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Metabolic reprogramming and altered isotope fractionation of cancerous cells of neural origin in the light of advanced atomic level mass spectrometry

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The author declare no conflict of interest

THE CANCER PUZZLES

- **microscopic studies** made the level of recognition and allow to classify cancer
  10^{-4}
- **molecular techniques** drilled down and made possible to summarize cancer hallmarks
  10^{-8}
- **INTERDISCIPLINARITY** allows to reach previously unknown an atomic level of recognition of pathological processes
  $10^{-10}/10^{-12}$

The puzzles Source: internet

Awarded the four Nobel Prizes Mass Spectrometry (MS) is the most advanced analytical method worldwide and it's the most versatile technique is **Isotope Ratio Mass Spectrometry (IRMS)** implemented even into forensics demanded by legal constraints.

IRMS METHOD

The isotope ratios of nitrogen $^{15}N/^{14}N$ and carbon $^{13}C/^{12}C$ were measured in with the use of a Sercon 20–22 Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS) coupled with a Sercon SL Elemental Analyzer for simultaneous carbon-nitrogen-sulfur (NCS) analysis

The ^{15}N and ^{13}C abundance was showed as delta (δ) values (in parts per mil, ‰), relative to international standards for nitrogen (atmospheric, air N2) and carbon (Pee Dee belemnite, V-PDB) according to the formula:

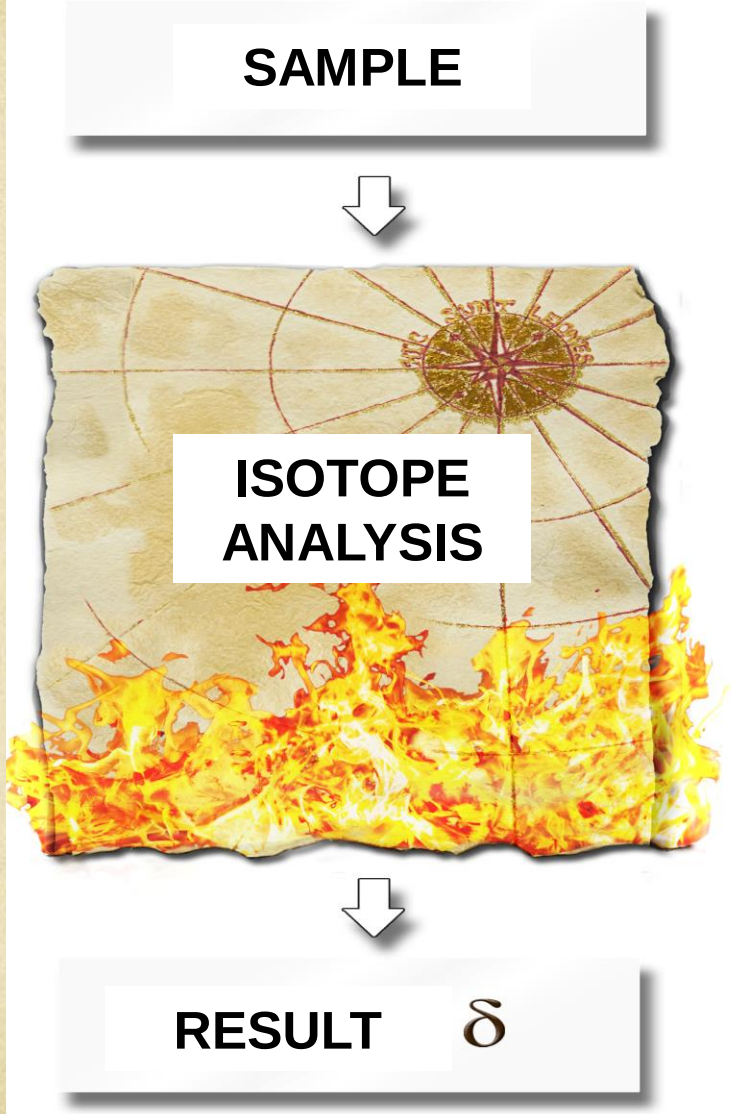
$$\delta(‰) = (R_{sample/Rstandard} - 1) * 1000,$$

where R_{sample} and $R_{standard}$ are heavy/light isotopic ratios for the sample and international standard, respectively.

