DECODING EARLY TRANSPLANT GRAFT BIOPSIES - A TRANSCRIPTOMIC ANALYSIS OF PANCREAS EARLY ACUTE REJECTION
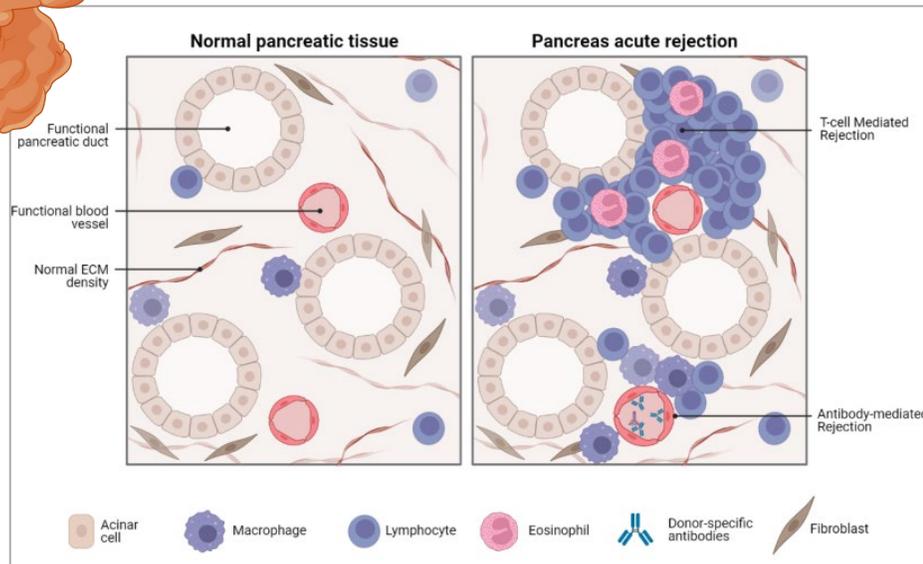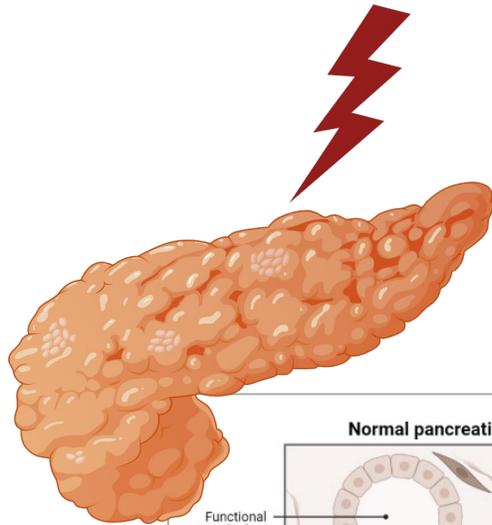
Charlotte Franken, Tim Swaab, Jordi Rovira, Ivan Archilla, Mª Angeles GarciaCriado, Joana Ferrer-Fàbrega, Fritz Diekmann, Maria Ramirez-Bajo, Pedro Ventura-Aguiar

LENIT – Laboratori Experimental de Nefrologia i Trasplantament

Pancreas transplant acute rejection

Normal pancreatic tissue | Pancreas acute rejection

- Functional pancreatic duct
- Functional blood vessel
- Normal ECM density
- T-cell Mediated Rejection
- Antibody-mediated Rejection

Acinar cell | Macrophage | Lymphocyte | Eosinophil | Donor-specific antibodies | Fibroblast

Incidence of acute rejection (AR)

