Conclusions: The ability to predict tumour invasion and recurrence after the initial surgery will decrease morbidity and mortality rate in patients with NFPAs. The selected profile of miR-106a, miR-17, miR-20 and miR-210 showed biomarker potential and could be used for future miRNA – based targeted therapies.

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53P Expression of sodium-dependent phosphate transporter NaPi2b is downregulated in malignant ovarian tumors after neoadjuvant chemotherapy

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Background: The membrane protein NaPi2b (SLC34A2 gene) is overexpressed in ovarian cancer and other malignancies including thyroid, lung, breast, and others. Currently, NaPi2b-specific therapeutic monoclonal antibodies XMT1536 and XMT-1592 are successfully undergoing clinical trials for the treatment of ovarian and non-small cell lung cancers, demonstrating safety and clinical efficacy. These humanized auristatin F (AF-HPA) conjugated antibodies are created on the dolaflexin and dolasynthen technology platforms respectively. We aimed to evaluate NaPi2b as a target for antibody therapy and molecular marker for diagnostics and predicting the course and outcome of ovarian cancer disease.

Methods: The analysis of SLC34A2 gene expression in 48 ovarian tumors was performed using real-time PCR, droplet digital PCR, and western blot analysis. Statistical analysis was performed taking into account various clinicopathological characteristics of the ovarian cancer patients, including the stage of the disease, the tumor grade, the presence of ascites and the applying of neoadjuvant chemotherapy which was predominantly carried out according to the TCb regimen (carboplatin and paclitaxel).

Results: It was shown that expression of the NaPi2b transporter is downregulated in tumors of patients who received neoadjuvant chemotherapy. This fact allowed us to suggest that ovarian cancer patients after neoadjuvant therapy may be not sensitive to targeted drugs directed against the NaPi2b transporter due to the loss of its expression. We found no relationships in the expression level of the NaPi2b transporter with the survival rate of ovarian cancer patients, as well as with tumor grade, and presence of ascites. The NaPi2b showed also a tendency to be downregulated at late stage of disease most likely due to low degree of differentiation of tumor cells.

Conclusions: Thus, the NaPi2b protein abundance is lower in tumor ovarian cells of patients who had received neoadjuvant therapy. This study suggests that the level of expression of the sodium-dependent phosphate NaPi2b transporter gene can serve as a potential marker for the monitoring and predicting responses to neoadjuvant and targeted therapy in patients with ovarian cancer.

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54P Identification of patient-specific T-cell neoantigens through HLA-agnostic genetic screens

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Background: Accumulating evidence suggests that the efficacy of cancer immunotherapies, such as immune checkpoint blockade, is to a large extent driven by T-cells that recognize cancer neoantigens — T-cell antigens that arise as a result of patient-specific nonsynonymous tumor mutations. Based on these data, a major effort has been initiated to develop approaches that can be used to specifically boost the activity of neoantigen-reactive CD8+ and CD4+ T-cells in individual patients. However, tumor mutations and their associated neoantigens are, with few exceptions, unique to individual cancer patients, underscoring the need for technologies that enable the comprehensive discovery of both CD4+ and CD8+ T-cell-recognized neoantigens in a truly personalized fashion.

Methods: We present HANSolo (HLA-Agnostic Neoantigen Screening), a high-throughput genetic platform for the personalized identification of CD4+ and CD8+ T-cell-recognized (neo)antigens. In this method, patient-matched, Bcl-6/xL-immortalized B cell lines are engineered to express large libraries of minigenes that encode candidate T-cell antigens. As the resulting B cells are fully MHC class I and class II proficient, this enables the unbiased screening of T-cell specificities across the complete MHC class I and class II genotypes of individual patients. To this purpose, antigen library-expressing B cells are co-incubated with patient T-cells, and antigen hits are identified by next-generation sequencing to measure the depletion of those B cells that express T-cell-recognized epitopes.

Results: We benchmark the feasibility and sensitivity of our genetic screening method, and illustrate the potential of our approach by profiling the neoantigen-specificities of patient-derived T-cell populations in a setting in which neoantigen-specific T-cells represent only a minor fraction of the T-cell population.

