Nowoczesna diagnostyka w transplantologii

-*Omiki* - narzędzia do monitorowania biorcy przeszczepu (Transplant*omics*)

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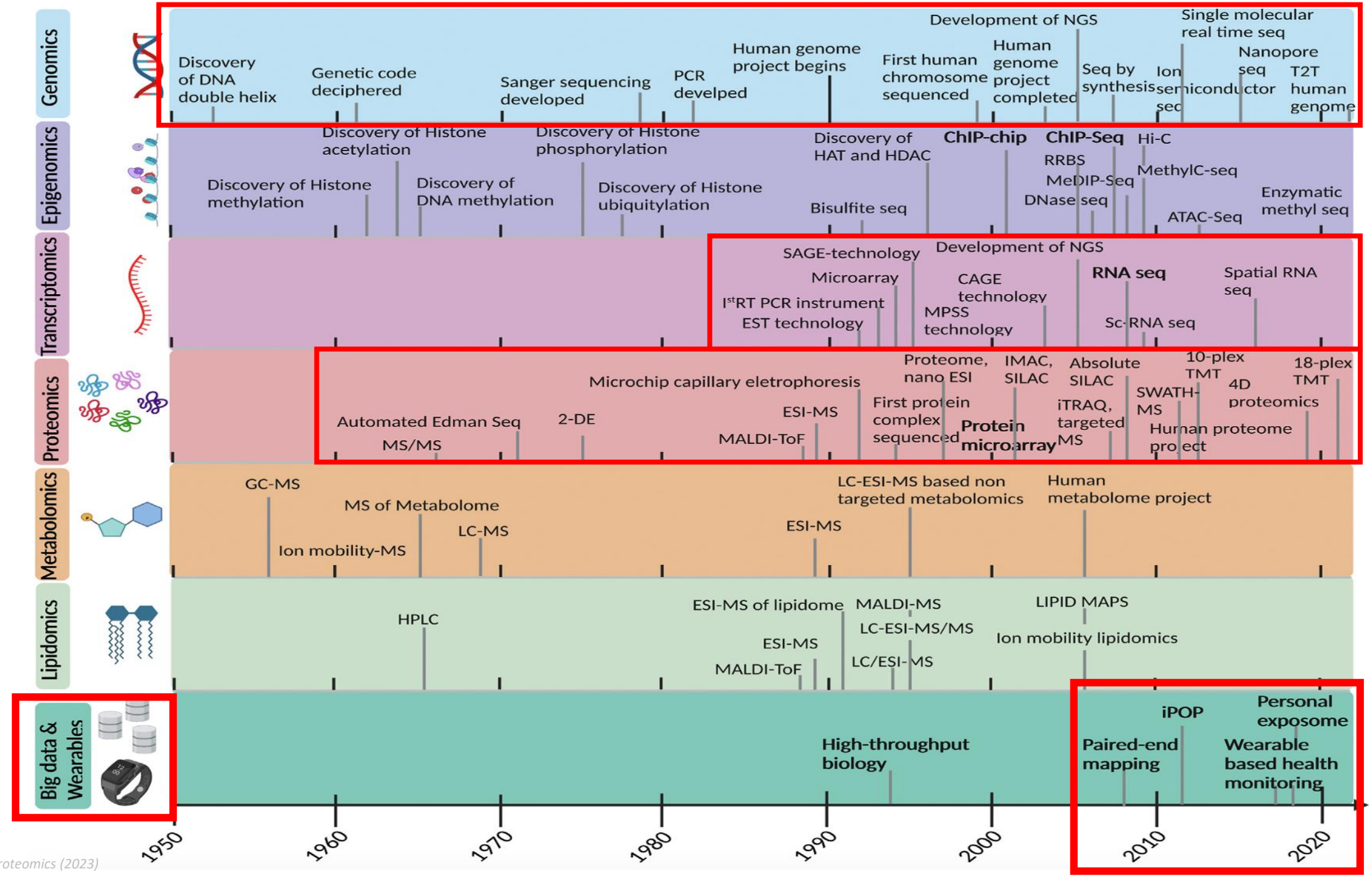
Warszawski Uniwersytet Medyczny

Zajęcia z Transplantologii Klinicznej
WUM – 2025/2026



Mol Cell Proteomics (2023)

‘Omics

Different sequencing technologies, including genomics, epigenomics, transcriptomics, proteomics

GENOMICS

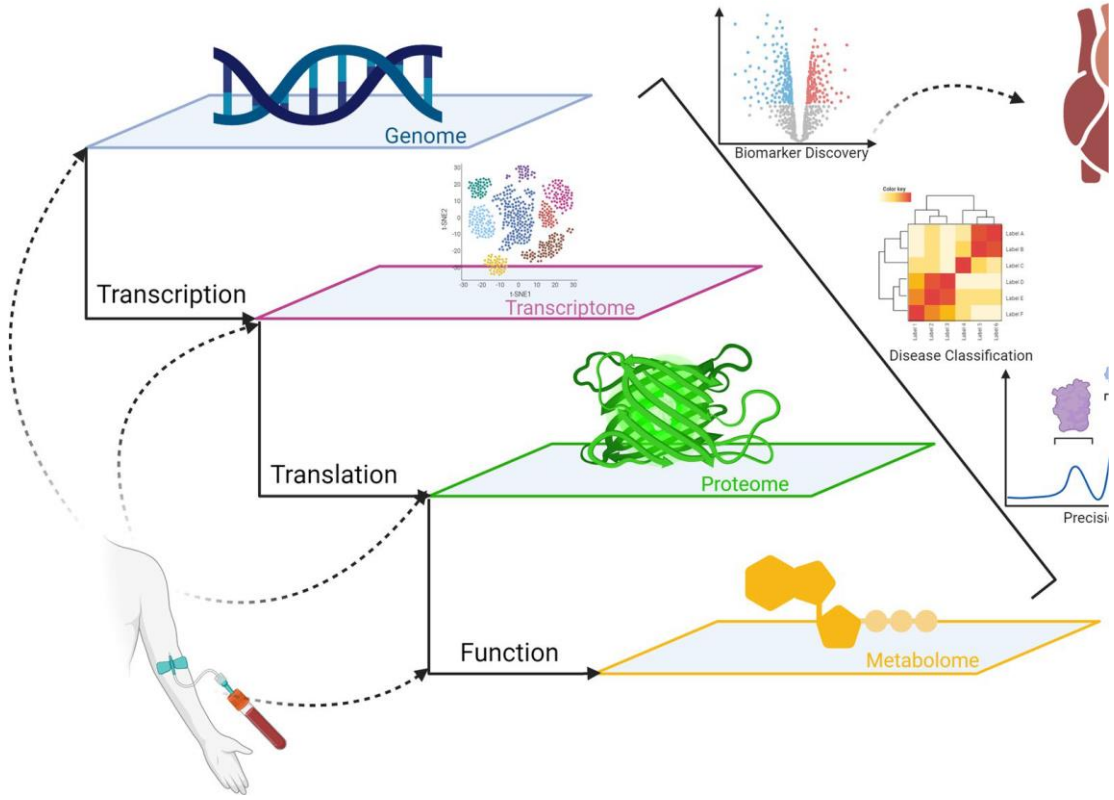
organism’s **complete genetic code**, including all genes, both **coding and noncoding**.

TRANSCRIPTOMICS

all **expressed genes** in the genome.

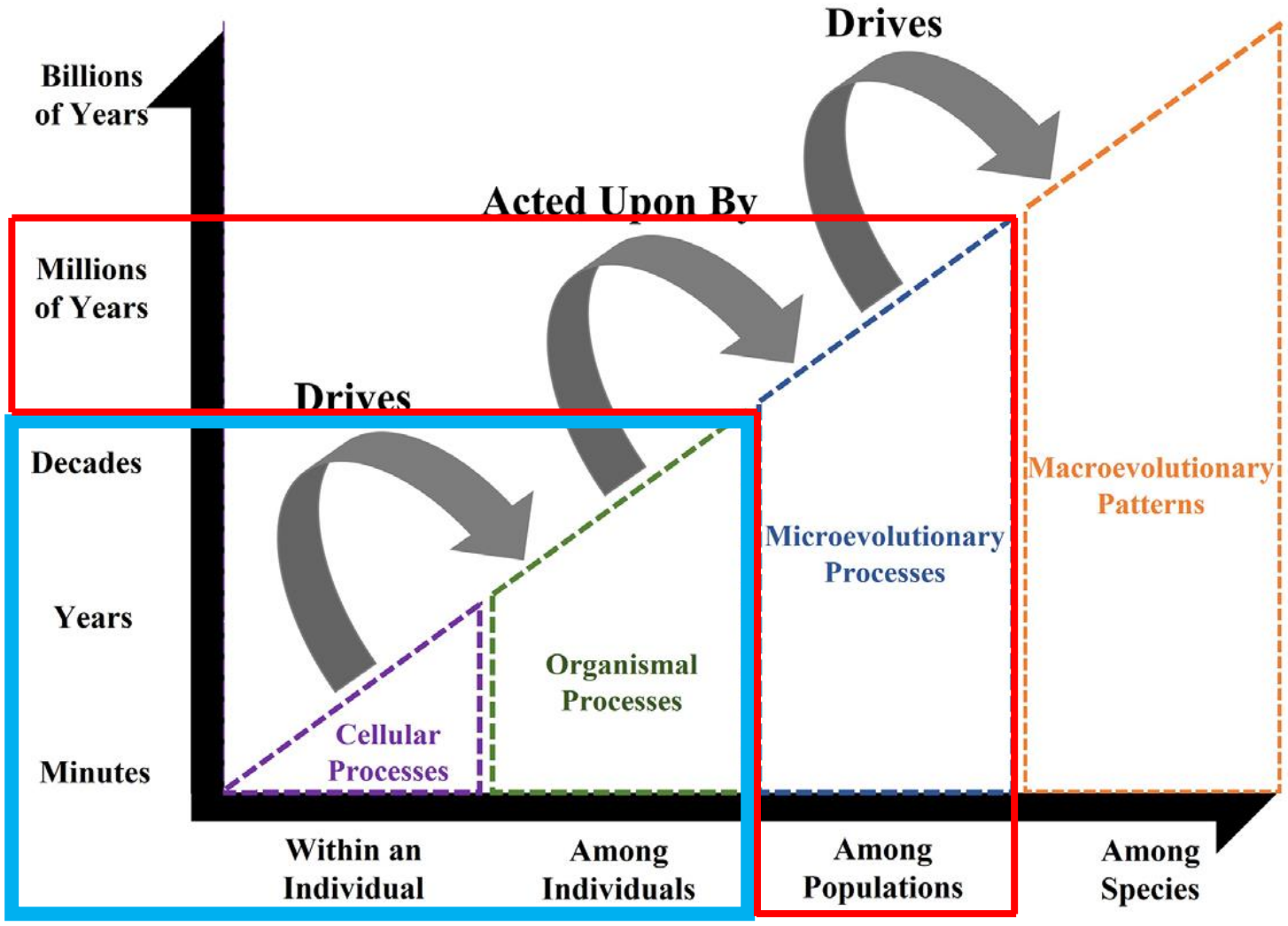
PROTEOMICS

all proteins produced or <u>modified</u>



A. A. Snead and R. D. Clark, Int&Comp Biol, 2022

Czas (od osoby → do gatunku)



