GENOMICS

FAMILIAL GASTRIC CANCER: RESULTS OF A MULTIPLE-GENE SEQUENCING PANEL FOR CANCER RISK ASSESSMENT.

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BACKGROUND:
The major gene involved in gastric cancer (GC) predisposition is CDH1, but other genes have recently emerged as possibly predisposing to the disease. The aim of our study was to assess the presence of predisposing mutations in Italian patients with GC family history by analyzing a panel of 94 genes involved in the main cancer syndromes.

METHODS:
We selected 67 patients with GC and 13 patients with lobular breast cancer with family history of GC. Genomic DNA was extracted from blood samples and analyzed by Next-Generation Sequencing, using Illumina Trusight Cancer on MiSeq. Results were analyzed by a customized bioinformatic pipeline.

RESULTS:
In 10 out of 80 patients (12.5%), we identified 10 CDH1 pathogenic mutations: 4 frameshift deletions, 4 nonsense mutations, 1 splicing mutation and 1 gross deletion. In 10 out of 80 patients (12.5%), we found 10 deleterious mutations in unexpected genes, including ATM, BLM, BMPR1A, BRCA1, BRCA2, PALB2, MSH2, PMS2 and PRF1. Four out of 10 mutations were frameshift deletions, 5 nonsense mutations and 1 gross deletion. In 60 out of 80 cases (75.0%) we did not find any clear deleterious mutation. Among all variants identified, the 60 patients showed 271 variants with frequency <1% in 1000Genomes, Esp6500 and Exac03: 93 synonymous variants, 173 missense mutations, 3 in-frame deletions and 2 in-frame insertions. To assess their possible role in cancer development, we evaluated the 156 unique missense variants by using PolyPhen/SIFT tools and 27 were classified as probably damaging by both tools.

CONCLUSIONS:
The majority of the deleterious mutations identified were in genes related to breast cancer or colorectal cancer, while others were in genes involved in susceptibility to multiple cancers, mainly leukemias and lymphomas. Further functional studies will confirm the role in GC development of the other 27 variants classified as probably damaging by bioinformatic tools.

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TARGETED GENOMIC PROFILING OF TRIPLE NEGATIVE BREAST CANCER FROM PATIENTS WITH RESIDUAL DISEASE AFTER NEOADJUVANT CHEMOTHERAPY TO IDENTIFY MARKERS OF THERAPEUTIC RESISTANCE.

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INTRODUCTION:
More than 50% of triple-negative breast cancer (TNBC) patients have residual disease after neoadjuvant chemotherapy (NAC) with associated risk of relapse after surgery. Hence, the identification of predictive alterations represents an unmet clinical requirement. In case of lack of pathological complete response following NAC, mutational analysis of pre- and post-treatment tumor tissues could lead to the identification of markers of acquired resistance to therapy.

METHODS:
Somatic mutations were identified using the Ion AmpliSeqTM Comprehensive Cancer Panel on matched tumor and normal samples from 14 TNBC women treated with an anthracycline/taxane-based NAC and surgery. The cohort included 3 responders (R) and 11 non-responders (NR). For NR patients both pre- and post-NAC tumor tissues were analyzed. Association between somatic mutations and clinical features were analyzed both at the gene and pathway level.

RESULTS:
We observed a very heterogeneous pattern of somatic mutations across all samples with TP53 mutated in 11 patients followed by a long tail of mutations occurring at low frequency. We did not find any significant association between the mutational load and NAC. There were no single gene mutations associated to NAC response but different mutations converging to immune-related pathways were more frequent in NR patients. Mutational load was significantly lower in post-NAC than in pre-NAC lesions ($p=0.01$). Clustering analysis showed that for 7 patients, mutational profiles of pre- and post-NAC tumors were very similar while for 3 patients they were divergent with few or no shared mutations. Among post-NAC samples TP53 and PIK3CA were the most frequently mutated genes.

CONCLUSIONS:
TNBC has a heterogeneous mutational spectrum. No somatic mutations associated to NAC response were found. Pathway-level analysis showed that NR patients carrying mutations in specific targetable pathways may potentially benefit from alternative therapies. Identification of mutations occurring in post-treatment lesions could identify actionable therapeutic targets in resistant tumors.

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WHOLE-EXOME SEQUENCING (WES) IN DESMOPLASTIC SMALL ROUND CELL TUMOR (DSRCT) A RARE, AGGRESSIVE, POORLY RESPONSIVE DISEASE.