Conclusions: Collectively, these data demonstrate the feasibility of personalized and HLA-agnostic discovery of $CD4^+$ and $CD8^+$ T-cell neoantigens from large genetic libraries. Thus, this technology should facilitate the development of personalized neoantigen-based cancer immunotherapies, such as neoantigen vaccines or neoantigen TCR gene therapies.

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55P Clinical landscape of LAG-3-targeted therapy

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Background: Lymphocyte-activating gene-3 (LAG-3) is a cell surface inhibitory receptor with multiple biological activities over T cell activation and effector functions. LAG-3 is the third inhibitory receptor to be exploited in human anti-cancer immunotherapies. LAG-3 is considered a next-generation target in cancer immunotherapy, right next to PD-1 and CTLA-4.

Methods: Several LAG-3 blockade immunotherapeutic models are being pursued at various stages of clinical and pre-clinical development. An extensive bibliographic research was performed using Pubmed and Clinicaltrials.gov databases to identify preclinical and clinical trials conducted up to date involving LAG-3 as a target. Here we summarize the current understanding of LAG-3 clinical applications.

Results: LAG-3 was first used in clinical trials in 2006 as a LAG-3-Ig soluble fusion protein, but as an immune stimulator. Nowadays, several LAG-3-antagonistic immunotherapeutic models are being evaluated at various stages of clinical and pre-clinical development. In addition, combinations blocking LAG-3 together with other immune checkpoints are also being characterized. A new generation of bispecific PD-1/LAG-3 blocking agents have shown strong capacities to specifically target PD-1+ LAG-3+ highly dysfunctional T cells and enhance their proliferation and effector activities.

Conclusions: LAG-3 is a key regulator of immune homeostasis and a highly important next-generation immune checkpoint. Anti-LAG-3 antibodies and combinations are being evaluated at preclinical and clinical levels. Indeed, the co-blockade of LAG-3 with PD-1 is showing encouraging results. A deeper understanding of the basic mechanisms underlying LAG-3 intracellular signaling will provide insight for further development of novel strategies for cancer targeted treatment.

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56P Continuation treatment with immunotherapy beyond two years in patients with metastasic non-small cell lung cancer: Retrospective analysis of optimal duration treatment

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Background: Immunotherapy (IO) with antiPD1/PD-L1 antibodies is a standard treatment for advanced non-small cell lung cancer (NSCLC), with higher benefits with PDL1+. However, optimal duration of treatment remains unknown. In some pivotal trials, IO was given until disease progression or toxicity, and others limited treatment to two years. This study aimed to assess if continuation therapy with antiPD1/PDL1 therapies in advanced NSCLC past two years is superior to shorter treatment.

Methods: Retrospective study of patients treated with antiPD1/PDL-1 therapies for advanced NSCLC in a single institution. Two groups were performed: patients treated past two years (group1); patients who stopped treatment after two years or before due to toxicity (group2). To evaluate survival, Cox regression analysis and Kaplan Meier curves with log-rank were performed.

Results: 27 patients fulfilled inclusion criteria: 7 patients (group1) and 20 patients (group2). Toxicities by which immunotherapy was suspended were pneumonitis (n=6), cutaneous (n=4), gastrointestinal (n=3), hypophysis (n=2), hepatitis and keratitis (n=1). Patients' characteristics: average age was 69, 96% were smokers or former smoker and 81.5% adenocarcinomas; 12 patients received immunotherapy as first and 13 as second-line. 3 complete response, 13 partial responses and 11 stable disease were achieved. Overall survival (OS) was 21.61 months (Group1) vs 11.78 months (Group 2), p=0.19. Progression-free survival (PFS) was 15.23 months (Group1) vs 11.86 months (Group2), p=0.206. Differences in OS were observed when the analysis was performed comparing treatment duration >1 vs ≤1year (OS: 21.96 vs 10.90 months, p=0.029;); not if compared >18 vs 18months (OS: 22.9 vs 11.97 months, p=0.091). Two patients from group 2 were retreated with immunotherapy at disease progression, colitis and pneumonitis reappeared requiring definitive suspension.

Conclusions: In our series, a favorable trend is observed in OS and PFS in patients who were treated beyond two years; differences were statistically significant for patients treated >1 year. Retrospective nature and small sample of patients condition our results and limit their interpretation.

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