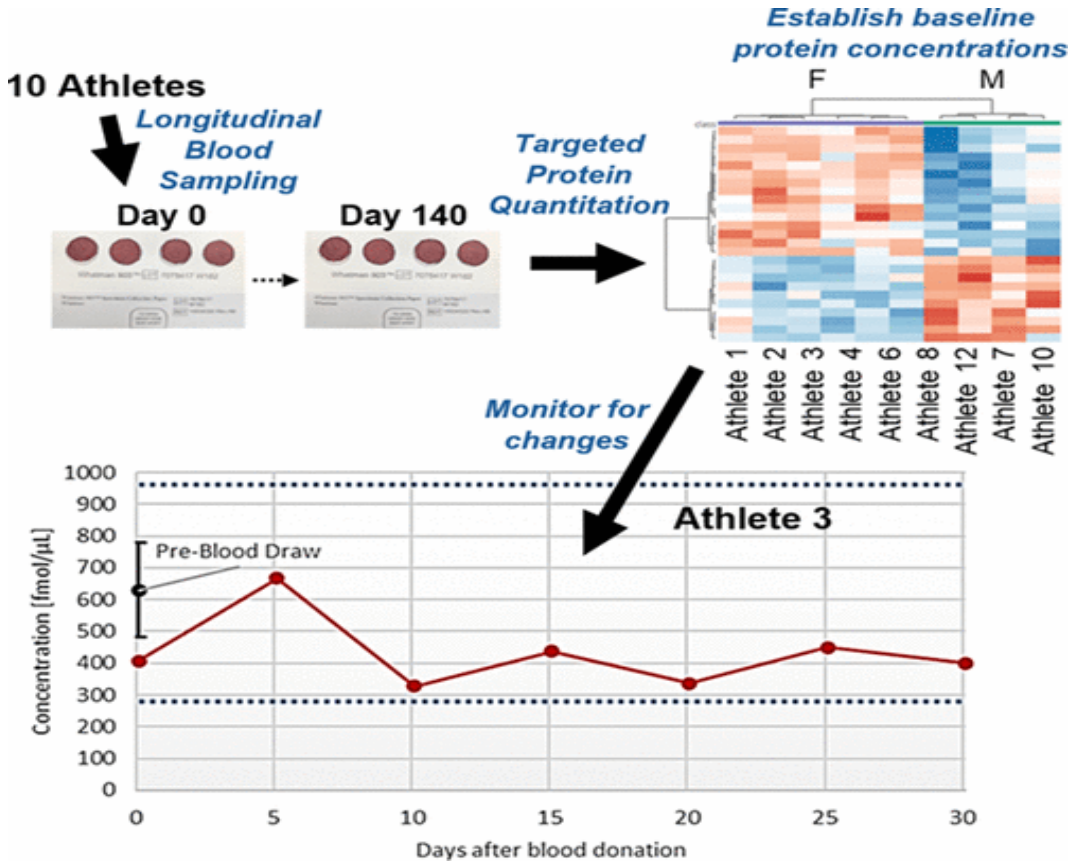
Pacjent po Tx nerki

- 15 - 25 - 35 lat follow-up
- 4 wizyty / rok

A. A. Snead and R. D. Clark, Int&Comp Biol, 2022

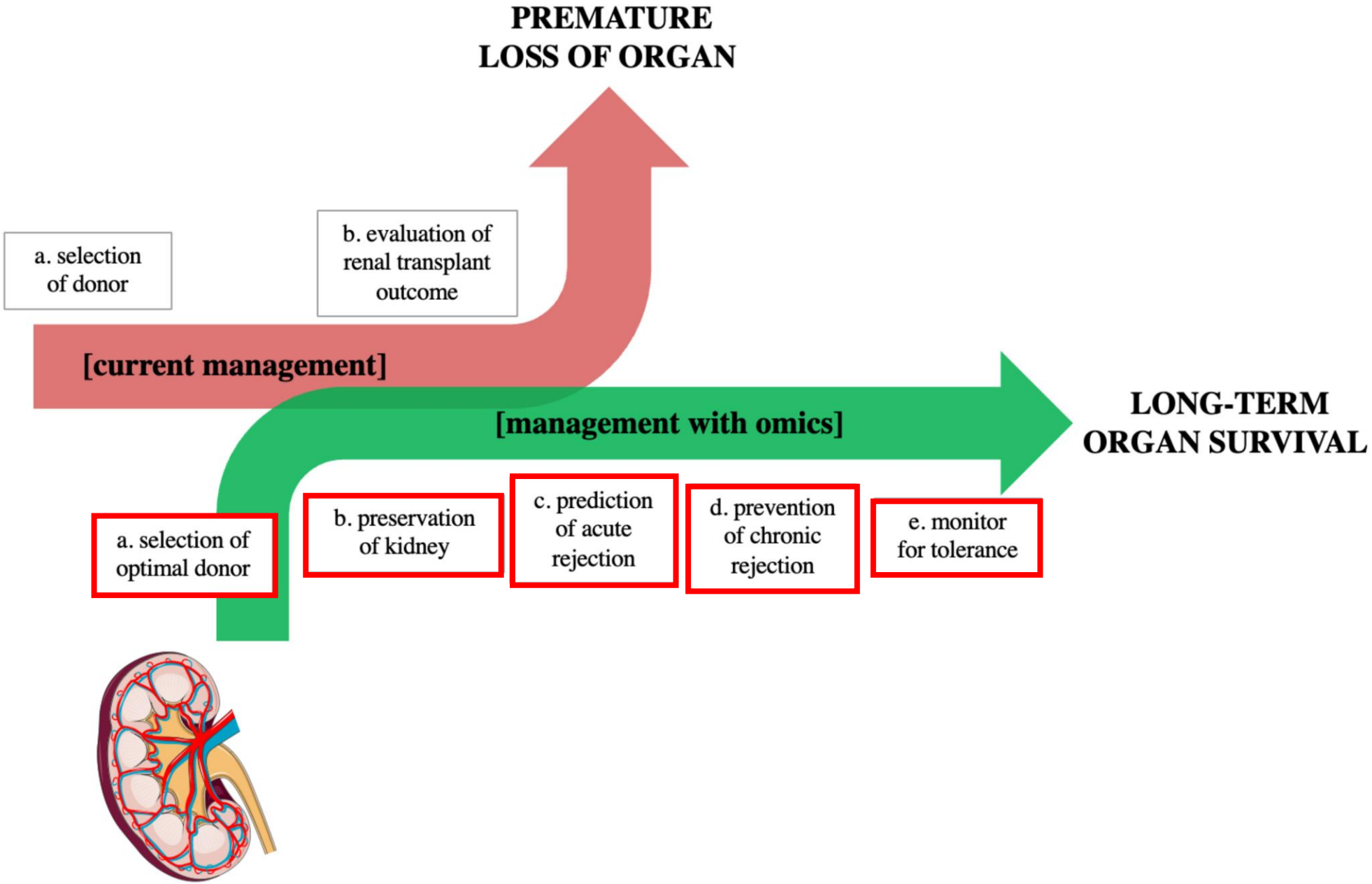
Czas (pobrania i badania)

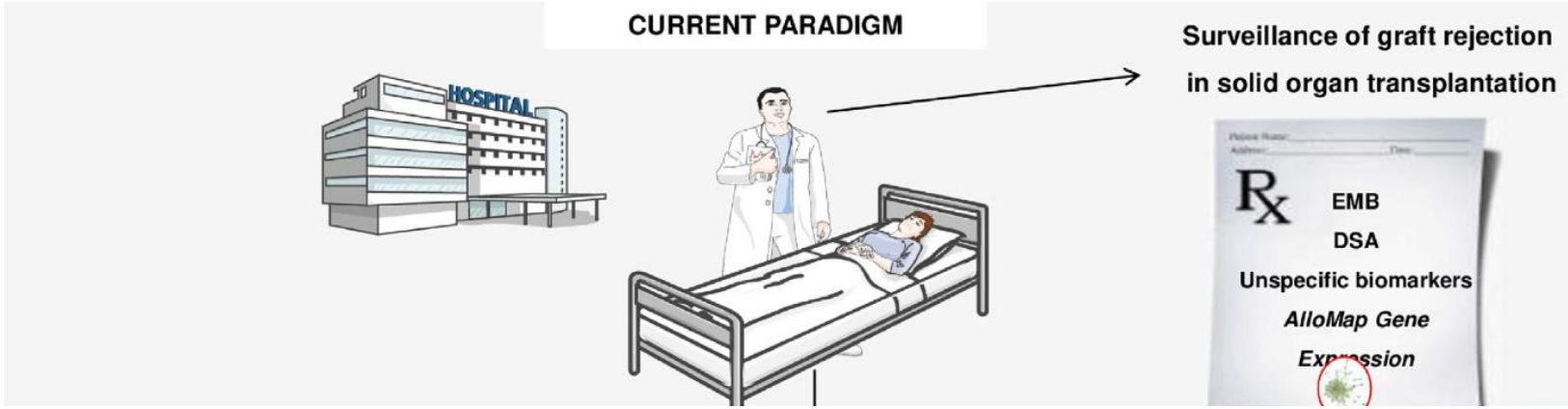
Genomika	**lata**
Transkryptomika	**dni - tygodnie**
Proteomika	**6 miesięcy**
Lipidomika	godziny - dni
Metabolomika	minuty - godziny



Proteome Res. 2024

-*Omiki* w transplantologii

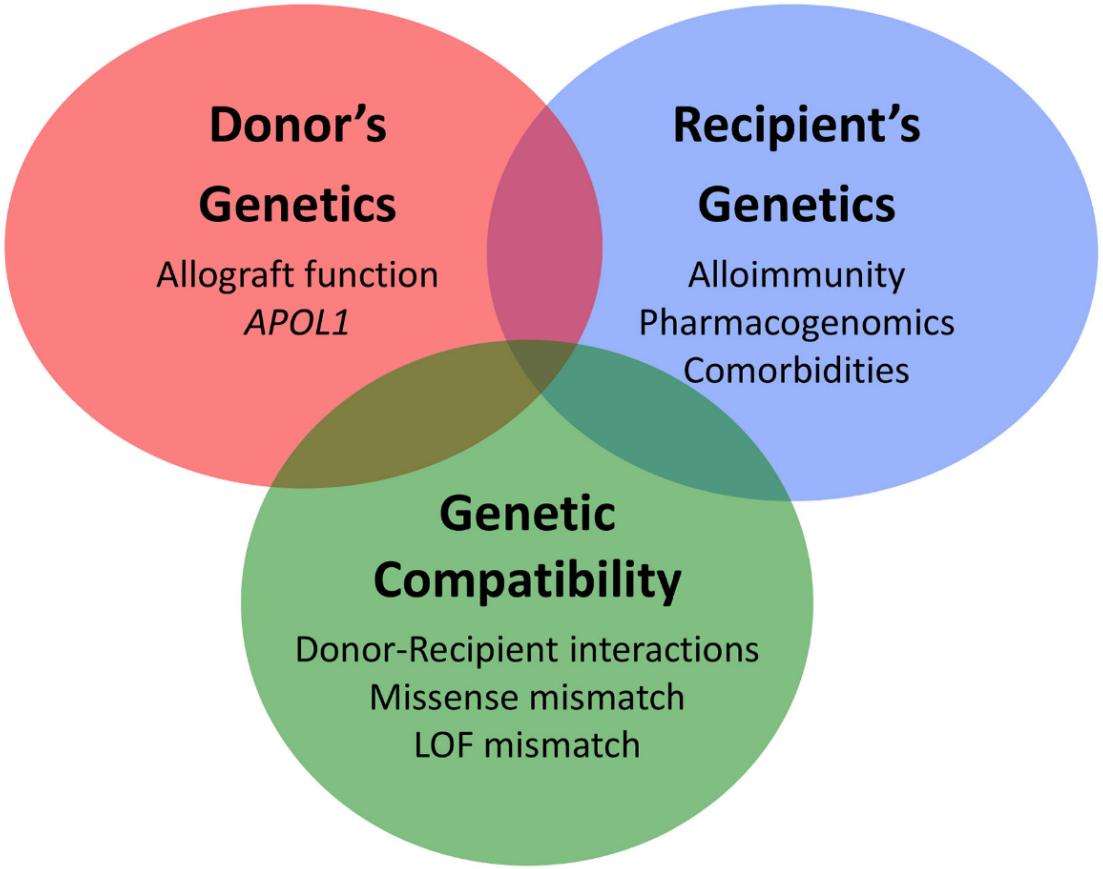




Benincasa G et al.., Human Immunology 2023

Genomika i Transkryptomika

Genetics of Transplant Outcomes



A donor-recipient genetic mismatch

- allosensitization = **REJECTION**

"**Genomic collision"**

genetic mismatch where homozygous gene-disrupting (loss-of-function) variants are present in the recipient but not in the donor, leading to allorecognition of the intact gene product when expressed in the allograft.

UNDERRECOGNIZED CAUSE OF REJECTIONS

Zanoni F, Kiryluk K, Curr Opin Organ Transplant, 2020

Farmakogenomika

Immunosuppressive agent	Gene	Effect on drug level	Other toxicities
CNI	*CYP3A5*3*	↑	
	*CYP3A*1*	↓	
	*POR*28*	↑	
	MDR1	↓	
	ABCBI/MDR1	↓	
MMF	*ABCC2*		Gastrointestinal Intolerance
	IMPDH2		Neutropenia
	HNF1A		Leukopenia
mTORi	*CYP3A5*1*	↓	

Truby LK et al. Current Heart Failure Reports 2023

Transplant*omics* – Tx nerki

Omics	Platform and sample source/size	Clinical outcome	Refs.
Kidney			
Transcriptomics	Microarray of 519 biopsy samples from 491 recipients	AMR and TCMR	[4]
	RNA-seq of whole-blood from 235 recipients	Acute rejection	[5]
	RNA-seq of urine samples from 53 recipients	AMR and TCMR	[6]
	RNA-seq of 37 peripheral blood and tissue biopsy samples	Acute AMR and acute TCMR	[7]
	RNA-seq of PBMCs and microarray of allograft biopsies in 136 rejection patients and 248 controls	AMR	[8]
	scRNA-seq of PBMCs in 2 rejection patients and 2 controls	Chronic AMR	[9]
	scRNA-seq of 3 biopsies of kidney cortex from 1 donor and 2 recipients	Chronic AMR	[10]
	Gene expression signatures from peripheral blood of 307 post-transplant patients	Subclinical acute rejection	[11]
Epigenomics	Whole-genome DNA methylation of 13 biopsies at preischemia, 13 biopsies at postischemia, and 5 biopsies at 3 or 12 months after transplant	Chronic rejection	[12]
	Whole-genome DNA methylation of PBMCs from AR-induced allograft dysfunction, graft-stable cohort, and healthy controls	Acute TCMR	[22]
	Microarray of whole blood from 12 patients undergoing transplantation and at 1 year follow-up	Kidney failure replacement therapy	[14]
	Bisulfite pyrosequencing of peripheral blood samples from 30 recipients	Operational tolerance	[15]
Metabolomics	NMR spectroscopy of 1230 urine samples and matched biopsies from 972 transplant recipients	Acute renal allograft rejection	[16]
	Mass spectrometry of urine samples from 56 recipients grouped as “stable”, under standard minimal immunosuppression, and “spontaneous tolerant”	Operational tolerance	[17]