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BACKGROUND:
DSRCT is a rare, ominous, poorly responsive, small round cell sarcoma mainly affecting young men and bears the EWS-WT1 translocation. Being the disease locally disseminated at time of diagnosis, the patients are medically treated (standard treatment) before surgery. We interrogate a small series of DSRCT by WES.

METHODS:
Six primary DSRCTs, surgically removed after combined chemotherapy between 2000 and 2016, were collected along with the clinical data, including follow-up data. The WES analysis was made using FFPE material obtained by dissection from ten 7 µm methylene blue-stained sections of non-necrotic tissue, representative of tumoral areas, paired by the corresponding adjacent normal tissue. DNA libraries were prepared according to the SureSelectXT2 protocol. Captured libraries were then sequenced on an Illumina NextSeq500.

RESULTS:
We identified genetic variants in 135 genes. No overlapping in individually mutated gene was observed, pointing to a high heterogeneity across the cases. Protein coding mutated genes (n=65) segregated into three main functional categories involving DNA damage response (DDR) network, MET/EMT, Cancer Testis Antigens (CTA). DDR network genes were the most abundant functional category (n=22), being mutated in all six patients. They included the core proteins ATR and TP53 as well as RNA binding proteins (RBPs) including splicing factors, epigenetic regulators, and other genes related to RNA machinery. In addition a high number of intronic mutations were recorded.

CONCLUSIONS:
Our analysis indicates that RNA processing factors, like RBPs and other DDR network genes are frequently mutated in DSRCT and may play a role in driving pathogenesis. Owing to the characteristics of our case material, we cannot, however, rule out a treatment effect on the mutational status. Further studies on untreated patients are needed to address this critical point. Finally, the results demonstrate the feasibility of WES profiling on FFPE material.

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(IDEA) TOWARDS CLINICAL DEPLOYMENT OF RNA-SEQ ANALYSIS FOR MOLECULAR STRATIFICATION OF BREAST CANCER.

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Over the last fifteen years, transcriptional analysis of breast cancer has led to the development of a variety of prognostic molecular tests based on gene expression. Recent studies have shown that these multigene assays may indicate low risk of recurrence, and spare adjuvant chemotherapy, in approximately 25% of patients with ER+ HER2- early-stage breast carcinoma. The most widely used tests are four: (i) MammaPrint (Agendia) measuring 70 genes by microarray; (ii) EndoPredict (Sividon), measuring eight genes by RT-qPCR combined with nodal status and tumor size; (iii) Oncotype DX (Genomic Health), measuring 21 genes by RT-qPCR; (iv) Prosigna (Nanostring), measuring 58 genes on the Nanostring platform. Notably, these tests are based on different methodologies, measure different genes and have been clinically validated in different patient cohorts. We conceived a project based on the principle that global expression profiling of breast cancer by RNA-seq could yield quantitative measurements for all expressed genes, and thus should allow reconstructing each of the above classifiers in the same sample, at a reasonable - and progressively decreasing - cost. At the same time, it could enable new class discovery efforts, to further improve breast cancer stratification in the future. In this view, we have generated RNA preparations from eight breast cancers, each divided in three portions: flash-frozen, fixed at room temperature and fixed at 4°C (which partially preserves RNA integrity). The 24 RNA samples are being profiled by seven different RNA-seq protocols from Illumina, Lexogen, Thermo and Diagenode. Classification by clinical tests will be performed for comparison. The approach providing the best cost-effectiveness in terms of consistency between frozen and fixed material, and with the clinical tests, will be selected for large-scale profiling of those breast cancer samples from the ACC network that are already classified by one of the above tests as a reference.

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SKIN MICROENVIRONMENT ENHANCES PROLIFERATION INDEX AND ACTIVATES MTORC 1 PATHWAY REVEALING NOVEL POTENTIAL THERAPEUTIC TARGETS FOR SEZARY SYNDROME TREATMENT.

