Protein and Metabolite Biomarkers of Graft Injuries in Renal Transplantation

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UMR 850 INSERM/Univ Limoges/CHU Limoges
DHU SUPORT
FP7 BIOMARGIN

DIA DEVELOP INNOVATE ADVANCE

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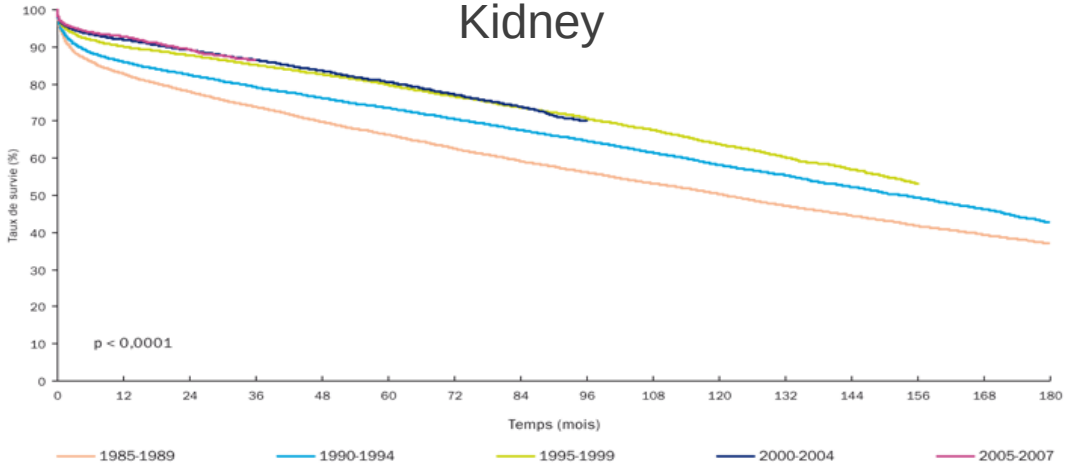
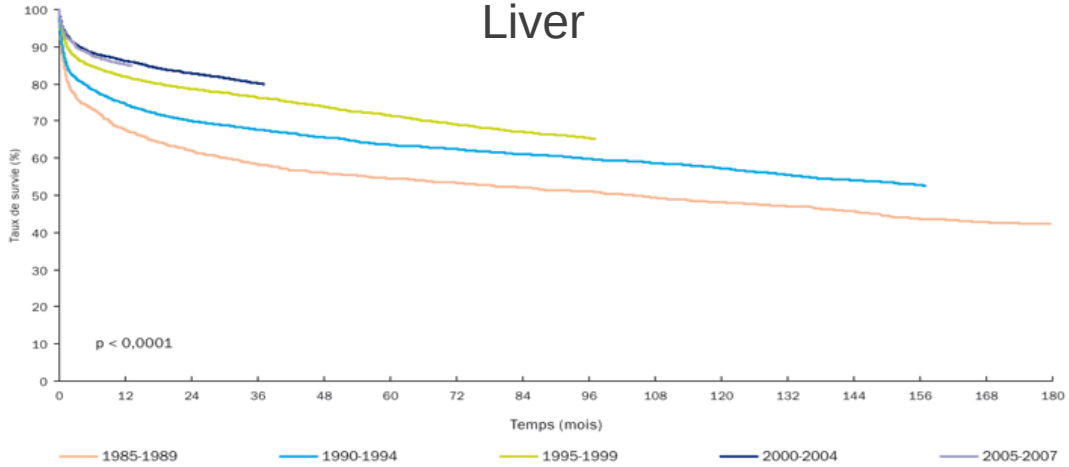
Clinical problems & needs in organ transplantation