AR / KTx

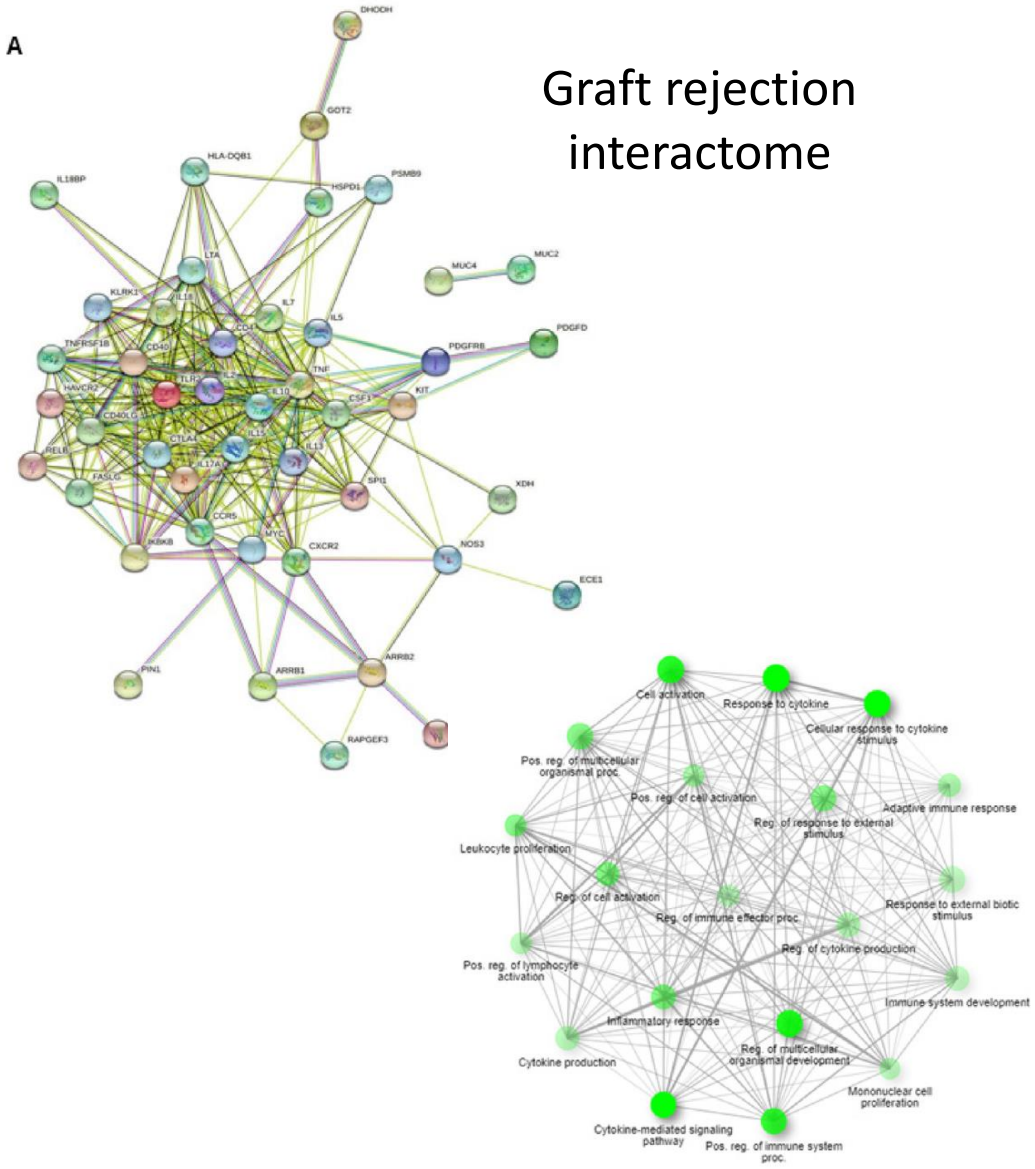
6–11%	**2020**
10–20%	początek 2000’s

Omics	Platform and sample source/size	Clinical outcome	Refs.
Heart, lung, and liver			
Epigenomics	cfDNA fragments carrying hepatocyte-specific methylation patterns from 18 transplant recipients	Graft survival	[13]
Transcriptomics	RNA-seq of heart biopsies from 617 transplant recipients	AMR	[18]
	RNA-seq of heart biopsies from 30 patients undergoing transplantation	AMR	[19]
Metabolomics	Mass spectrometry of 50 donor lung biopsies	Early lung transplant outcomes	[20]
	Nuclear magnetic resonance of 42 liver grafts at the time of ex vivo preparation and 36 native livers	Early allograft dysfunction	[21]

Benincasa G et al.., Human Immunology 2023

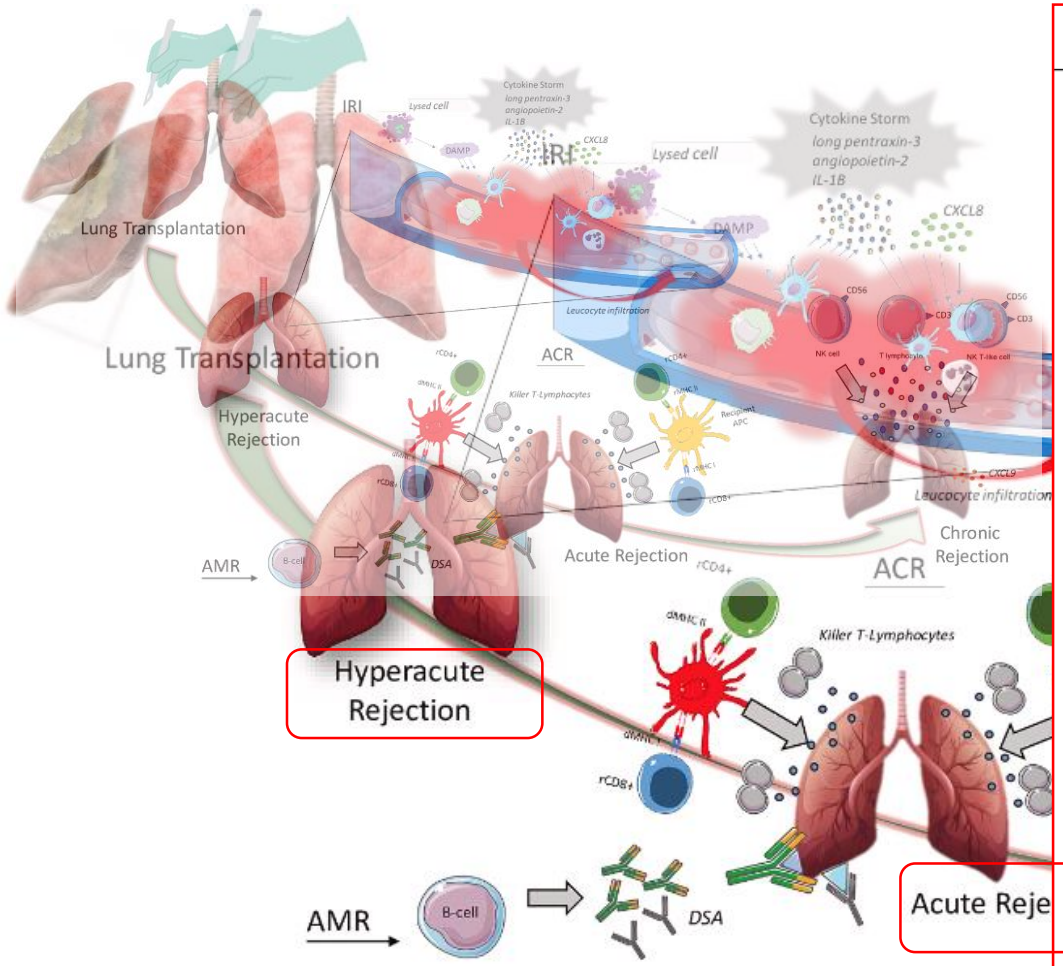
Genomika/Transkryptomika – Tx nerki

Gene	Gene_id	UniProt	Gene_Full_Name
CD40LG	959	P29965	CD40 ligand
PSMB9	5698	P28065	proteasome 20S subunit beta 9
PIN1	5300	Q13526	peptidylprolyl cis/trans isomerase, NIMA-interacting 1
PDGFRB	5159	P09619	platelet derived growth factor receptor beta
NOS3	4846	P29474	nitric oxide synthase 3
MYC	4609	P01106	MYC proto-oncogene, bHLH transcription factor
MUC4	4585	Q99102	mucin 4, cell surface associated
MUC2	4583	Q02817	mucin 2, oligomeric mucus/gel-forming
ARRB2	409	P32121	arrestin beta 2
ARRB1	408	P49407	arrestin beta 1
RELB	5971	Q01201	RELB proto-oncogene, NF-kB subunit
SLC5A7	60,482	Q9GZV3	solute carrier family 5 member 7
SPI1	6688	P17947	Spi-1 proto-oncogene
CD40	958	P25942	CD40 molecule
CD4	920	P01730	CD4 molecule
HAVCR2	84,868	Q8TDQ0	hepatitis A virus cellular receptor 2
PDGFD	80,310	Q9GZP0	platelet derived growth factor D
XDH	7498	P47989	xanthine dehydrogenase
TNFRSF1B	7133	P20333	TNF receptor superfamily member 1B
TNF	7124	P01375	tumor necrosis factor
TLR2	7097	O60603	toll like receptor 2
TBXA2R	6915	P21731	thromboxane A2 receptor
LTA	4049	P01374	lymphotoxin alpha
KIT	3815	P10721	KIT proto-oncogene, receptor tyrosine kinase
IL18	3606	Q14116	interleukin 18
GOT2	2806	P00505	glutamic-oxaloacetic transaminase 2
GFER	2671	P55789	growth factor, augmenter of liver regeneration
KLRK1	22,914	P26718	killer cell lectin like receptor K1
ECE1	1889	P42892	endothelin converting enzyme 1
DHODH	1723	Q02127	dihydroorotate dehydrogenase (quinone)
CTLA4	1493	P16410	cytotoxic *T*-lymphocyte associated protein 4
CSF1	1435	P09603	colony stimulating factor 1
CCR5	1234	P51681	C—C motif chemokine receptor 5 (gene/pseudogene)
RAPGEF3	10,411	O95398	Rap guanine nucleotide exchange factor 3
HLA-DQB1	3119	P01920	major histocompatibility complex, class II, DQ beta 1
HSPD1	3329	P10809	heat shock protein family D (Hsp60) member 1
IKBKB	3551	O14920	inhibitor of nuclear factor kappa B kinase subunit beta
IL17A	3605	Q16552	interleukin 17A
IL15	3600	P40933	interleukin 15
IL13	3596	P35225	interleukin 13
IL10	3586	P22301	interleukin 10
CXCR2	3579	P25025	C-X-C motif chemokine receptor 2
IL7	3574	P13232	interleukin 7
IL5	3567	P05113	interleukin 5
FASLG	356	P48023	Fas ligand
IL2	3558	P60568	interleukin 2
IL18BP	10,068	O95998	interleukin 18 binding protein



