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# Novel diagnostic/prediction systems and combination immunotherapies for pancreatic cancer.


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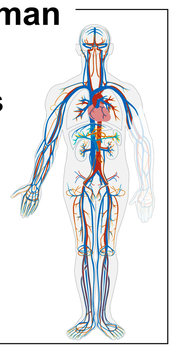
**Keywords**: Peripheral blood, Gene expression profile, Longitudinal study, Immune system, Physiological changes


## Background: Circulatory human system, and specifically blood features, reflect the body physiological and pathological condition

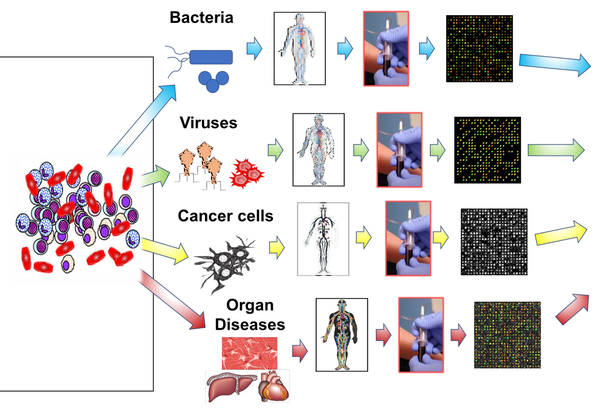
**Circulatory human system**

Blood circulates continuously throughout the body via the peripheral circulatory system



**Blood cells**

In blood there are red blood cells, platelets and leukocytes. White blood cells consist of phenotypically and functionally miscellaneous cells and protect the body from harmful exogenous pathogens as well as endogenous emerging disease such as cancer.

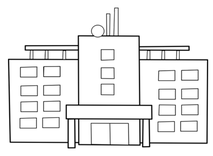



Blood cells will respond to the miscellaneous alterations of the physiological condition of the body; hence the features of the blood will change over-time.

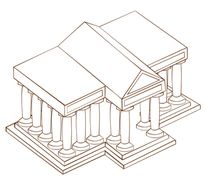
**The objective** is to assess the individual's biological characteristics, the presence of "silent" diseases that do not result in detectable clinical signs/symptoms and identifying treatment response prediction markers.

## Method Study ❶: Eight-year longitudinal study of whole blood gene expression profiles in individuals undergoing long-term medical follow-up

Participants were enrolled in the study in 2008, and every 4 years, from 2008 to 2016, regularly visited the Public Central Hospital of Matto Ishikawa for medical check-ups.



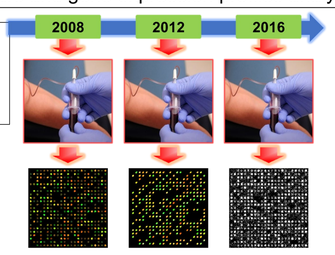
**Matto - Ishikawa Public Central Hospital**



**Kanazawa University**

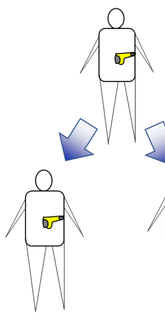
Whole blood was collected from the same individuals every 4 years and gene expression profiles analyzed.

**Average age of participants at registration: 50 years**



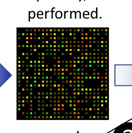

## Method Study ❷: Bioinformatics methods for the identification of treatment response prediction immunomarkers in pancreatic cancer

**Patient with pancreatic ductal adenocarcinoma**

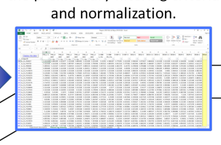


Blood sample withdrawn **before Gemcitabine (GEM) chemotherapy**

mRNA amplified and labeled, cRNA hybridized on a Chip Array, readings performed.

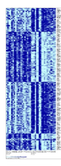


The software BRB-ArrayTools used for gene expression analysis. **20356 genes** passed the preliminary filtering criteria and normalization.