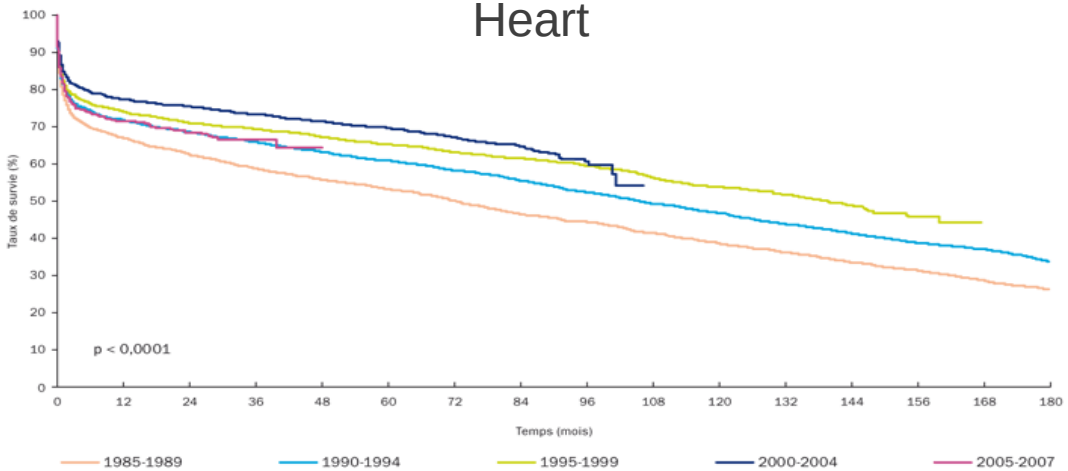
Kidney



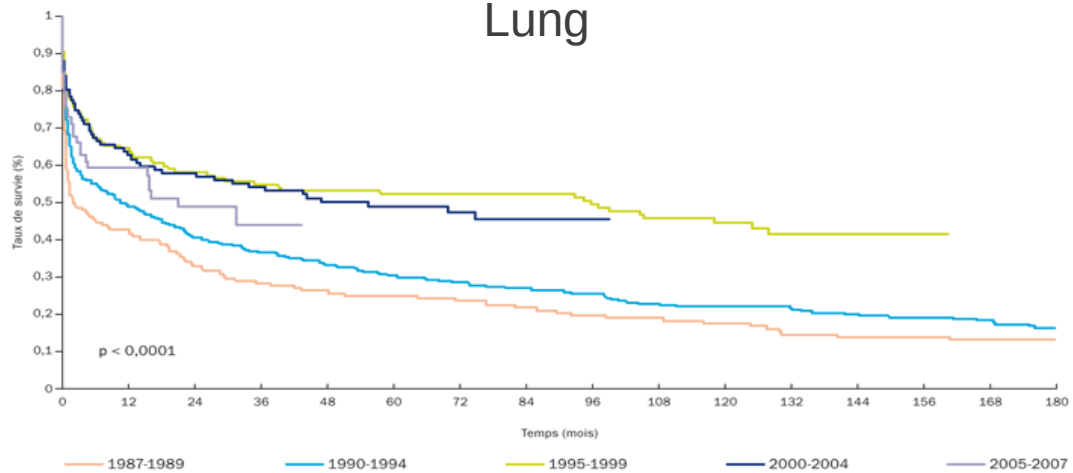
Liver



Heart



Lung



Possible biomarkers in Tx and associations

- **Types of biomarkers**
  - Nucleic acids (DNA, mRNA, micro-RNA)
  - Proteins, peptides
  - Lipids, metabolites
- **Matrix:** preservation solution, machine perfusate, urine, blood … or biopsy
- **Possible association with:**
  - specific lesions (including ischemic lesions) in the graft
  - primary non-function (PNF)
  - delayed graft function (DGF)
  - graft function in the stable phase
  - graft survival (GS) or graft failure (GF)

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1 – Pre-transplantation biomarkers

Characterization of the perfusate proteome of human donor kidneys

[Snoeijs et al. *Ann Clin Biochem* 2013; 50: 140–146]

- Study of 18 kidney grafts kept in hypothermic conditions in a perfusion machine
  - Samples collected after 1h perfusion
  - 2D-DIGE separation of **proteins**
  - In-gel trypsin digestion
  - MALDI-TOF/TOF analysis
  - Mass spectra interpretation with MASCOT® algorithm and comparison with the Swissprot® database
  - 32 protein spots → 19 proteins identified

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- In kidneys with vs. without ischaemic lesions
  - 2 unidentified protein spots significantly up-regulated
  - haptoglobin significantly down-regulated
- In kidneys with vs. without primary non-function :
  - 2 unidentified protein spots up-regulated
- In kidneys with vs. without delayed graft function :
  - α1-antitrypsin up-regulated

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Targeted analysis of 6 biomarkers in the perfusate of 306 renal grafts

[Moers et al. *Transplantation* 2010;90: 966–973]

- Kidneys from donation after brain death (231) or cardiac death (75)
- 230 kidneys developed immediate function, 7 PNF and 76 DGF
- Targeted biomarkers: LDH, ASAT, total GST, Ala-AP, NAG, H-FABP (i.e., **proteins**)
- Enzymatic and ELISA assays