Benincasa G et al.., Human Immunology 2023

Transplant*omics* – Tx płuca

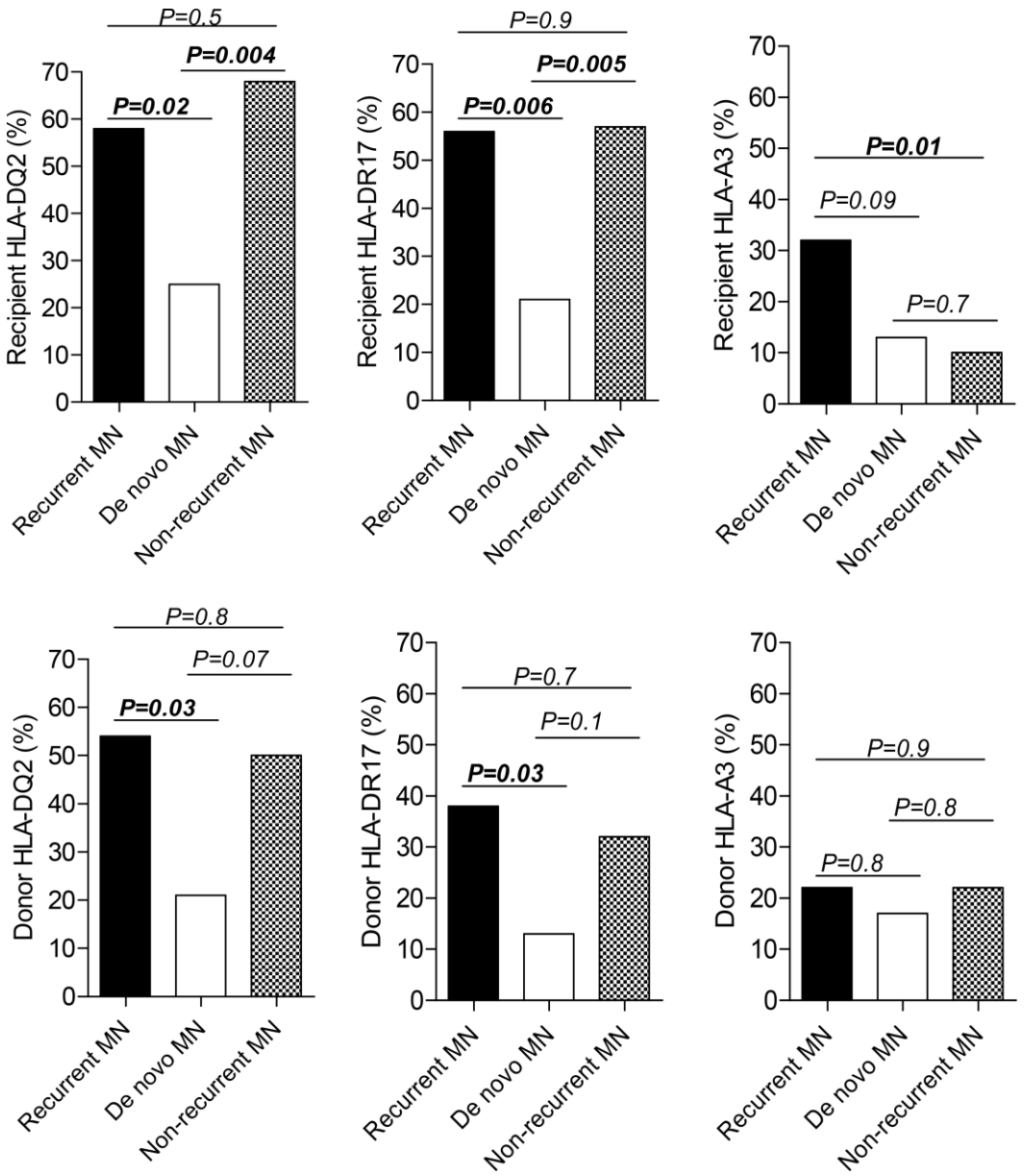


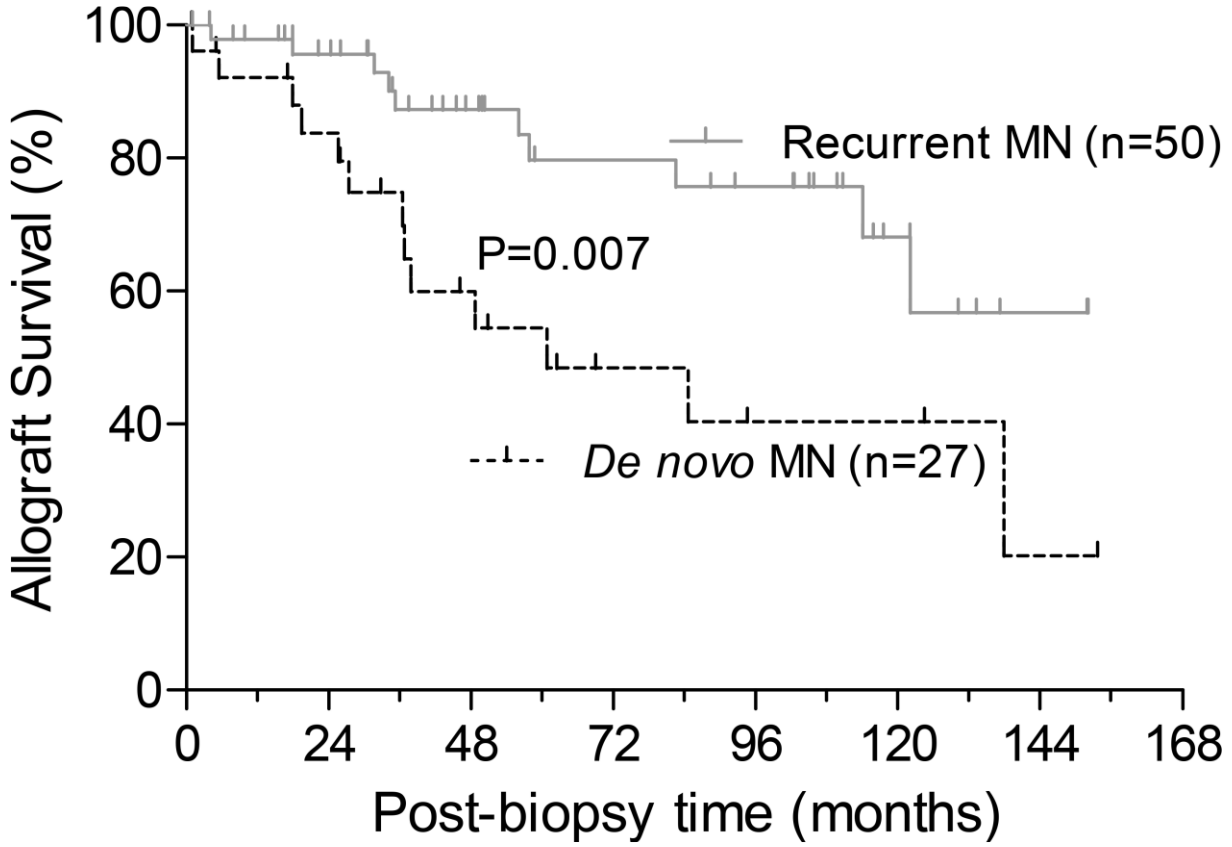
CLAD - 50% w ciągu 5 lat od Tx

Platform	Sample size	Molecular platform	Results
BALF			
Genomics	A total of 60 lung transplant recipients grouped in stable ($n = 20$), BOS (n = 20) or RAS (n = 20)	ELISA	Combination of cfDNA and CXCL10 levels as diagnostic biomarkers of CLAD
Bulk Transcriptomics	A total of $n = 219$ lung transplant recipients with A-grade ACR (n = 61), lymphocytic bronchiolitis (n = 58), infection (n = 41), or no rejection/infection (n = 59)	RNASeq	A panel of n = 72 immune-related genes as biomarkers of A-grade ACR
Single-cell Transcriptomics	A total of $n = 46$ study participants grouped inn = 4 lung transplant recipients versus n = 42 healthy subjects	Single cell-RNASeq	Most AMs in lung transplants are peripherally derived
Exosomes	A total of $n = 12$ lung transplant recipients grouped in free of lung allograft injury ($n = 6$) vs. biopsy-proven acute rejection (n = 6)	Ultracentrifugation Flow-citometry, RNA-Seq	A panel of pro-inflammatory genes as biomarkers of AR
	A total of $n = 30$ lung transplant recipients grouped in stable ($n = 10$), acute rejection (n = 10), and BOS (n = 10)	Ultracentrifugation Flow cytometry, miRNA array	Exosomes expressing donor HLA, SAgs and immune-related miRNAs as biomarkers of BOS
Blood/plasma/serum			
Genomics	A total of 71 lung transplant recipients	AlloSure Lung kits	dd-cfDNA% as a biomarker of AR
	A total of $n = 170$ lung transplant recipients grouped in stable ($n = 70$), AMR/ACR ($n = 37$), infection ($n = 43$), and CLAD (n = 20) phenotypes	NGS	dd-cfDNA% as a biomarker of AR
Torque Teno Virus	A total of $n = 143$ lung transplant recipients	qRT-PCR	Useful biomarker for identification of the efficacy of immunosuppression after lung transplantation
Bulk Transcriptomics	A total of $n = 107$ lung transplant recipients grouped in $n = 49$ recipients with stable function for at least 3 years, n = 32 patients at least 6 months before BOS diagnosis, and n = 26 patients at or after BOS diagnosis	Microarray	Downregulation of *POU2AF1*, *TCL1A*, and *BLK* genes as early predictive biomarkers of BOS
Exosomes	A total of n = 67 lung transplant recipients grouped in symptomatic upper- and/or lower-tract RVI ($n = 35$) vs. no RVI diagnosis (n = 32)	Ultracentrifugation, ELISA, Immunoblot	Exosomes expressing both SAgs and viral as biomarkers of viral respiratory infections
Telomere lenght	A total of n = 82 patients with pulmonary fibrosis undergoing lung transplant	qRT-PCR	Reduced telomere length (<10th percentile) as biomarker of poor post-transplant survival
	A total of n = 262 PF lung transplant recipients	NGS	Rare variants in the telomere-related genes *TERT*, *RTEL*1, or *PARN* are associated with higher risk of death and CLAD
	A total of n = 69 patients undergoing lung transplantion	qRT-PCR	Short telomeres as early biomarkers of post-transplant leukopenia and reduced CLAD-free survival
Airway brushes and FFPE tissue blocks			
Bulk Transcriptomics	Cohort 1: large airway brushes from n = 6 LB cases and 18 post-transplant recipients. Cohort 2: eight biopsies for each pathology subtype were matched with pathology-free biopsies from the same subject (totaling 48 samples from 24 subjects)	RNASeq, digital RNA counting	LB-associated "metagene" as biomarker of *E*-grade and A-grade AR
Telomere lengtht	A total of $n = 120$ patients undergoing lung transplantion	qRT-PCR	Telomere length in the lung allograft airway is not a strong predictor of the future CLAD

Napoli C et al., Transplant Immunology 2024

MN i re-MN po KTx





HLA-DQ2 and HLA-DR17
more common in recipients with re-MN

Genomika i Transkryptomika

Farmakogenomika | Odrzucanie | Tolerancja | Przeżycie graftu | Nawrót KZN

Genomika i Transkryptomika

Farmakogenomika | Odrzucanie | Tolerancja | Przeżycie graftu | Nawrót KZN

Proteomika

December 3, 2024
Proteomics

Clinical proteomics, quo vadis?

Harald Mischak, Joost P. Schanstra, Antonia Vlahou, and Joachim Beige

Proteomic research aimed to identify biomarkers to assess disease onset and progression, decode disease mechanisms, and discover drug targets with precision. However, a retrospective look at clinical proteomics reveals that, despite substantial scientific output, its direct impact on patient outcomes has very much fallen short of initial expectations.

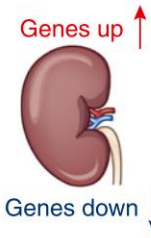
A medline search for "clinical proteome" retrieves well over 40 000 publications to date, with > than 16 000 of these also included when using the term "biomarker".

At the same time, **at best a handful of examples for actual clinical application can be found today, and none of these applied in wide use.**

This prompts a critical examination of both obstacles and accomplishments within the field and defining potential paths forward that could better fulfill its early promises, which still hold true today.

Proteomika KTx – TCMR or AMR (RNA – nerka i mocz)

Kidney allograft RNA-seq signature vs. urine RNA-seq

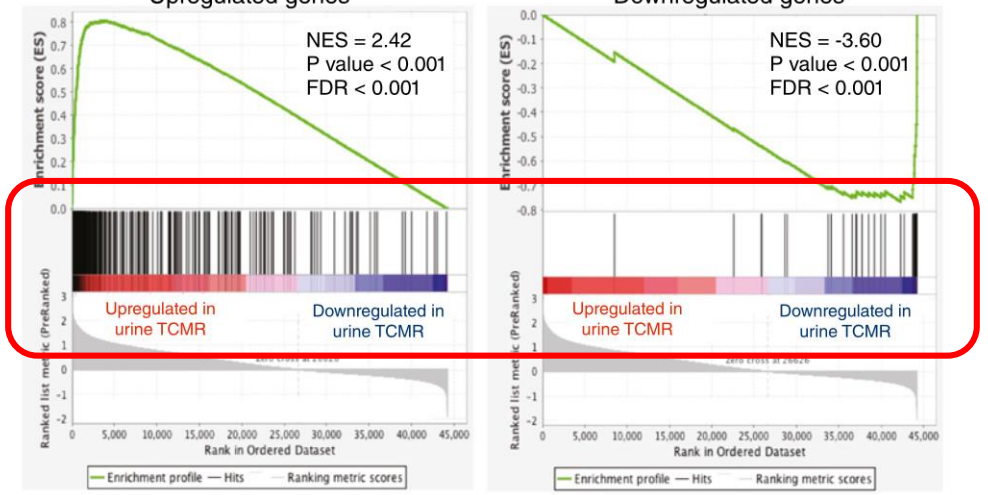


Tissue allograft RNA-seq
Gene signature
TCMR vs. no rejection
AMR vs. no rejection
(FDR < 0.05 & $\log_2$FC ≥ 2)