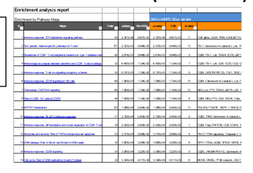
**Differentially expressed genes were identified between two stratified groups ($P < 0.05$; SD vs PD)**

❶

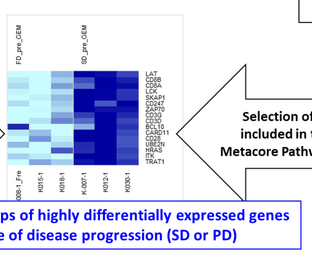


**Metacore analysis of upregulated 1489 genes in the group of SD versus group PD**

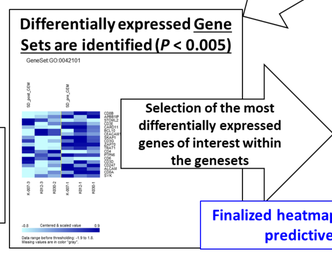
**Metacore Pathway maps enrichments results ($P < 0.00054$)**



**Selection of genes included in the top Metacore Pathways Maps**



**Selection of the most differentially expressed genes of interest within the genesets**



**Differentially expressed Gene Sets are identified ($P < 0.005$)**

❷

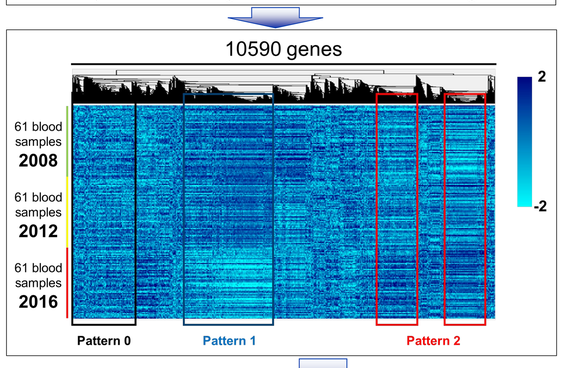
**Finalized heatmaps of highly differentially expressed genes predictive of disease progression (SD or PD)**

**Stable disease (SD):** no significant decrease or increase in the size of target lesions

**Progressive disease (PD);** increase of at least 20% in diameter of lesion or metastasis

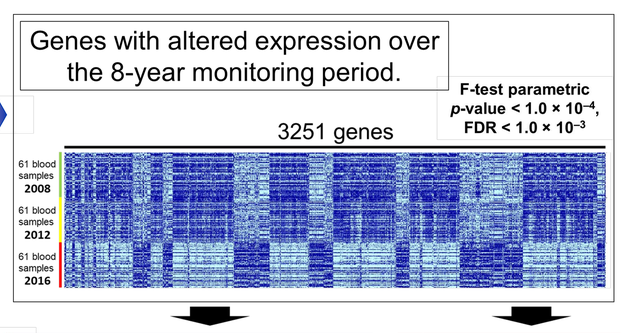
## Results Study ❶: Overall whole blood gene expression profiles of the same 61 participants over the 8-year period

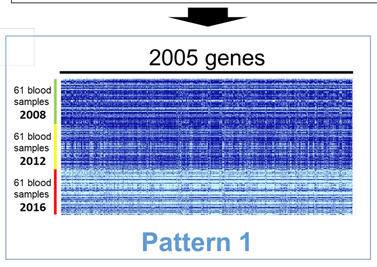
Quality check and gene filtration of 10590 genes

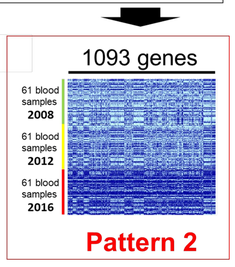



Genes with altered expression over the 8-year monitoring period.

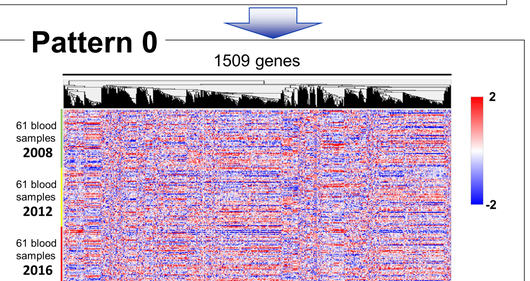
**F-test parametric $p$-value < $1.0 \times 10^{-4}$, FDR < $1.0 \times 10^{-3}$**