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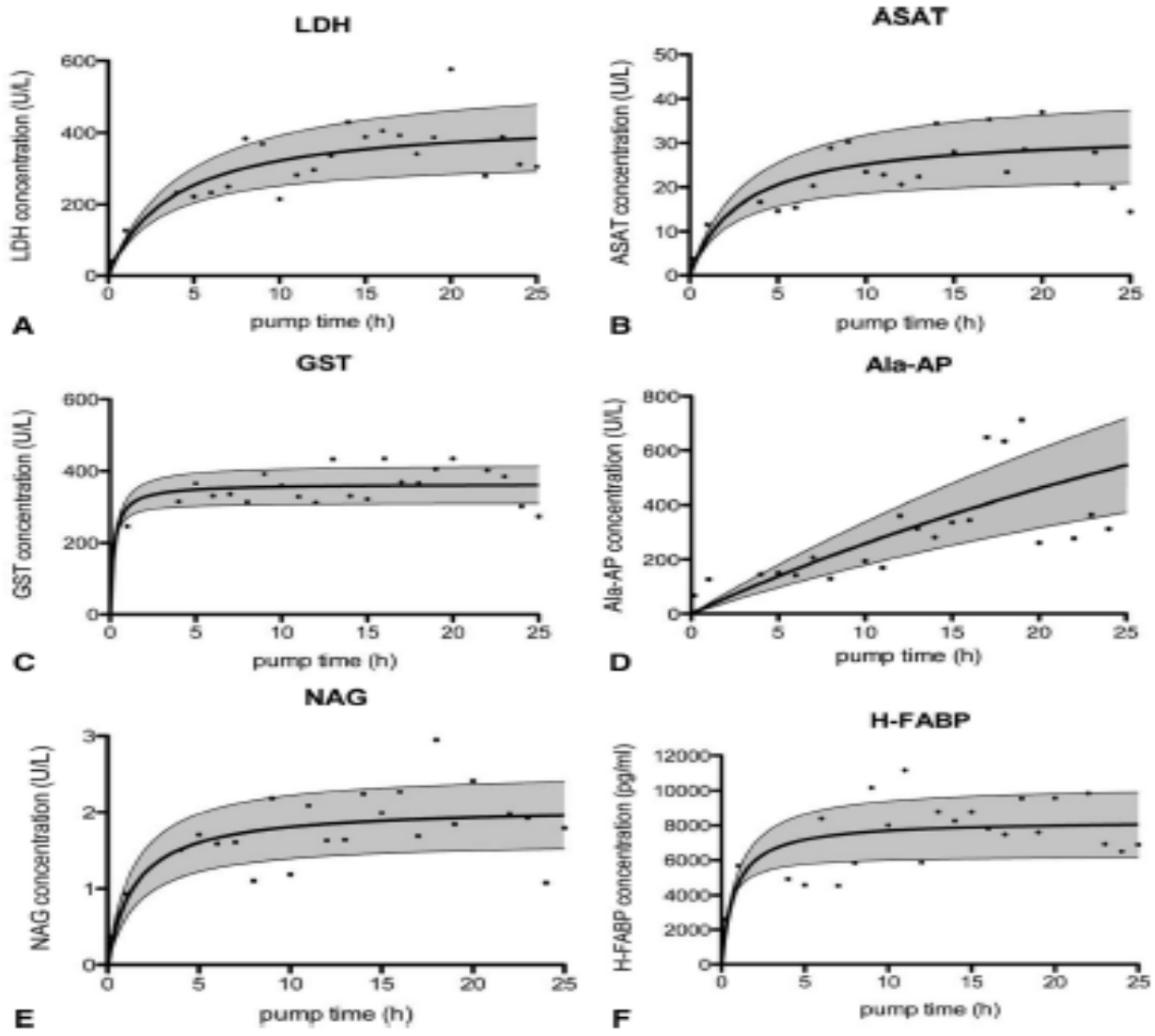
Evolution of each biomarker's perfusate concentration in time



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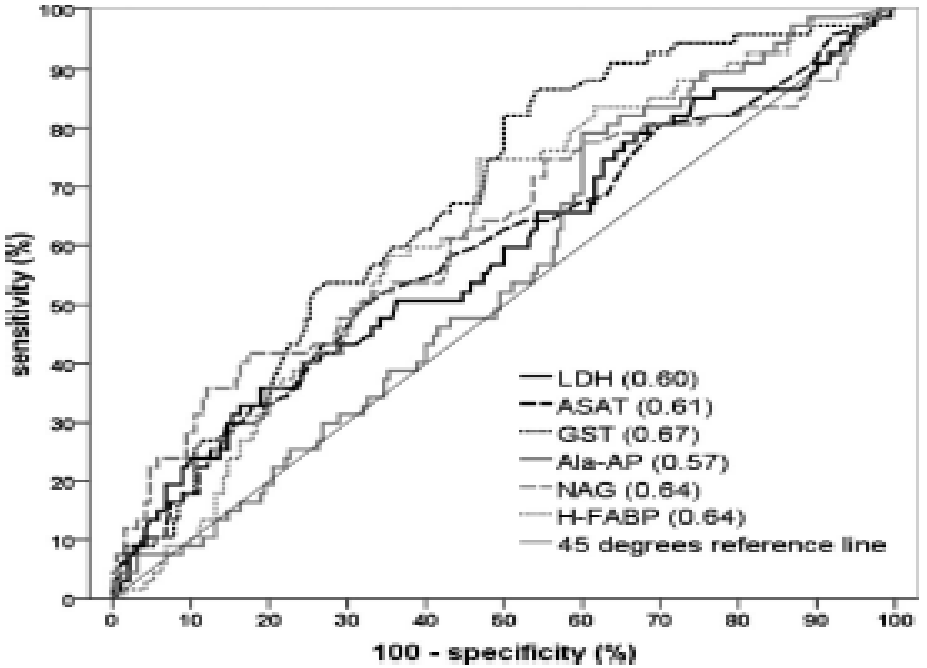
- Statistical analysis adjusted on known risk factors
- Only GST, NAG and H-FABP concentrations measured at the end of machine perfusion (*but no sooner*) were independent predictors of delayed graft function
  - However, all AUROC < 0.7
  - Poor sensitivity & specificity
  - No clear cut-off values