Urine RNA-seq
Genes changes
TCMR vs. no rejection
AMR vs. no rejection

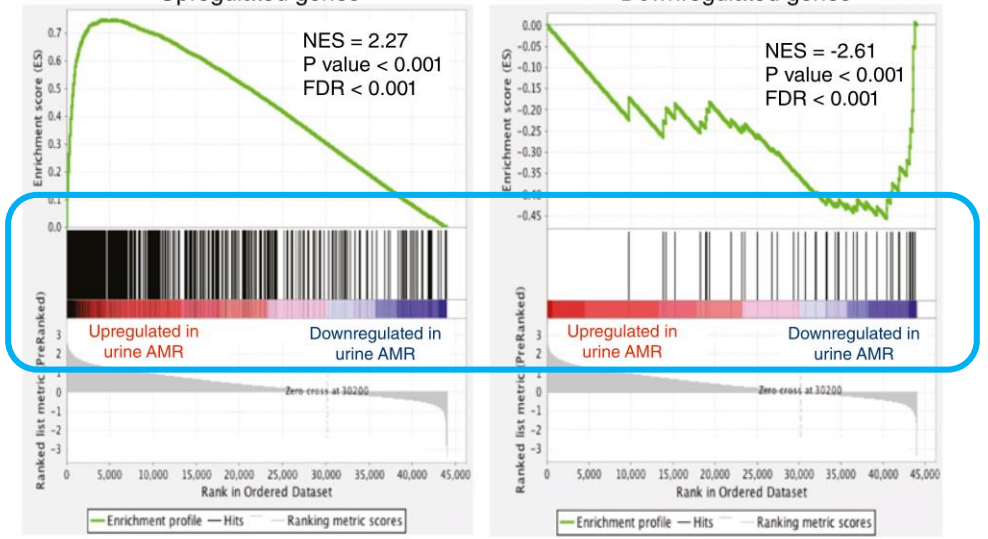
Gene set enrichment analysis

TCMR: Kidney biopsy signature is enriched in urinary cells

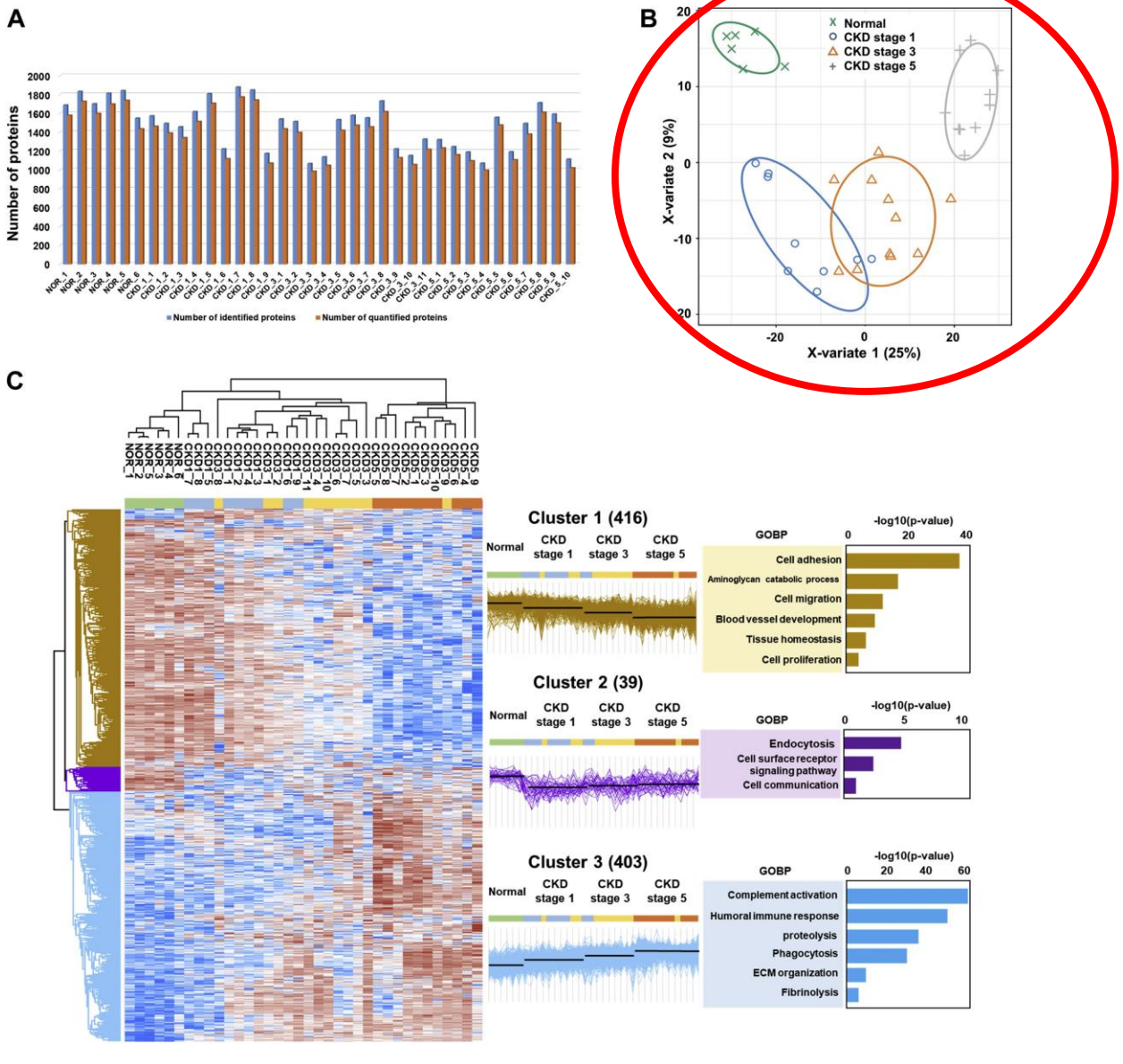


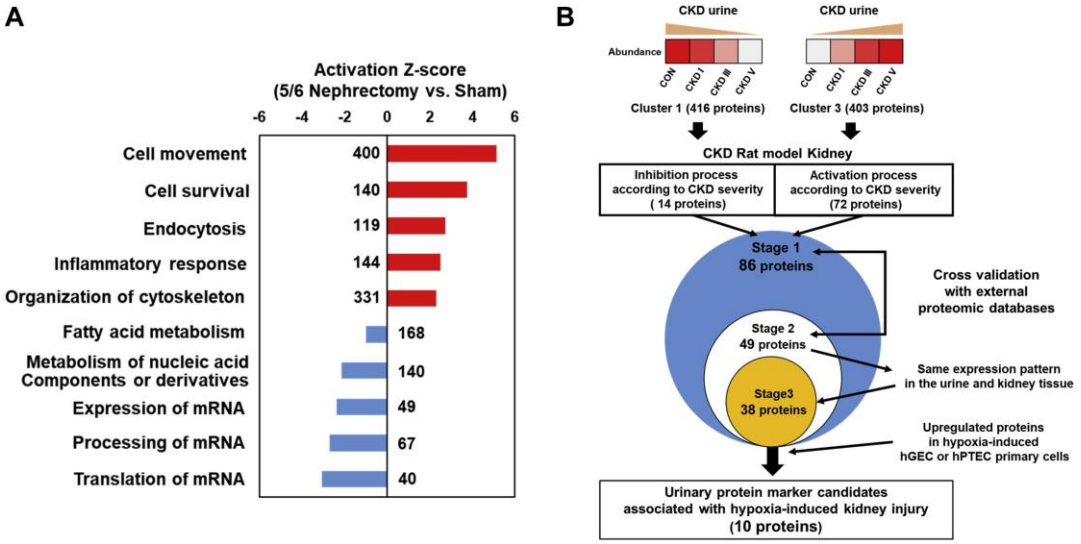
C

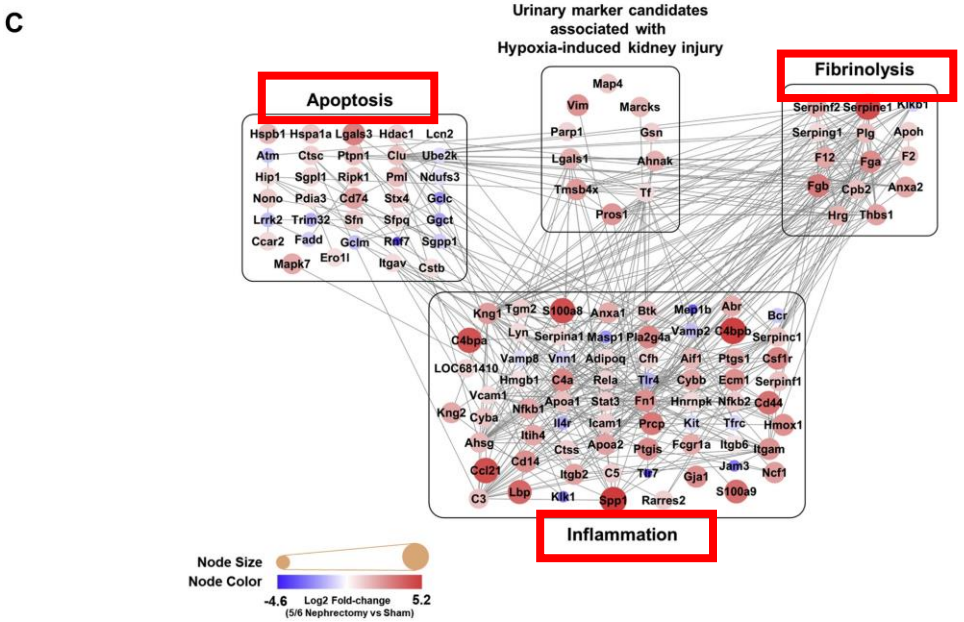
AMR: Kidney biopsy signature is enriched in urinary cells