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Introduction Sézary Syndrome (SS) is a rare and aggressive variant of Cutaneous T Cell Lymphoma characterized by the presence of malignant lymphocytes named Sezary (SS) cells in the skin, lymph nodes, and peripheral blood. To study the role of skin in SS pathogenesis we compared the proliferation index (PI) and activation levels of PI3k/AKT pathway of matched skin and blood derived SS cells. Using our previous SNP array data we also verified the genomic status of members of this pathway in a large cohort of SS patients. Methods Expression of Ki67 proliferation marker was evaluated in perfect-paired blood and skin-paraffin biopsies obtained from eleven SS patients by flow cytometry and immunohistochemistry, respectively. Phosphorylation levels of members of PI3k/AKT pathway were compared between matched circulating and skin resident SS cells proteins derived from 2 SS patients using an AKT kinase array. Affymetrix SNP6.0 arrays was used to investigate the copy number (CN) status of members of mTORC1 pathway in a cohort in 64 SS samples derived from 43 SS patients and 3 cell lines. Results Skin derived SS cells showed a significant higher PI respect to circulating SS cells (12%±11 vs 1,24%+1,18; P=0,00025) as well an enhanced phosphorylation of members of PI3K/AKT/mTOR cascade as PRAS40 (FC=2,4), mTOR (FC=2,1), BAD (FC=3,5) and PDK1 (FC=1,8). SNP6 array revealed CN variations of multiple genes leading toward an increased oncogenic activity of this pathway. In particular, we found CN loss of PTEN in 17 of 43 (40%), of STK11 in 8 of 21 (38%) and PDCD4 in 15 of 43 (35%) and CN gain of P70S6K in 13 of 43 (30%) samples studied. Conclusion: Our data demonstrate that skin microenvironment enhances SS cell proliferation index and activates PI3K/AKT/mTOR, an unbalanced pathway revealing novel therapeutic targets for SS treatment.

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INTEGRATED BIOINFORMATIC APPROACHES TO DEFINE IMMUNO SUBTYPES OF TUMORS: BEYOND IMMUNOCHECKPOINT INHIBITORS.

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Recent progress in cancer immunology and the development of ImmunoCheckpoint Inhibitors (ICI) have been truly remarkable. The relation between mutational burden, immune infiltration, tumor neoantigens and responsiveness to ICI has been showed in many studies. However, each of these parameters alone is not able to summarize the immune activity against tumor and there is an urgency to consider them as a whole. Next generation sequencing data, the development of bioinformatics tools and their integration into pipelines could allow to gain insight of the immune microenvironment of tumors in relation to its genomic background. It could be possible to reach the aim of defining the "immune-subtypes" of tumors. The comprehensive computational study of the tumor-immune cell interactions in tumors using existing data could allow to perfom massively analyses. In detail, immune infiltration, mutational load, T-cell receptor repertoire, HLA type, umor neoantigens and their affinity to MHC will be investigated. Peptide-based cancer vaccine is another attractive approach to evoke anti tumor immune activities, especially those of Cytotoxic T Lymphocytes. Then, the identification of cancer neoantigens could lead to the design of vaccines targeting them, which are recognized as "non-self", leading to less disappointing clinical effects. Many clinical trials on immunotherapy are ongoing and we are collecting samples to perform validation of the bioinformatic results. Moreover, we designed also two custom panels (DNA and RNA), including 45 and 95 genes respectively, involved in the most important immune and inflammation pathways. Our preliminary results on targeted sequencing has revealed the heterogeneity both in expression and in mutational status in genes involved in immune response. In conclusion, the use of existing datasets and the validation of results in "in-house" cohorts, could lower the cost of high-throughput analyses and the timing to reach our aim.

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SNUFFLE: SEMANTIC AND DISTRIBUTED FRAMEWORK FOR THE ANALYSIS AND STORAGE OF ACC DATA.

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IRCCS Casa Sollievo della Sofferenza.

Next-generation sequencing has quickly become a standard procedure for cancer genome studies and clinical investigation. As for preceding initiatives, the need to integrate different data types and to elaborate the huge quantity of data generated by ACC, may require including new centers devoted to data analysis, which work back-to-back with the working groups (WCs) to help researchers delving into their data and mining new knowledge out of them. These will fasten data analytics and co-develop software prototypes. They will guarantee reproducibility and comparability of results by standardizing and sharing all the analytical procedures. This is in fact essential for pan-cancer studies as well. Any produced data will then demand efficient storing and a reasoned query modality. Traditional database management systems (DBMS) proved to run 100 times slower than array-based DBMS on a class of problems, which includes Genomics. These can work in any number of dimensions, unlike the relational DBMS models, and can map perfectly to the need to manage genetic data, which are intrinsically multi-dimensional, in order to make a systemic view of data and infer multi-layer molecular relationships. In this view, we present our analytical platform that combines an ontology-based workflow management system, which drives the composition of semantically correct software pipeline for the analysis of cancer data, to a distributed array-based DBMS for storing and querying data. A higher programmatic interface will be implemented on top of the DBMS to make reasoning on data flexible and extendable, even for other domains. A task will be any executable program, e.g., MuTect, TopHat, FusionCatcher and seqCNA, which will be run in a distributed environment. Our platform will be prototyped and expanded to improve the management, analysis and sharing of ACC data, as well as to help answering relevant questions, peculiar to the working groups' clinical environments.