- None of the 6 biomarkers could predict graft failure within the first year post-transplant

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Targeted analysis of 6 biomarkers in the perfusate of 335 renal grafts

[Hoogland et al; *Transplantation* 2013;95: 603-610]

- Partly the same team as in previous study
- Partly same kidneys? Observation period 1997-2008
- All kidneys from donation after cardiac death (DCD)
- 63 kidneys developed immediate function, 67 primary non-function and 205 delayed function
- Targeted biomarkers: LDH, total GST, H-FABP, redox-active iron, IL-18 and NGAL (i.e., **proteins** and one **metabolite**)
- Enzymatic and ELISA assays for the proteins, “bleomycine detectable iron assay” for redox-active iron

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Biomarker concentrations at T4h MP

TABLE 3. Multivariate analysis for PNF, DGF, and graft failure

	OR (95% CI)	*P*
Risk of PNF (biomarker concentration at T_4)		
GST	1.004 (0.998–1.009)	0.161
LDH	1.001 (1.000–1.002)	0.005
H-FABP	1.002 (0.998–1.007)	0.280
Redox-active iron	1.462 (0.974–2.195)	0.067
IL-18	1.001 (1.000–1.002)	0.003
NGAL	0.999(0.980–1.018)	0.919
Risk of DGF (biomarker concentration at T_4)		
GST	1.006 (0.999–1.013)	0.112
LDH	1.002 (1.001–1.004)	0.007
H-FABP	1.007 (1.000–1.014)	0.064
Redox-active iron	1.532 (1.045–2.245)	0.029
IL-18	1.003 (1.001–1.004)	0.002
NGAL	1.000 (0.982–1.018)	0.994

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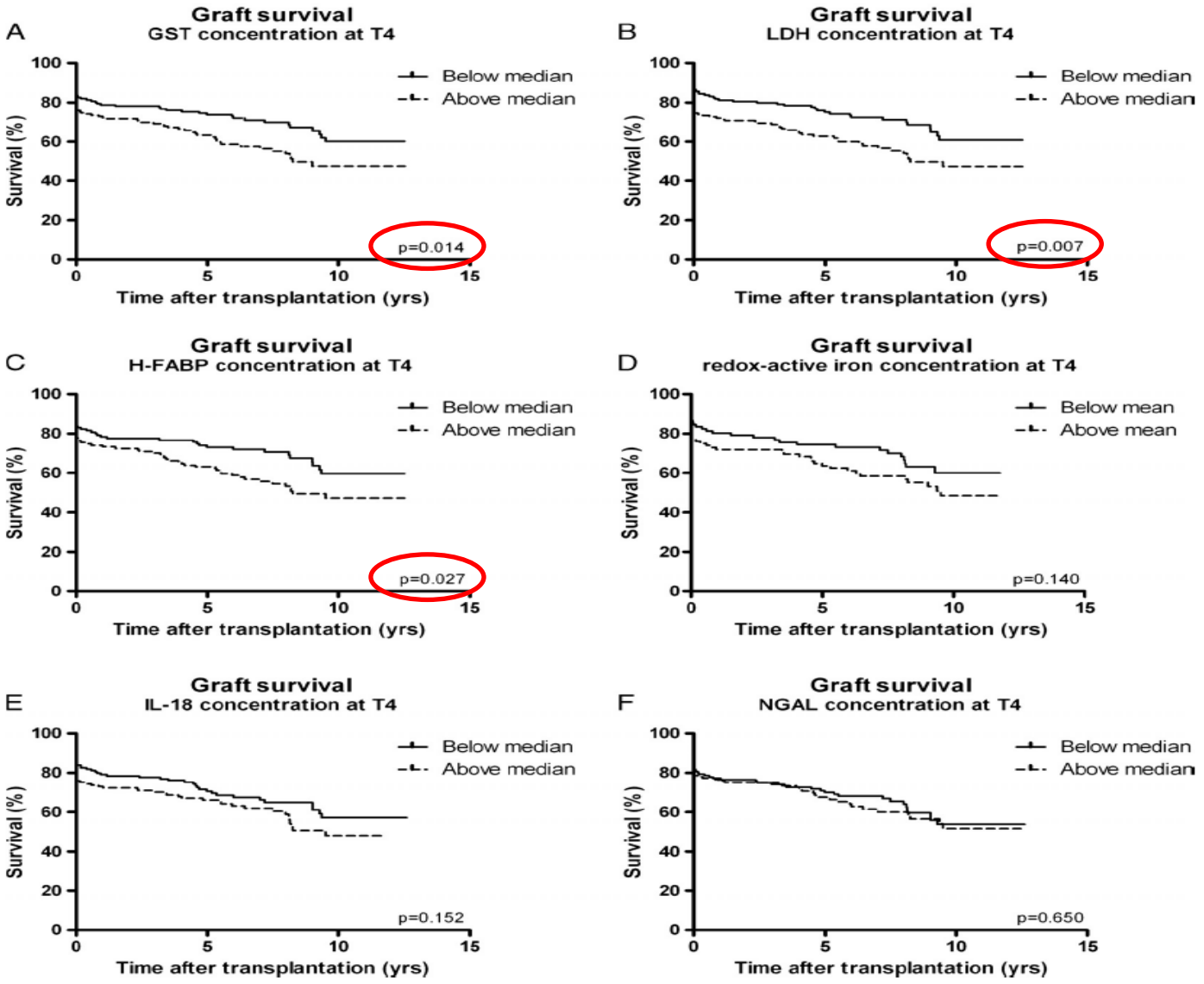
Kaplan-Meier graft survival curves