Proteomika moczu / Markery uszkodzenia zależne od ºCKD







Ji Eun Kim et al. 2021, Mol Cell Proteomics

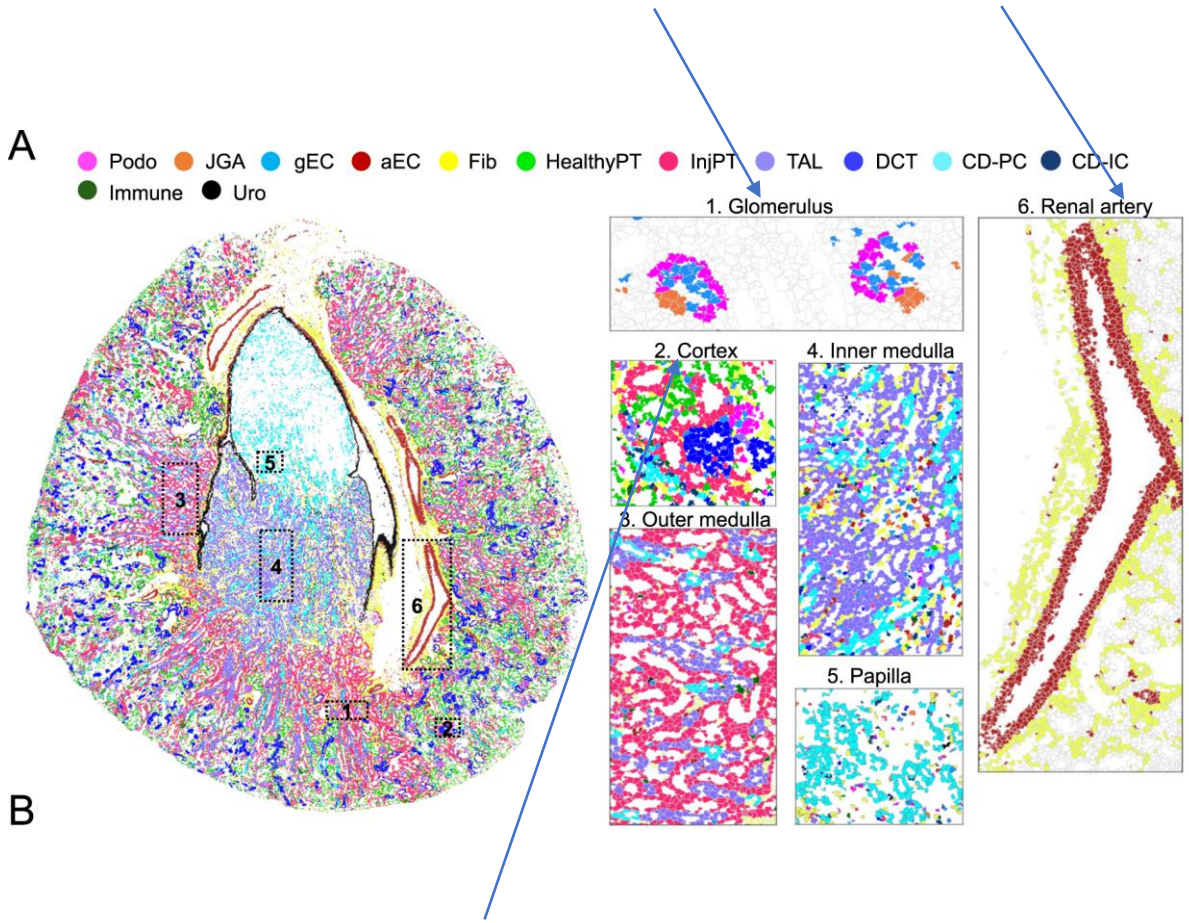
Genomika i Transkryptomika

Farmakogenomika | Odrzucanie | Tolerancja | Przeżycie graftu | Nawrót KZN

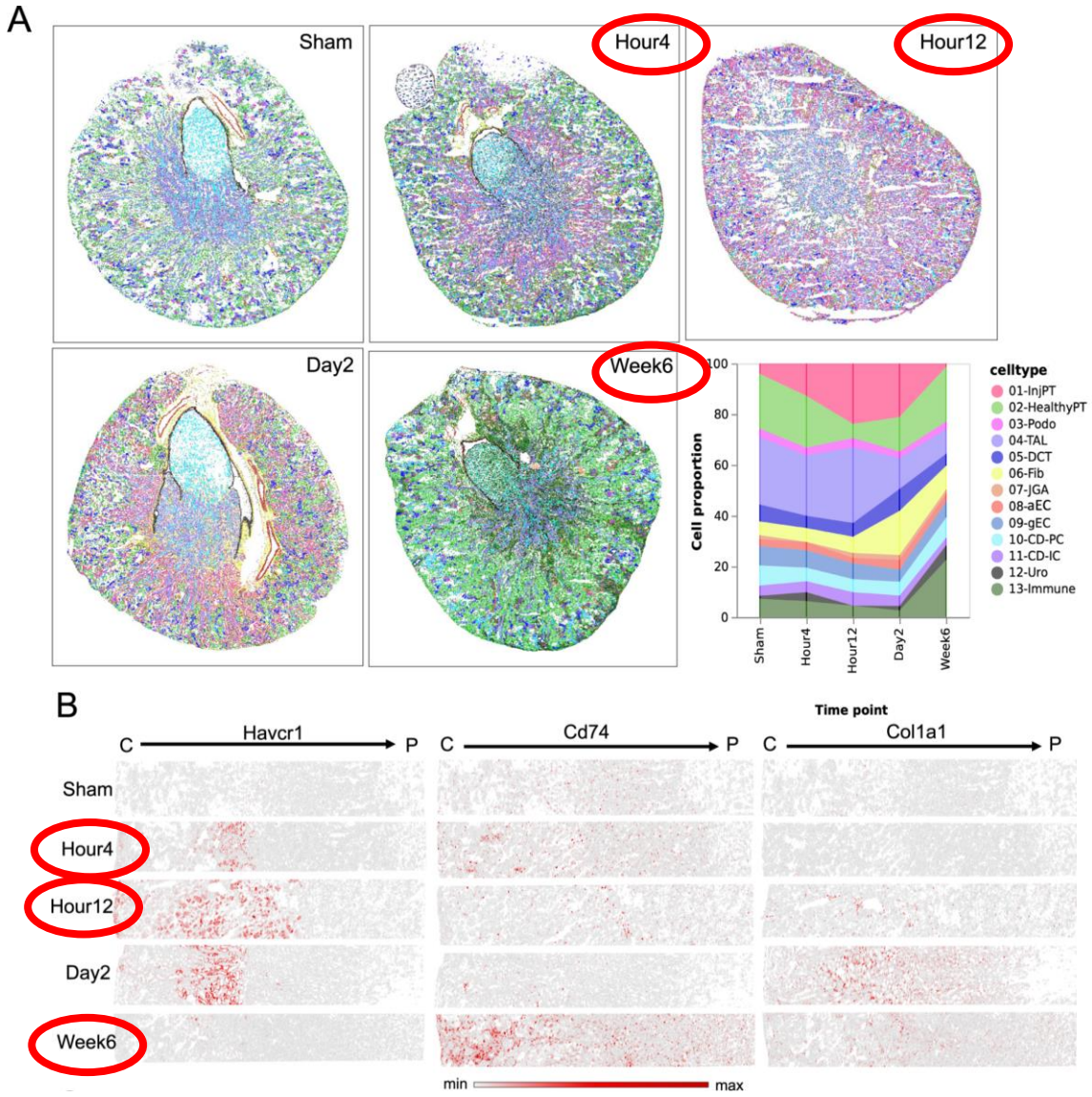
Proteomika

MultiOmics

MultiOmics – spatial cell/ genes & proteins distributions

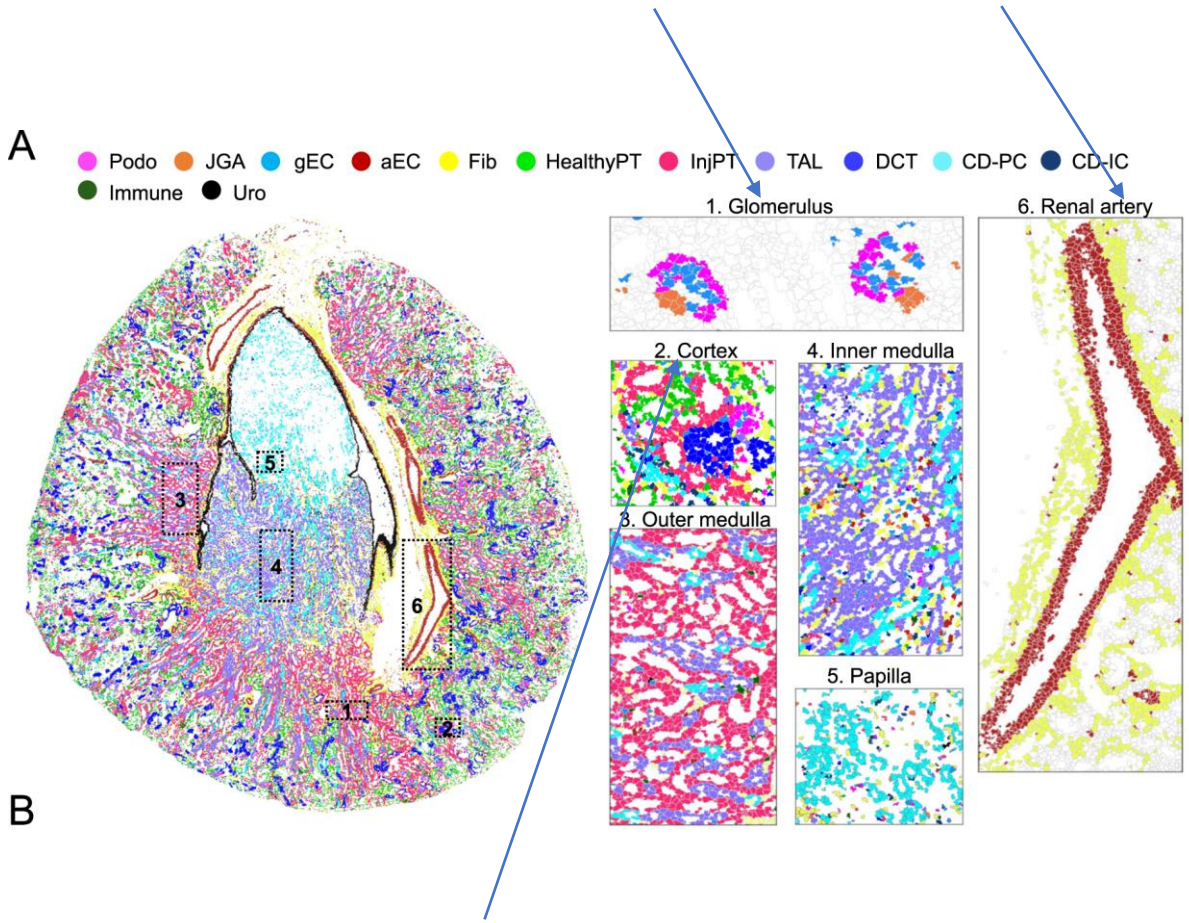


AKI – cell/proteome composition in timecourse

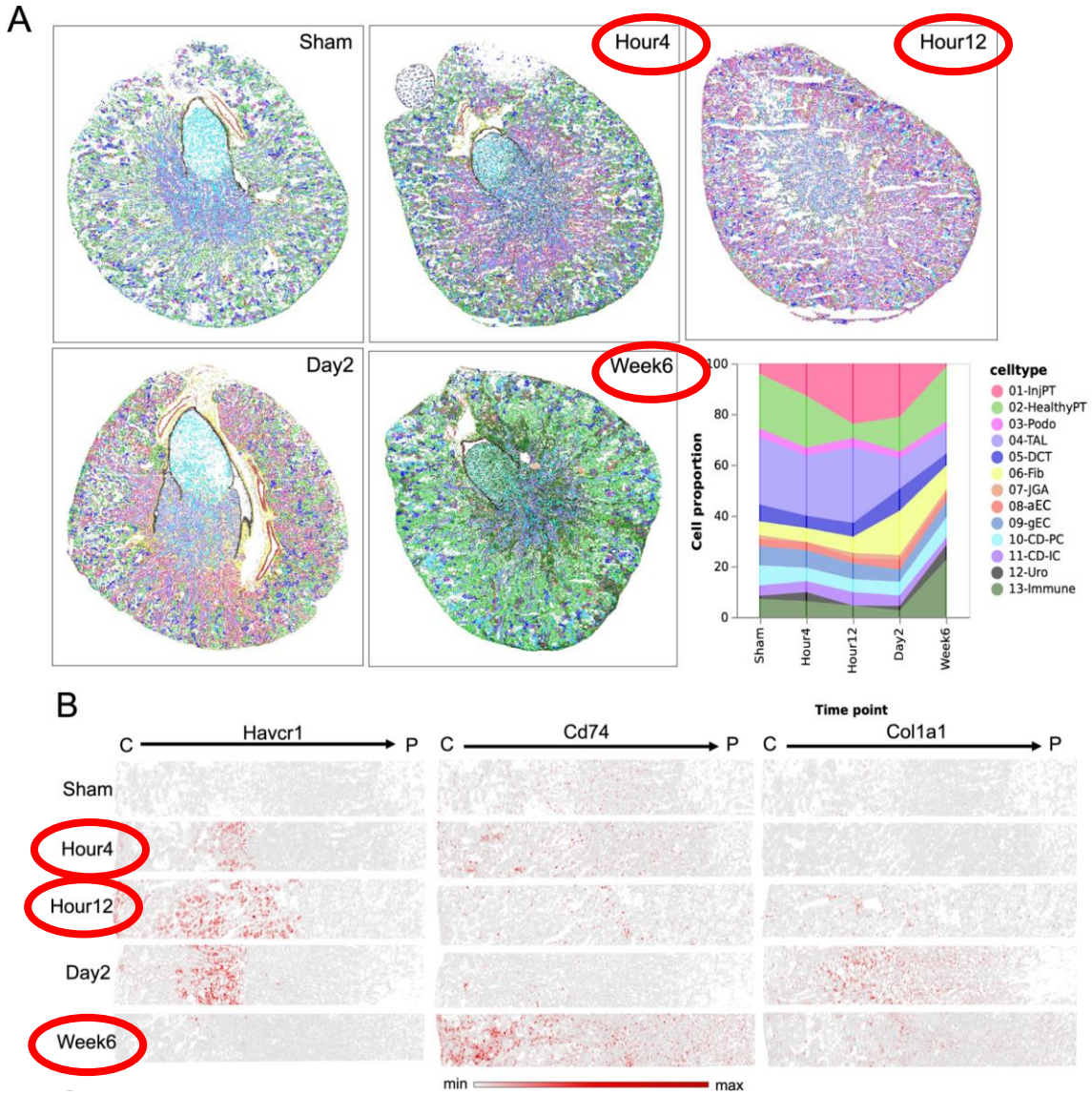


Wu, H et al. Nat Commun 2024

MultiOmics – spatial cell/ genes & proteins distributions



AKI – cell/proteome composition in timecourse



Wu, H et al. Nat Commun 2024

Genomika i Transkryptomika

Farmakogenomika | Odrzucanie | Tolerancja | Przeżycie graftu | Nawrót KZN

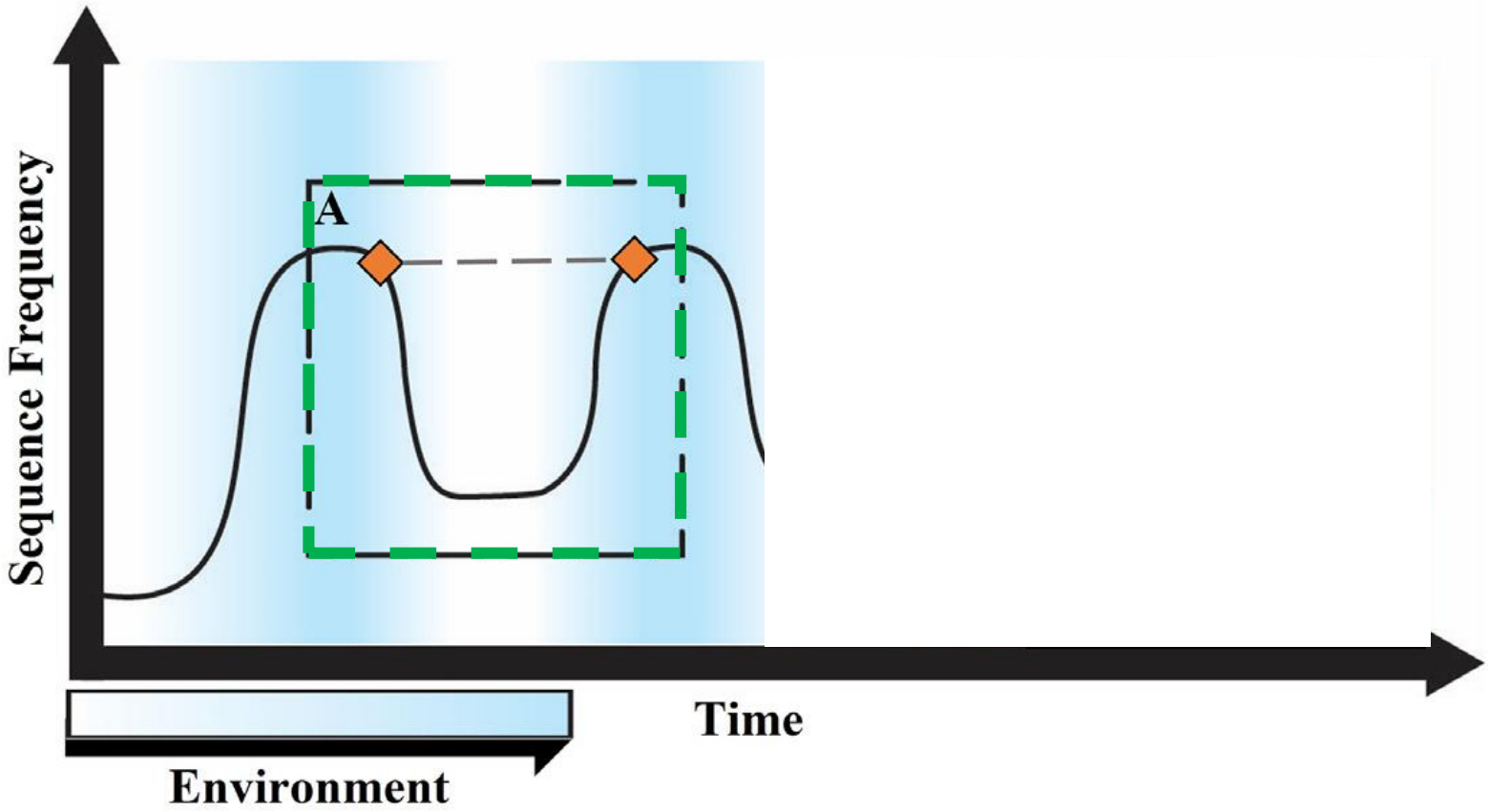
Proteomika

MultiOmics

Pułapki


World Wide Web
4%

1. Timing = **czas i środowisko** vs. zmiany metylacji, białek, metabolitów



A - No change

even though environmental change results in sequence fluctuations (e.g. seasonal gene expression)