10 – 35 % in first 12 months post-Tx

Risk factors for rejection

- Transplant type: SPK < PAK < PTA
- Donor age
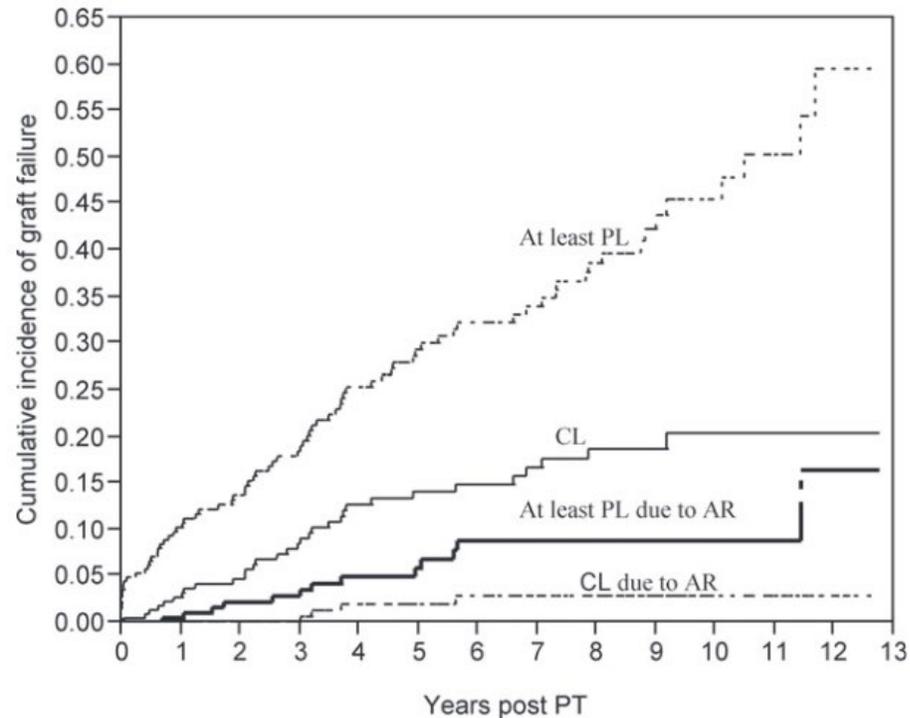- DSA presence
- Previous AR episode

AR = Acute rejection; Tx = transplant; SPK = Simultaneous pancreas-kidney transplant; PAK = pancreas after kidney transplant; PTA = pancreas transplant alone

Pancreas transplant acute rejection

Acute Pancreas Allograft Rejection Is Associated With Increased Risk of Graft Failure in Pancreas Transplantation

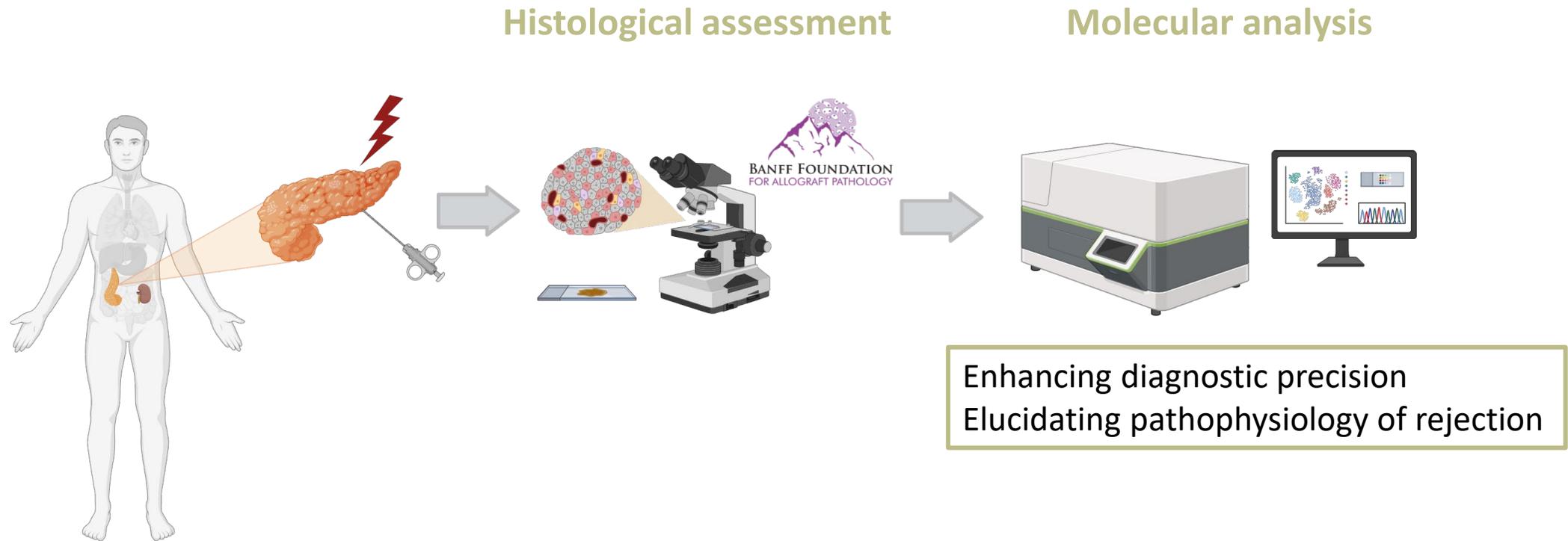## Incidence of acute rejection (AR)