- GST, LDH & H-FABP significantly linked with graft survival
- Not redox-active iron, IL-18 or NGAL

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In Summary: pre-transplantation biomarkers

- 1 very preliminary untargeted study of the proteome
- Candidate biomarkers:
  - Ischemic lesions: haptoglobin
  - PNF: **LDH**, **GST** ± redox iron and IL-18 after 4h MP
  - DGF: **LDH**, **GST** ± NAG and H-FABP after 12h MP, redox iron and IL-18 after 4h MP ± α1-antitrypsin
  - Plasma creatinine level at M3: Glutamate after 4h MP; Valine, alanine, glycine, glutamate and total glutathione after 12h MP
  - Graft survival: **LDH** ± GST & H-FABP after 4h MP

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2 – Post-transplantation biomarkers

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Post-transplantation biomarkers of renal graft injuries

- Several teams have searched for biomarkers of renal graft lesions, such as:
  - mRNAs, micro-RNAs
  - peptides, proteins
  - (metabolites)
- Urine > plasma > graft
- Mostly biomarkers of “acute rejection” (*vs.* “normal”)
- Little cross-fecundation of these different “omics” approaches

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- Regarding proteomics/peptidomics:
  - Untargeted approaches → large diversity of mass spectrometry settings (historically SELDI-TOF, moving to MALDI-TOF/TOF, electrospray-orbitrap, ESI-Q/TOF, etc.)
  - Targeted approaches → mostly immunoassays (e.g., ELISA), sometimes LC-MS/MS in the MRM mode

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Some untargeted proteomic results

Reference	Biomarker candidates	Sample type	Method	Sample numbers	Outcome
Quintana et al. 2009	14 ions in urine (proteins and peptides not identified)	Urine	MALDI-TOF	50	IFTA, AbMR
Bañón-Maneus et al. 2010	SERPINA1, α1BG, AGT, TFR2, GSN, anti-TNFα antibody light chain, B2M, heparin sulfate proteoglycan, leucine-rich, α-2-glycoprotein-1	Urine	2D-DIGE + MALDI-TOF and CE-MS/MS	24 training set 8 validation set	IFTA
Sigdel et al. 2010	UMOD, PEDF and CD44, among 22 up-regulated and 23 down-regulated proteins	Urine	Capillary LC-ESI- linear ion trap MS	60	AR
Wu et al. 2011	NF-κB, STAT1, STAT3 and 63 other proteins	Plasma	iTRAQ + preparative HPLC + offline ESI-Q/TOF	13	AR
Loftheim et al. 2012	IGFBP7, VASN, EGF, LG3BP	Urine	2D-LC-ESI-Orbitrap (IDA mode)	12	AR
Sigdel et al. 2014	AR: HLA-DRB1, FGB, FGA, FGG, KRT14, HIST1H4B, ACTB, KRT7, DPP4, BKVN: KRT18, SUMO2, STMN1, CFHR2, KRT8, KRT19, RPL18, KRT75, FAM3C, HIST1H2BA CAI: CALR, FAM151A, SERPINA2P, FAM3C, DAG1, KITLG, LUM, FABP4, AGT, LRG1	Urine	iTRAQ + 2D-LC-ESI-LTQ-Orbitrap	108 discovery set 154 validation set	AR, BKVN, CAI