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IDENTIFICATION OF MECHANISMS INVOLVED IN THE SQUAMOUS-CELL CARCINOMA INITIATION AND PROGRESSION IN AGED SKIN.

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Aging and cancer may be considered related endpoints of accumulating cell damages. The accumulation of senescent cells in aged tissues may be due to synergic contribution of the genomic instability, senescence-associated secretory phenotype (SASP) dysregulation, and decline in the immune system function. It may worsen the senescence response efficacy in tumor suppression and induce a chronic inflammatory status that might increase cancer incidence and progression. Thus, aging is considered a major risk factor for tumorigenesis. Squamous-cell carcinoma (SCC) is the most common age-associated malignancy. UV radiation is the major etiologic factor as damages DNA, suppresses cutaneous antitumor immunity and inhibits DNA repair. SCC has metastatic potential and may originate from an early lesion (actinic keratosis or AK) arisen from the field cancerization (FC), a chronically injured skin area in which clinical and subclinical lesions coexist. Most of AKs do not progress into SCC, even if keratinocytes harbor genetic or epigenetic changes. Differential evolution of FC lesions might be due to changes in stroma secretion that fuel the uncontrolled proliferation of these mutated keratinocytes. The presence of multiple subclinical lesions in FC often reduces the efficacy of SCC surgical treatment allowing AK persistence or SCC recurrence. However, chemoprevention and chemical AK treatment seem beneficial from patient outcomes. The objectives of the study are: -to study mechanisms involved in the SCC initiation following the aging of either epidermal or dermal compartments; -to investigate mechanisms of specific chemopreventive agents to counteract SCC initiation; -to identify specific markers predictive of FC molecular changes to provide new knowledge about AK/SCC recurrence and define new strategies in SCC prevention and early diagnosis. Our proposal may have relevant positive socio-economic consequences for the SSN. Indeed, SCC can be considered an occupational disease and an increasing economic burden as the proportion of elderly is steadily growing.

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WHOLE-EXOME SEQUENCING IN RADICALLY RESECTED GASTRIC CANCER (GC): ANALYSIS OF PATIENTS (PTS) WITH POOR PROGNOSTIC FACTORS FROM THE ITALIAN TRIAL OF ADJUVANT CHEMOTHERAPY ADENOCARCINOMA (ITACA-S) TRIAL.

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BACKGROUND:
At present, the clinical management of resected GC is based on risk stratification according to the AJCC staging. Aim of this work was to identify, through genomic profiling, novel risk biomarkers in radically resected primary tumors from an homogeneous group of GC patients enrolled in the ITACA-S trial and considered at poor prognosis according to nodal involvement (pT3/4pN3 AJCC 7th edition).

METHODS:
Matched formalin-fixed, paraffin embedded (FFPE) tumor/normal GC specimens collected from 12 patients were subjected to whole-exome sequencing using TruSeq Exome technology and Illumina NextSeq500. Somatic mutations and copy number variations were compared between 7 patients with recurrence (cases) and 5 patients without recurrence (controls) with the same follow-up time (60 months).

RESULTS:
The median number of somatic mutations per patient was 52 (range 6-222) with a total number of 859 mutated genes. Of these, 121 were found mutated in TCGA GC samples and 47 were in Cancer Gene Census (https://cancer.sanger.ac.uk/census). Despite not significant ($p=0.09$), controls had a higher number of mutations (100±70) compared to cases (33±23). They also displayed significant amplifications of 19p13.13, 20q13.33 and 22q11 chromosomal regions and mutations in genes belonging to specific molecular pathways. In particular, genes affecting the unfolded protein response (UPR), an essential adaptive survival pathway, were mutated in 4/5 controls and in none of the 7 cases ($p=0.01$).

CONCLUSIONS:
Our data indicated that, among categories of GC driver mutations, those in p53 and in genes affecting chromatin remodeling and receptor tyrosine kinase pathway are also mutated in our cohort. Although a validation analysis in an independent and bigger cohort of GC patients is required, our results further suggest that deregulation of the UPR pathway might identify patients with lower risk disease and that, as for other cancers, interference with this pathway could provide a novel anticancer strategy.