The studies were approved by The Bioethics Committee of The Medical University of Lodz.

Isotope Ratio Mass Spectrometry
IRMS advanced approach

- ✓ to perform a complex isotopic tumor profiling in neuroblastoma group of tumors (286 Isotopic Ratio Measurements and 143 C/N Mass Ratio Estimations)
- ✓ followed by interdisciplinary analysis, in relation to the standards of contemporary oncology and needs of further coming personalized treatment strategies
- ✓ to reveal for the first time an atomic level of cancer disease and prospective clinical impact of isotopic measurements



THE MOST IMPORTANT RESULTS*

1.

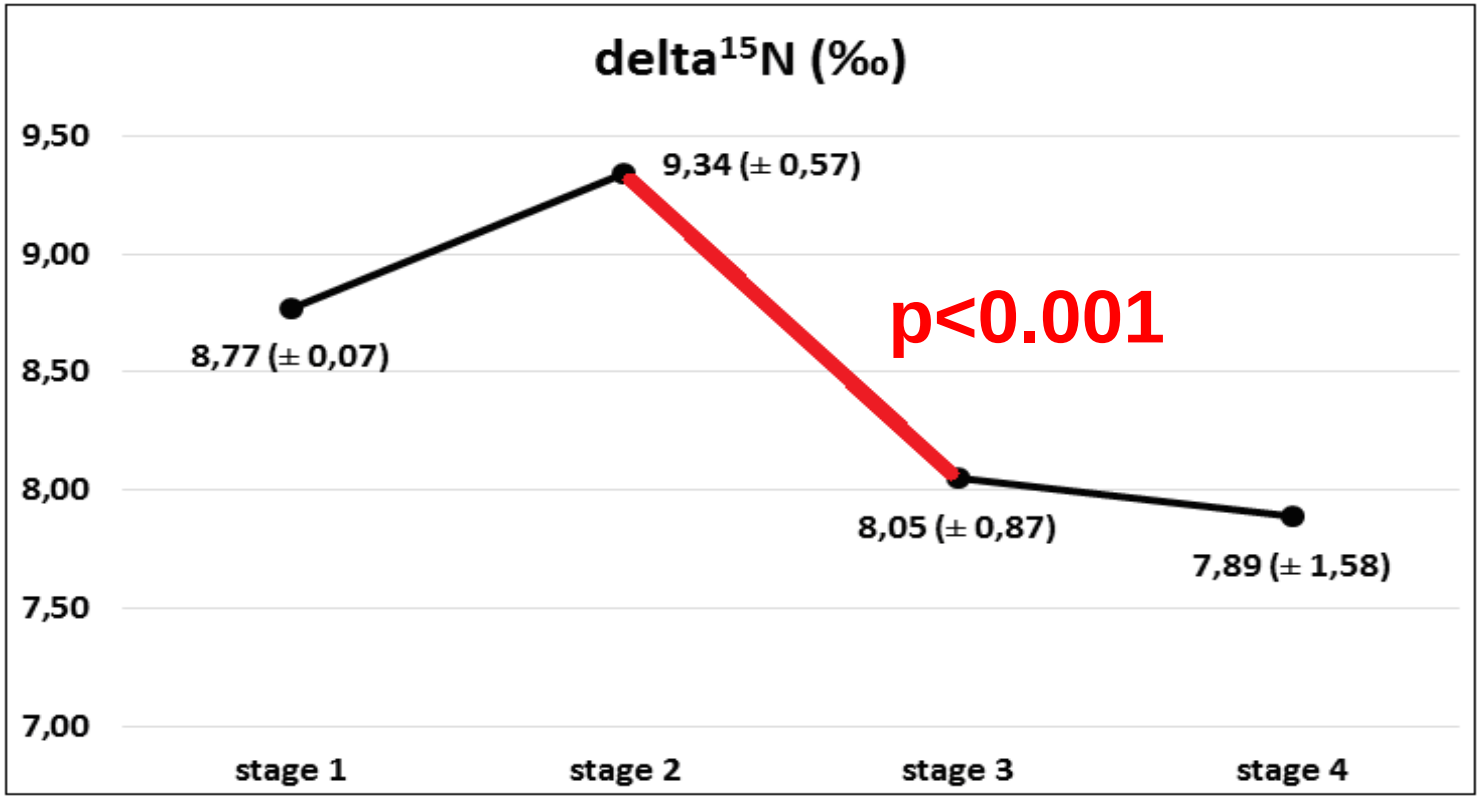
Nitrogen enrichment of tumor tissue, p=0.048 (an increase of heavy nitrogen concentration in cancer tissue, against the natural tendency to use energetically preferable light isotopes)

C/N mass ratio, p=0.006

*Taran, K. Isotope Ratio Mass Spectrometry (IRMS) as a new tool in evaluating pathomechanisms of neoplastic disease with regard to isotope map of children's organisms in Lodz region (in Polish). ***Academic thesis of habilitation, Medical University of Lodz***, 2015.