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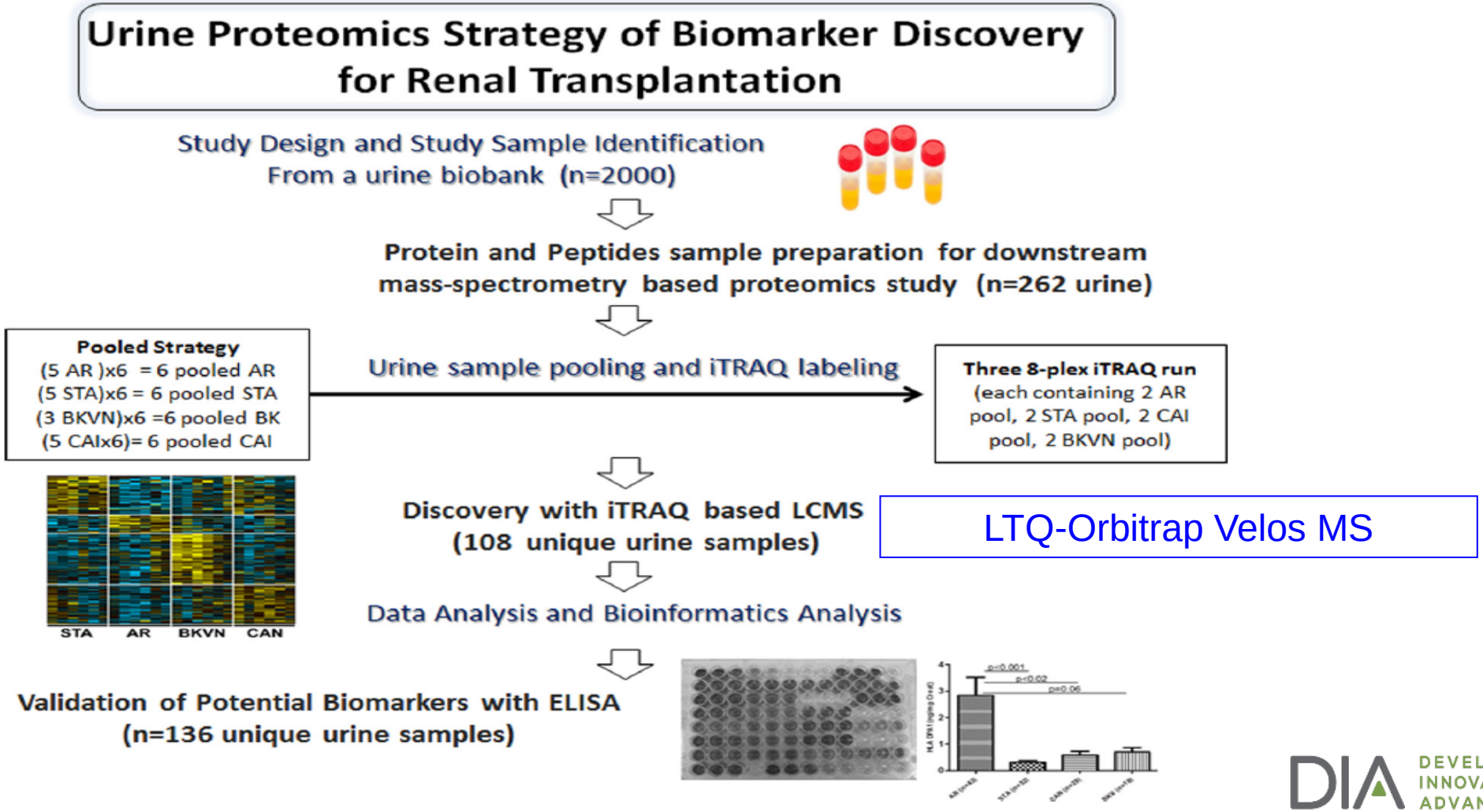
Some targeted proteomic results

Biomarker	Sample type	Sample numbers	Outcome	Method	AUROC	Reference
NGAL	Urine	91	DGF	Immunoassay	0.82	**Hall et al. 2010**
	Urine	182 and 11 for validation	AKI	Immunoassay (screening and validation)	0.92	**Heyne et al. 2012**
	Urine	577	Graft failure	Immunoassay	0.63	**Nauta et al. 2011**
CXCL9	Urine	91	Subclinical tubulitis	Immunoassay	0.78	**Schaub et al. 2009**
	Urine	204	AR	q-PCR (for mRNA) Immunoassay (for proteins)	0.856	**Hricik et al. 2013**
	Urine	156	AR and BKVI	Immunoassay	0.86 sensibility 0.80 specificity	**Jackson et al. 2011**
CXCL10	Urine	91	Subclinical tubulitis	Immunoassay	0.79	**Schaub et al. 2009**
	Urine	91	IFTA	Immunoassay	0.845	**Ho et al. 2011**
	Urine	122	Acute and chronic injuries	Screening: Luminex multiplex Validation: Luminex quaduplex	0.93	**Hu et al. 2009**
	Urine	156	AR and BKVI	Immunoassay	0.80 sensitivity 0.76 specificity	**Jackson et al. 2011**

An integrated proteomic approach

(Sigdel et al. *Mol Cell Proteomics* 2014)