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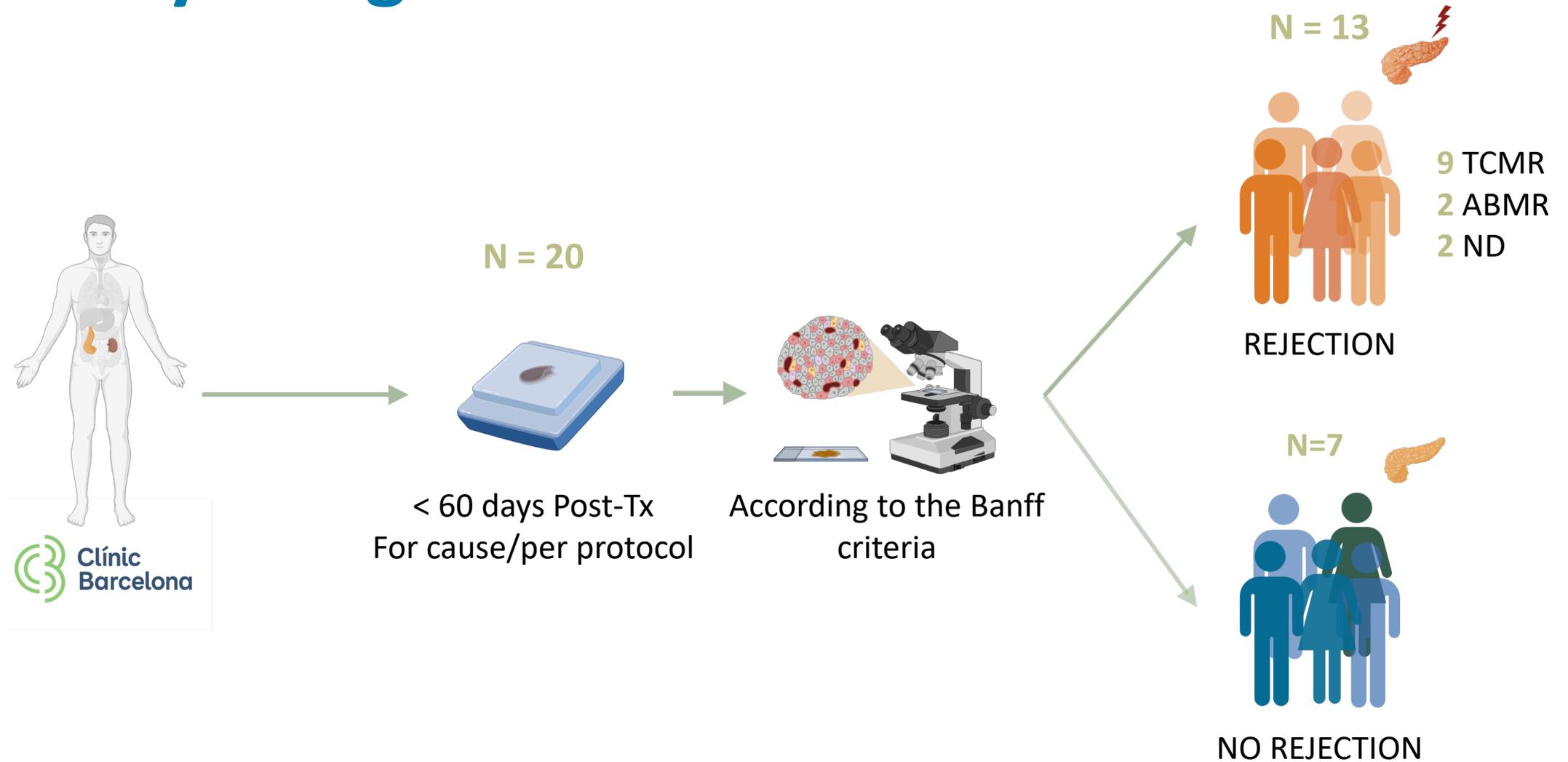
- Donor age