THE MOST IMPORTANT RESULTS cont.

2a. Isotopic composition of cancer tissue is not stable at the course of cancer disease

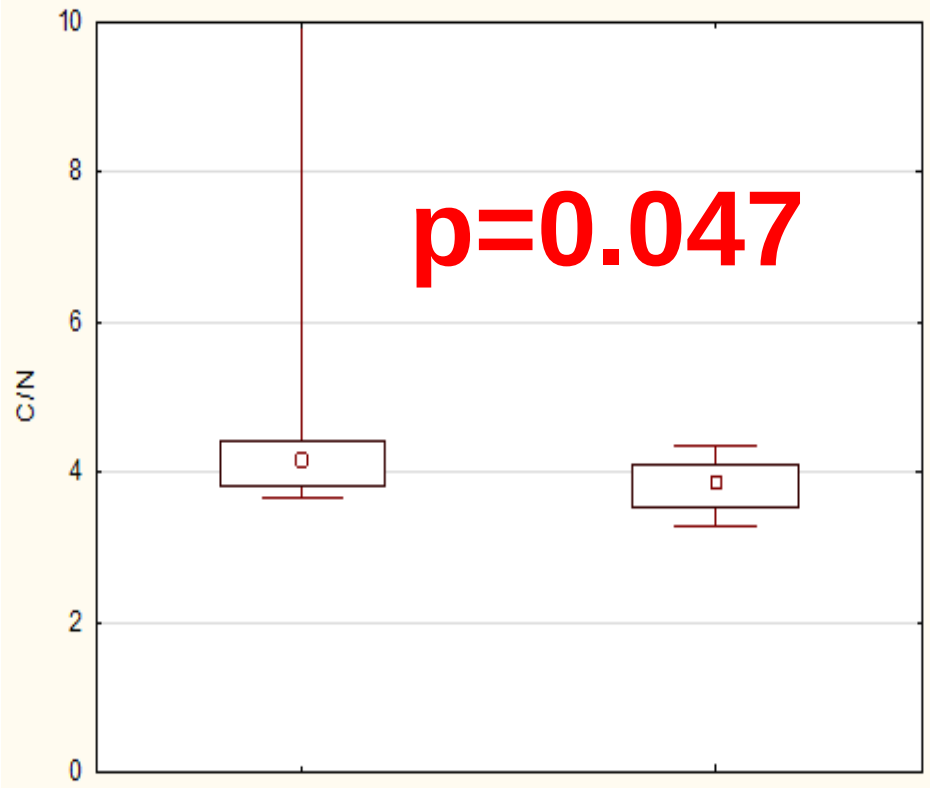


2b. Statistically significant breakthrough (red line) in nitrogen isotope ratio (nitrogen depletion) at the key moment of cancer dissemination, p<0.001