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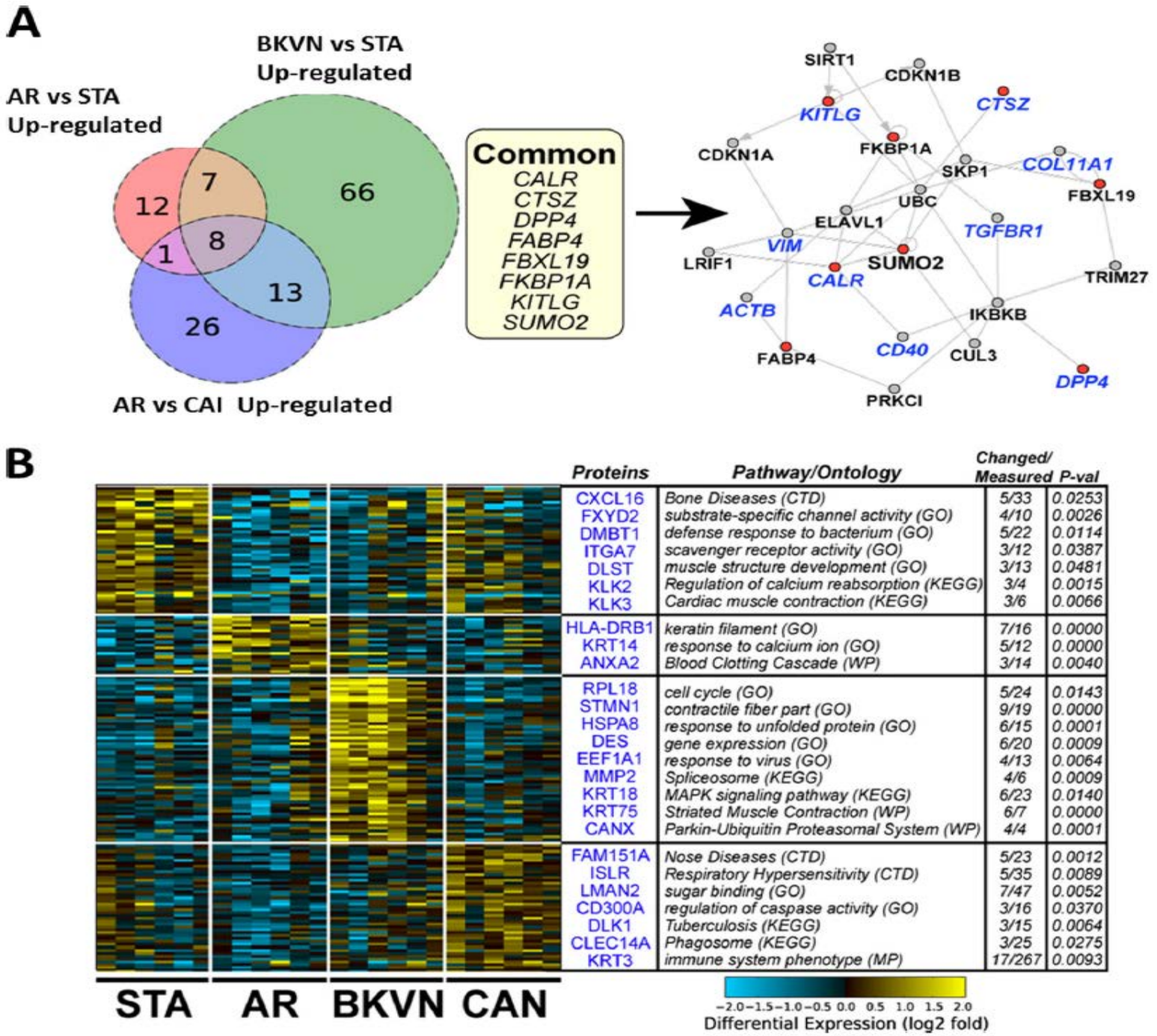
- Specificity of the candidate biomarkers



Proteins	Pathway/Ontology	Changed/ Measured	P-val
CXCL16	Bone Diseases (CTD)	5/33	0.0253
FXYD2	substrate-specific channel activity (GO)	4/10	0.0026
DMBT1	defense response to bacterium (GO)	5/22	0.0114
ITGA7	scavenger receptor activity (GO)	3/12	0.0387
DLST	muscle structure development (GO)	3/13	0.0481
KLK2	Regulation of calcium reabsorption (KEGG)	3/4	0.0015
KLK3	Cardiac muscle contraction (KEGG)	3/6	0.0066
HLA-DRB1	keratin filament (GO)	7/16	0.0000
KRT14	response to calcium ion (GO)	5/12	0.0000
ANXA2	Blood Clotting Cascade (WP)	3/14	0.0040
RPL18	cell cycle (GO)	5/24	0.0143
STMN1	contractile fiber part (GO)	9/19	0.0000
HSPA8	response to unfolded protein (GO)	6/15	0.0001
DES	gene expression (GO)	6/20	0.0009
EEF1A1	response to virus (GO)	4/13	0.0064
MMP2	Spliceosome (KEGG)	4/6	0.0009
KRT18	MAPK signaling pathway (KEGG)	6/23	0.0140
KRT75	Striated Muscle Contraction (WP)	6/7	0.0000
CANX	Parkin-Ubiquitin Proteasomal System (WP)	4/4	0.0001
FAM151A	Nose Diseases (CTD)	5/23	0.0012
ISLR	Respiratory Hypersensitivity (CTD)	5/35	0.0089
LMAN2	sugar binding (GO)	7/47	0.0052
CD300A	regulation of caspase activity (GO)	3/16	0.0370
DLK1	Tuberculosis (KEGG)	3/15	0.0064
CLEC14A	Phagosome (KEGG)	3/25	0.0275
KRT3	immune system phenotype (MP)	17/267	0.0093

(Sigdel et al. *Mol Cell Proteomics* 2014)

An integrated, European initiative

"Biomarkers of renal graft injuries in kidney allograft recipients"

(FP7-HEALTH.2012)