B - Change

a slight increase in sequence frequency is detected that could be misattributed to a different environmental condition not visualized in the figure

A. A. Snead and R. D. Clark, Int&Comp Biol, 2022

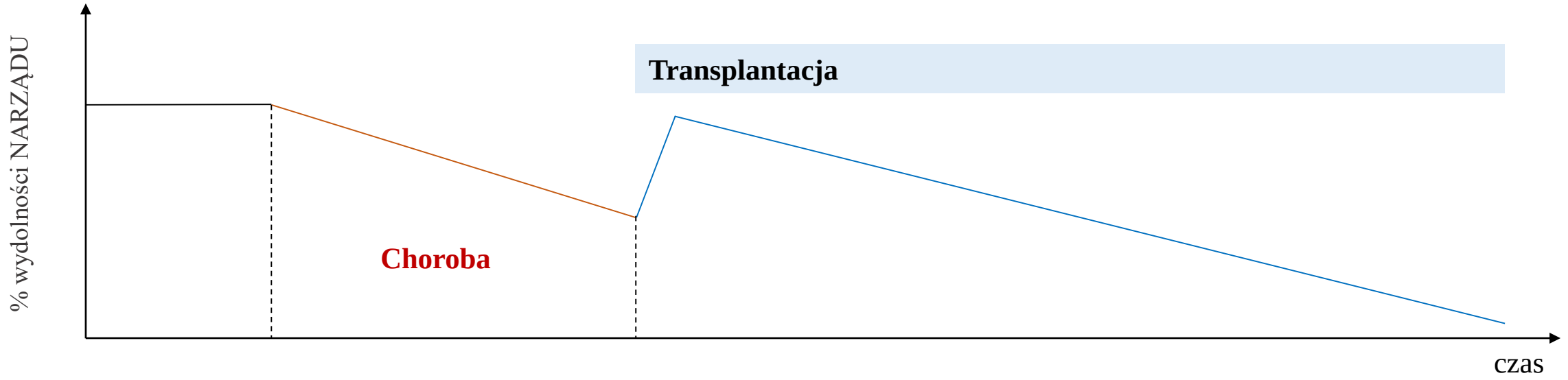
1. Timing - SOT

Genomika (lata)

Transkryptomika (tygodnie)

Proteomika (6 miesięcy)

G_{ch1} T_{ch1} P_{ch1} T_{ch2} P_{ch2} T_{ch3} G_{Tx1} T_{Tx1} P_{Tx1} T_{Tx2} P_{Tx1} G_{Tx2} T_{Tx3} P_{Tx1} T_{Tx4} .....

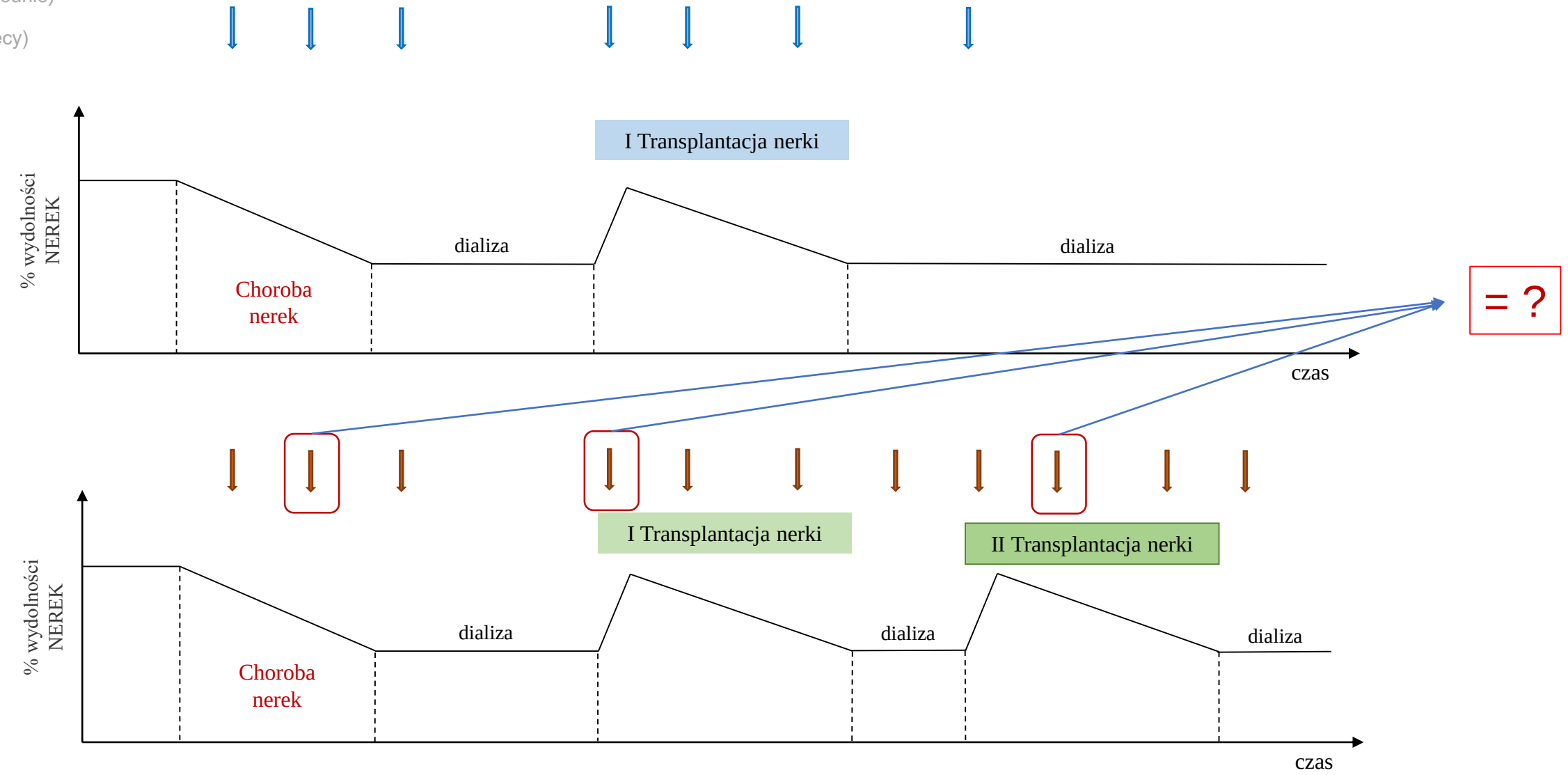


1. Timing - KTx

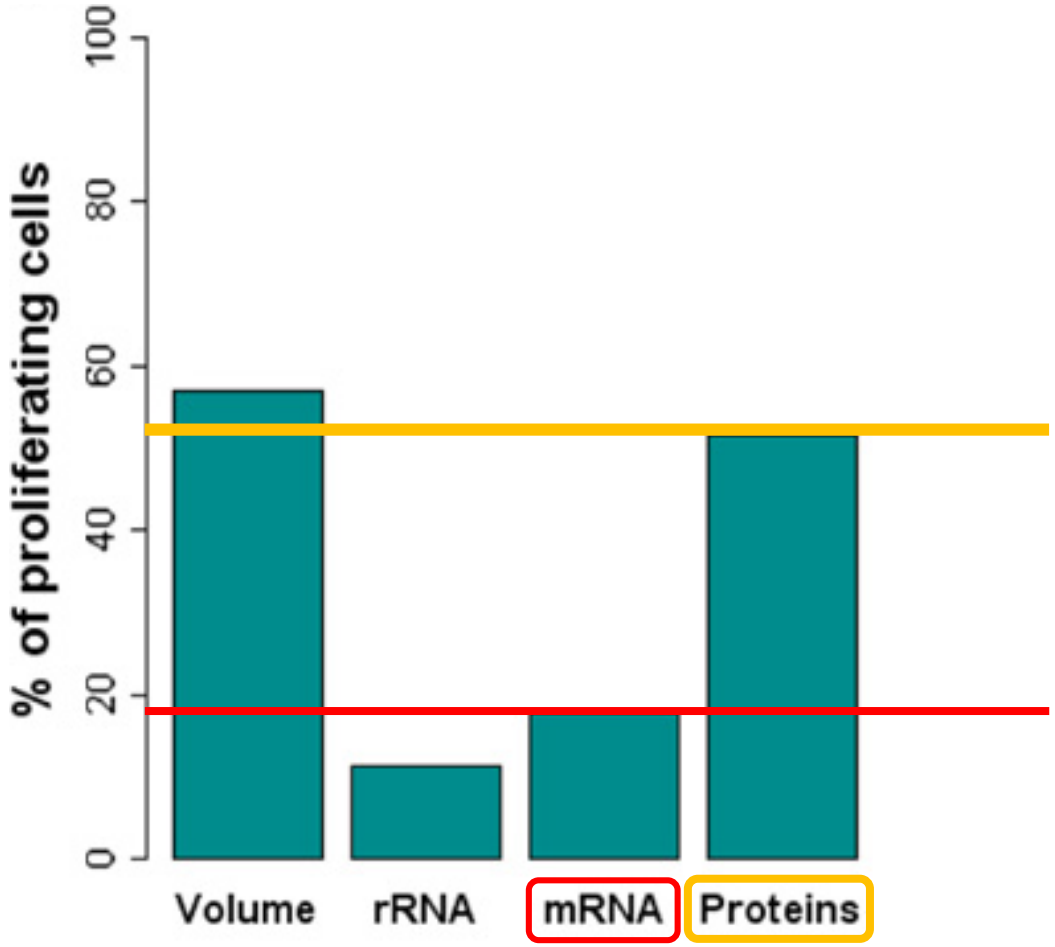
Genomika (lata)

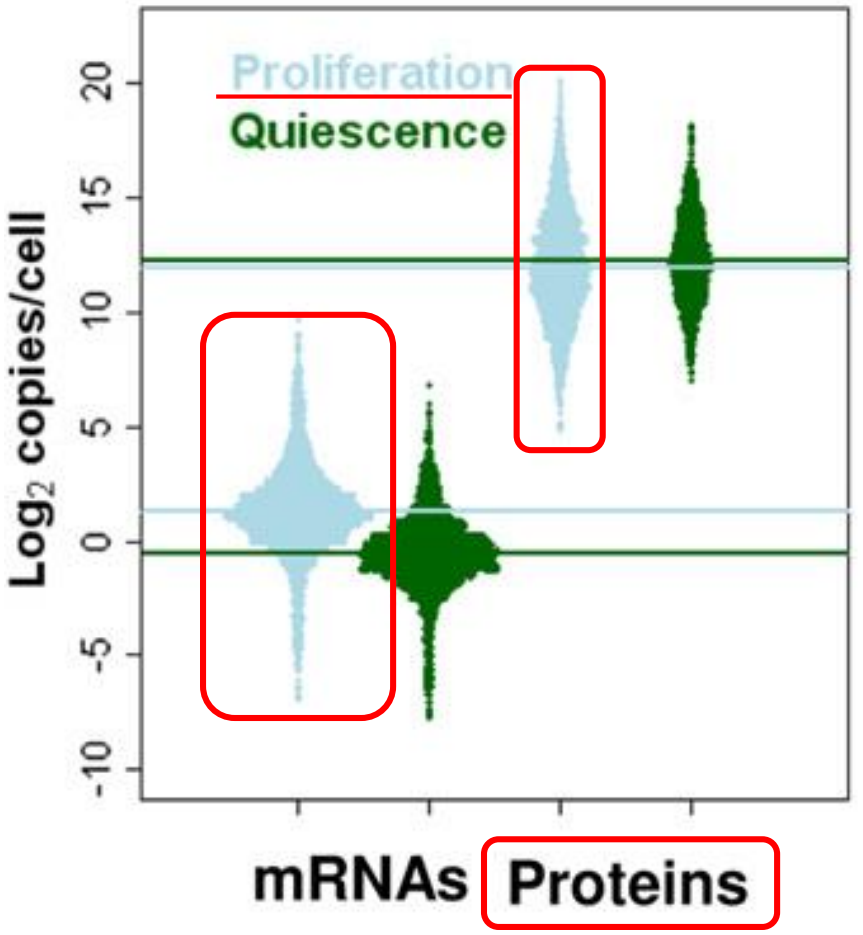
Transkryptomika (tygodnie)