- DSA presence

- Previous AR episode

➡ AR is associated with increased risk of **graft failure**

AR = Acute rejection; Tx = transplant; SPK = Simultaneous pancreas-kidney transplant; PAK = pancreas after kidney transplant; PTA = pancreas transplant alone

Pancreas graft rejection analysis

Histological assessment

Molecular analysis

Enhancing diagnostic precision
Elucidating pathophysiology of rejection

➡ **Need:** Development of **Pancreas-specific** genesets

Study design

N = 13

9 TCMR
2 ABMR
2 ND

REJECTION

N = 20

< 60 days Post-Tx
For cause/per protocol

According to the Banff
criteria

N=7

NO REJECTION

Tx = transplant; Bx = biopsy; TCMR = T cell-mediated rejection; ABMR = Antibody-mediated rejection; ND = undetermined

Methods

- Samples: FFPE pancreas biopsies taken up to 60 days post-Tx (n = 20)
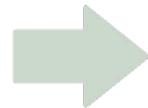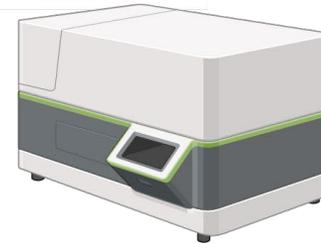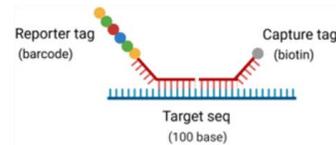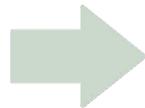- B-HOT panel: 770 genes associated with graft injury

Workflow

Data analysis

Pancreas biopsy

FFPE samples

FFPE = formalin-fixed, paraffin-embedded; Tx = transplant; B-HOT = Banff-Human Organ Transplant

Study population characterisation

	Overall (n=20)	No rejection (n= 7)	Rejection (n= 13)	P value
Recipient age (years; mean±SD)	39±8,3	43,1±9	36,8±7,4	0,11
Donor age (years; median (IQR))	33,5 (21,3 – 45,0)	36 (19,0 – 46,0)	31 (22,5 – 45,0)	0,84
Time between PTx and Bx (days, mean±SD)	28,6±14,6	28,7±10,8	28,5±16,6	0,97
Transplant type (n, %): SPK	16 (80%)	6 (85,7%)	10 (76,9%)	1,00
PAK	4 (20%)	1 (14,3%)	3 (23,1%)	
cPRA (%, median (IQR))	0 (0,0 – 50,3)	0 (0,0 - 51)	0 (0 – 49,5)	0,86
Total HLA mismatches (median (IQR))	5 (4 – 6)	6 (5 – 6)	5 (4 – 5)	0,15
CIT (h, median (IQR))	8,5 (7,1 – 10,5)	9 (7,9 – 12,0)	8 (6,9 – 10,4)	0,21