**Pattern 0**



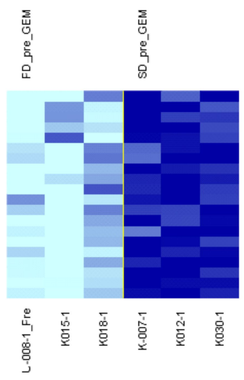

1,509 genes did not change over time, and they were defined as the most representative stably expressed genes

**F-test parametric $p$-value > 0.2**

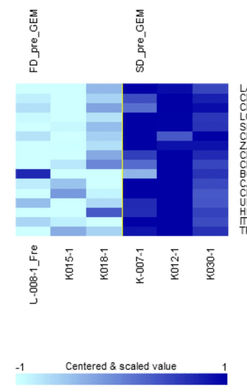

Sakai Y, Nasti A, Takeshita Y, et al. Eight-year longitudinal study of whole blood gene expression profiles in individuals undergoing long-term medical follow-up. Scientific Reports. 2021 Aug 16;11(1):1-0. (CC BY).


## Results Study ❷: Highly differentially expressed genes organized for biological processes predictive of disease progression

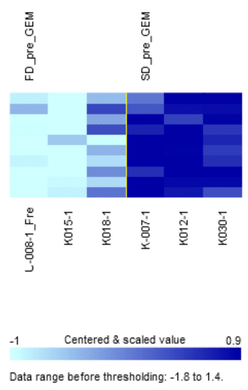
**T cell immunosurveillance**



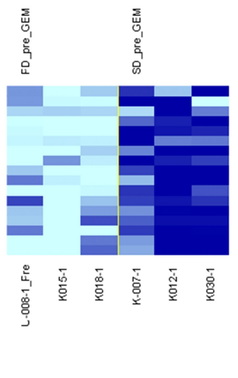

**TCR alpha beta signaling pathway**




**Chemotaxis: CXCR3-A signaling**




**Cytokines/chemokines and receptors**

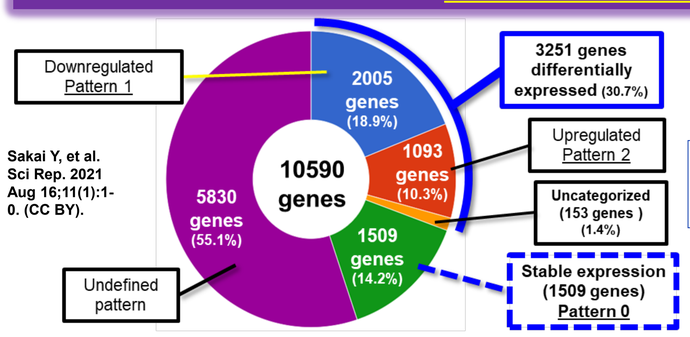




酒井佳夫, 金子周一, 宮澤正樹, ナスティアレッサンドロ, 関晃裕. 膵癌・胆道系癌の化学療法剤の奏効性を血液遺伝子発現によって予測する奏効性予測マーカー、及び奏効性予測キット [Patent in Japanese] [Markers and kits for predicting the response to chemotherapeutic agents for pancreatic and biliary tract cancers by blood gene expression]. JP 2021-126107, 2021.


Key biological processes and associated genes which are significantly different between the Stable disease group and Progressive disease group, the identified genes are related to the prediction of anti-tumor immune response.

## Conclusion Study ❶




Sakai Y, et al. Sci Rep. 2021 Aug 16;11(1):1-0. (CC BY).

**Blood gene expression analysis**

**3251 genes whose expression levels were changed over the 8 years:**

Genes involved to development, signal transduction, cell cycle, apoptosis, survival, chemotaxis, as well as immune response pathways.

**1509 genes showed stable expression levels over the 8 years:**

Genes related to immune system pathways, including antigen cell presentation and interferon-related signaling.

**The physiological and structural homeostatic characteristics of living organisms change over time, while the central immune system, antigen presentation and related immune cells are preserved.**

## Conclusion Study ❷

**The mRNAs genes levels are differentially expressed among pancreatic cancer patients, this variation of expression levels was successfully quantified. In future, prediction kit for pancreatic cancer will be established to predict the responsiveness or unresponsiveness to chemotherapy.**