Proteomika (6 miesięcy)

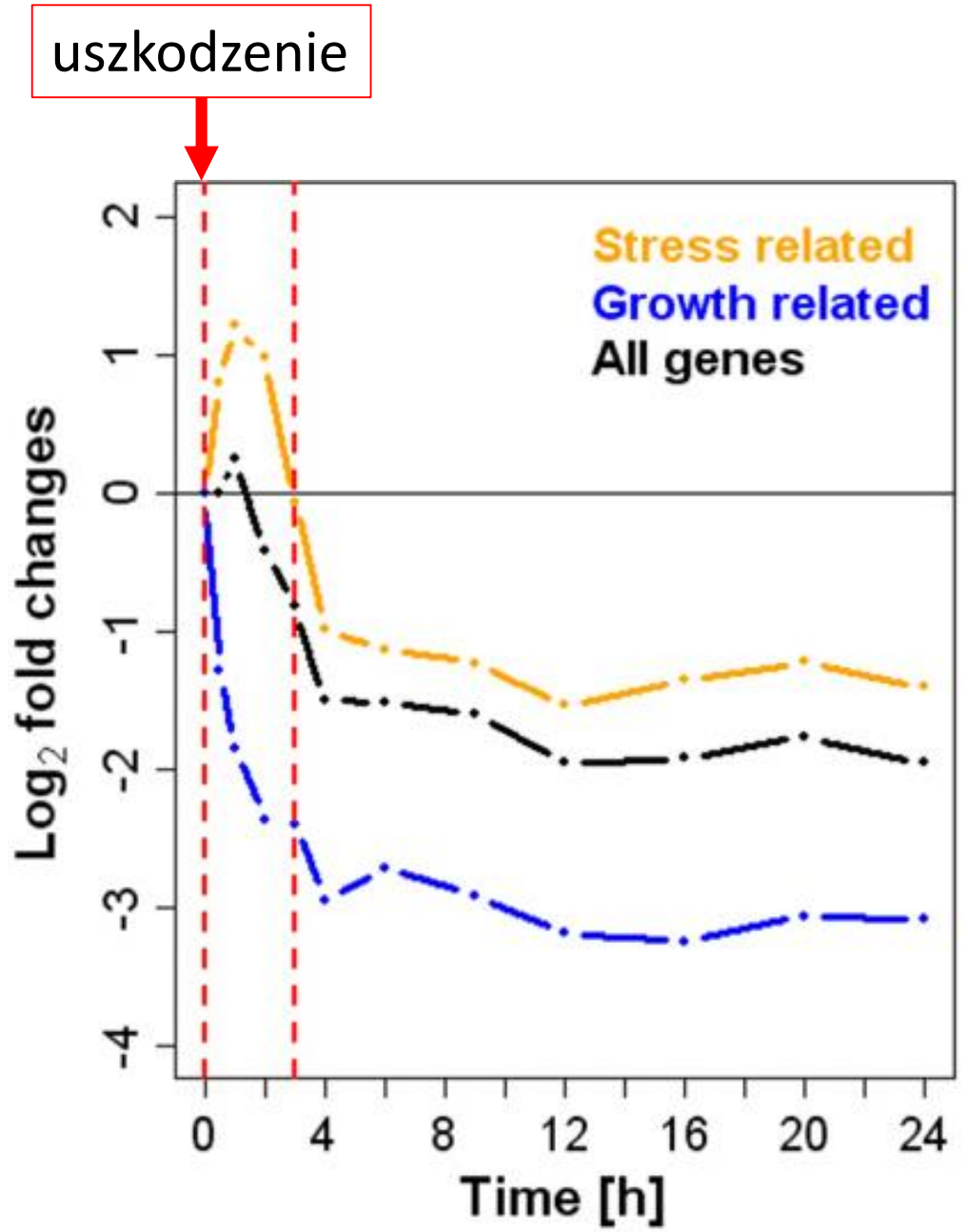


2. Status komórki = pacjenta (odrzucanie, infekcja etc.)





2. Status komórki = pacjenta (odrzucanie, infekcja etc.)



A. A. Snead and R. D. Clark, Int&Comp Biol, 2022

3. Czy wszystko badamy ?

Kiedy badać?

1. Pierwsza wizyta ?
2. Follow-up ?
3. Pierwsza poranna próbka ?
4. Druga poranna próbka ?
5. 24-h zbiórka moczu ?
6. ♀ ile dni przed/po miesiączce ?
7. ……
8. ……

Lab - Methodology

1) Cut-off 10 kDa

+

2) Adsorbtion

+

3) Degradation

Jak dużo białek tracimy ?

„Deep & Dark" Proteomics

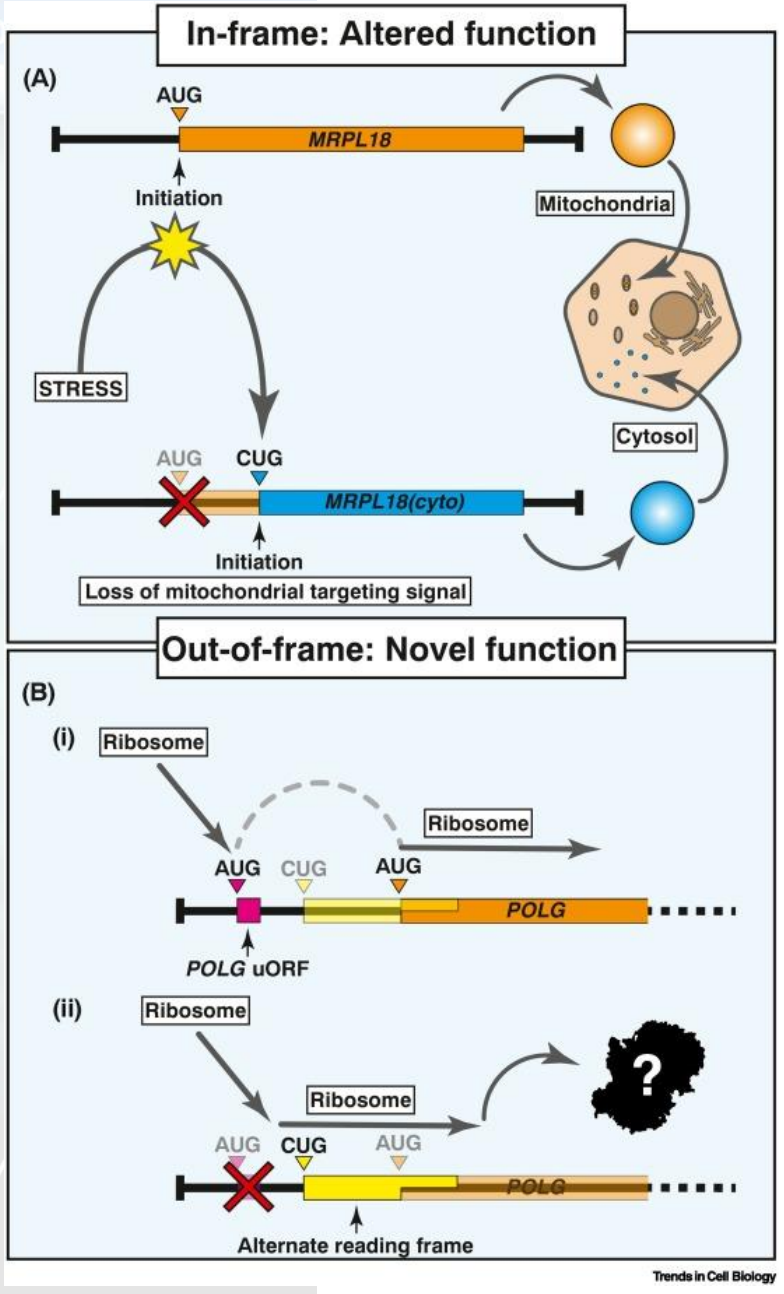
1. **Non-canonical ORFs:** Proteins translated from upstream ORFs (uORFs), downstream ORFs (dORFs), overlapping ORFs, and other non-standard coding regions.

2. **Small Open Reading Frames (sORFs):** Short sequences that can encode bioactive peptides, often overlooked in traditional gene annotation methods.

3. **Alternative Translation Products:** Proteins resulting from translation initiation at non-AUG codons, internal ribosome entry sites (IRES), or cryptic translation start sites.

4. **De novo Genes:** New genes that arise from previously non-coding regions of the genome through evolutionary processes.

10-30% of the proteome depending on the biological kingdom

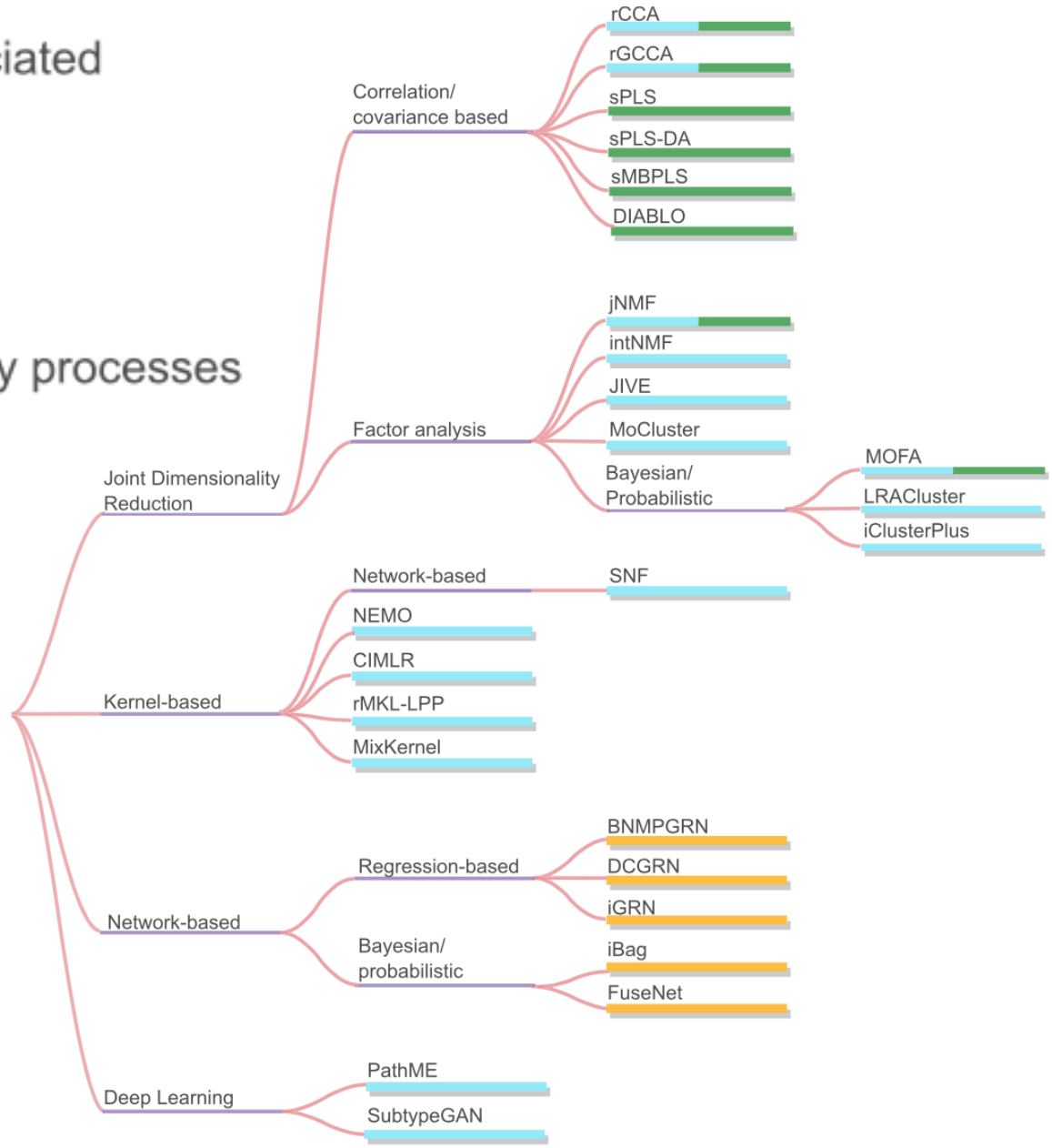


4. Multiomics = Multimetodologia (metody integracji danych)

Detect disease-associated molecular patterns

Subtype Identification

Understand regulatory processes



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Dziękuję za uwagę