13 partners (Belgium, France, Germany, Sweden)

Funding: 6 M€

SEVENTH FRAMEWORK PROGRAMME

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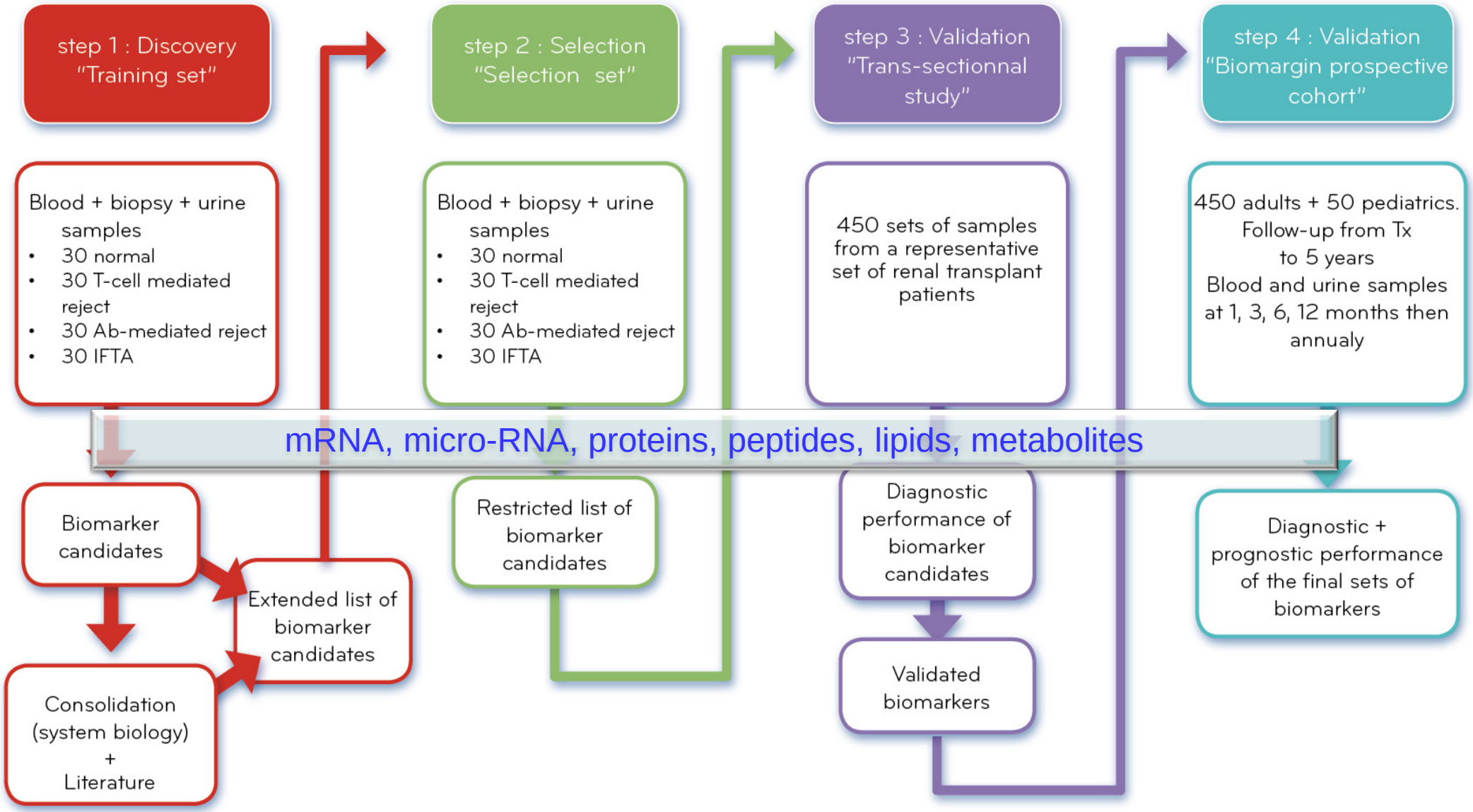
Detect, select and validate:

- Non-invasive, diagnostic and prognostic biomarkers of kidney graft lesions
  - ✓ early biomarkers of acute or chronic lesions, as well as of graft loss-of-function
  - ✓ by combining transcriptomics, proteomics and metabolomics, in blood and urine
- Mechanism-based tissue biomarkers, to improve the histological analysis and interpretation of graft biopsies



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In summary

- Already many proteomic biomarker candidates for kidney allograft lesions … at least for AR! The lists are increasing with progress in mass spectrometry.
- However, all non-targeted studies have reported new candidates → have the best been discovered?
- BIOMARGIN:
  - is seeking new candidates, using in parallel CE-TOF, MALDI-TOF/TOF, LC-ESI-Q-TOF and LC-ESI-Orbitrap!
  - will then evaluate and rank as many candidates as possible, using LC-MRM
  - In the context of systems biology and all the other known predictors
- To be adopted clinically, it should be proven that biomarkers improve long-term graft survival and patients' quality of life (need for randomized, controlled clinical trials)

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Ask