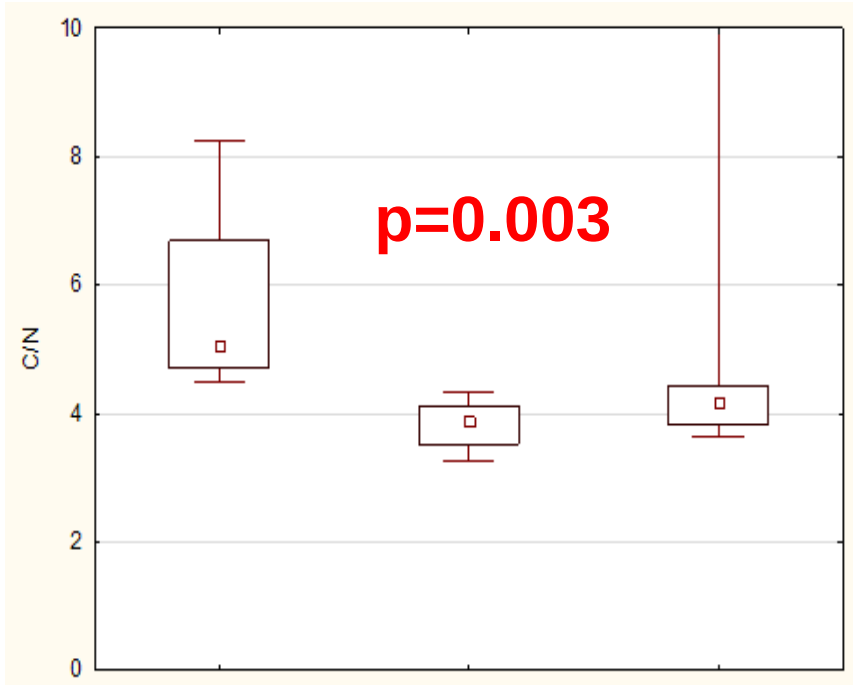
Isotopic profiles of nitrogen $^{15}N/^{14}N$ (delta ^{15}N) in subsequent stages of cancer disease in neuroblastoma group of tumors according to International Neuroblastoma Staging System Committee (INSS, HR-NBL-1.5/SIOPEN Protocol) with statistically significant changes of nitrogen isotopic composition in tumor tissue, p=0.009.

THE MOST IMPORTANT RESULTS cont.

3. Cancer tissue composition reflects prognostic parameters including two the most universal, the stage of cancer disease and histological type of the tumor.



Box plot of C/N mass ratio in neuroblastoma group of tumors, non-mature / malignant tumors (ganglioneuroblastoma and neuroblastoma) – left, mature / benign tumors (ganglioneuroma) – right.



Supported by ANOVA rang (normal core of adrenal gland vs. mature / benign (ganglioneuroma) and non-mature / malignant tumors (ganglioneuroblastoma and neuroblastoma))

TUMOR ENVIRONMENT INTERACTIONS

4. Nitrogen enrichment and carbon depletion of surrounding fibrous and fatty (!) tissue.

THE MAJOR CONCLUSION & HIGHLIGTS

from the studies in neuroblastoma group

The obtained results from interdisciplinary analysis of "in vivo" cancer cells, for the first time:

- ✓ **show the atomic level of metabolic reprogramming of cancer cells**
- ✓ **identify heavy nitrogen enrichment as the new isotopic hallmark of cancer**
- ✓ **alight the structural atomic mechanisms of cancer disease and tumor - environment interaction**
- ✓ **reveal the first measurement to predict cancer dissemination**

UNIVERSALISM OF THE OBTAINED RESULTS

- ✓ **IRMS**

Most of isotope effects associated with biosynthesis and metabolism exhibit different values depending of particular mechanisms of reactions that take place.

The IRMS results pointed amino acids metabolism as the source of the observed changes.

Nitrogen enrichment is known to result from gluconeogenesis in nutrient deficiency states.

- ✓ **PATHOLOGY**

Neuroblastoma group represent the complexity of cancer disease.

Glutamine become the major source of lipogenic acetylo-CoA through reductive carboxylation.

The anaplerotic pathway glutamin metabolism is known to be unregulated in many cancer cells.

The Nitrogen ($^{15}N/^{14}N$) and Carbon ($^{13}C/^{12}C$) Stable Isotopes Prognostic Database (NCSI Prognostic Database)

covers the results of isotope measurements in relation to the established prognostic parameters

PAN CANCER CLINICAL - SCALE ANALYSIS

confirms in pan cancer clinical scale analysis the results from neuroblastoma studies

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THE REVOLVE(R) HEAVY NITROGEN THEORY

shows the two faces of heavy nitrogen in life-or-death duality

THE REVOLVE(R) HEAVY NITROGEN THEORY, when referred to cancer explains persistent proliferation and metastasizing of cancer cells as the result of their specific pattern of isotopic fractionation as well as cancer cells support by tumor-environment interactions with fibrous and fatty tissue on an atomic level.

The method of the atomic level studies of pathological tissues looks beyond traditional meanings of cancer investigation and offers an innovatory concept for cancer identification and targeted personalized management via stable isotope fractionation processes.

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