*All recipients received standard induction therapy, consisting of tacrolimus, prednisone, and mycophenolate mofetil (MMF)
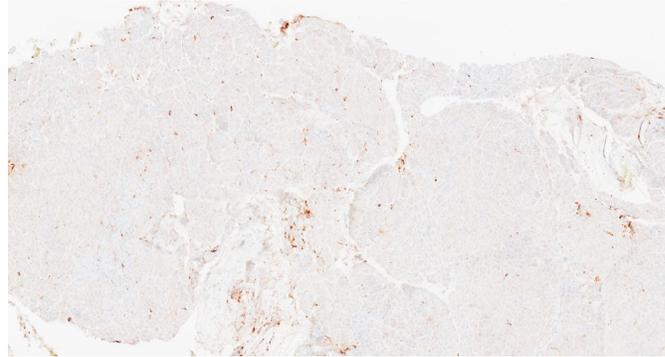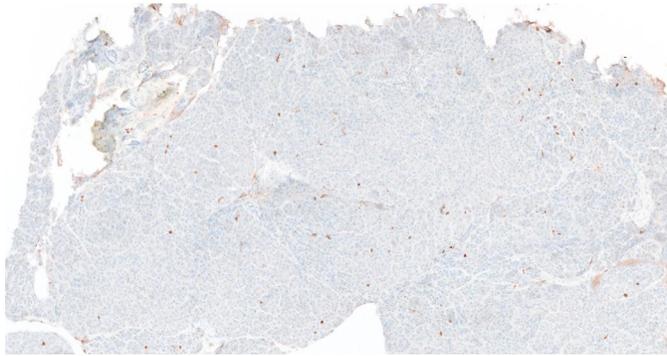
Histological characterisation

	Overall (n=20)	No rejection (n= 7)	Rejection (n= 13)	P value
Septal inflammation (n, %): 1+	14 (70%)	3 (42,9%)	11 (84,6%)	0,03
2+	1 (5%)	0 (0%)	1 (7,7%)	
Ductal inflammation (n, %): 1+	11 (55%)	0 (0%)	11 (84,6%)	< 0,001
2+	1 (5%)	0 (0%)	1 (7,7%)	
Acinar inflammation (n, %): 1+	6 (30%)	1 (14,3%)	5 (38,5%)	0,43
2+	1 (5%)	0 (0%)	1 (7,7%)	
Arteritis (n, %): +	1 (7,1%)	0 (0%)	1 (10%)	1,00
Venulitis (n, %): +	7 (35%)	0 (0%)	7 (53,8%)	0,04
Isleitis (n, %): 1+	2 (10%)	0 (0%)	2 (15,4%)	0,68
2+	1 (5%)	0 (0%)	1 (7,7%)	
C4d: +	1 (5%)	0 (0%)	1 (7,7%)	1,00
Fibrosis: +	2 (10,5%)	1 (14,3%)	1 (8,3%)	1,00

Histological characterisation – IHQ

CD3 CD68

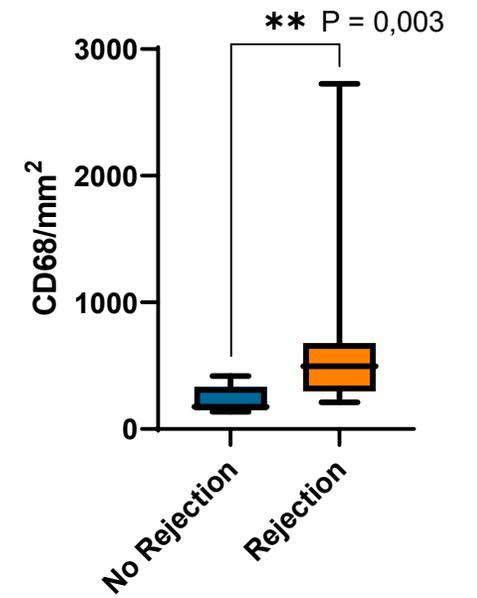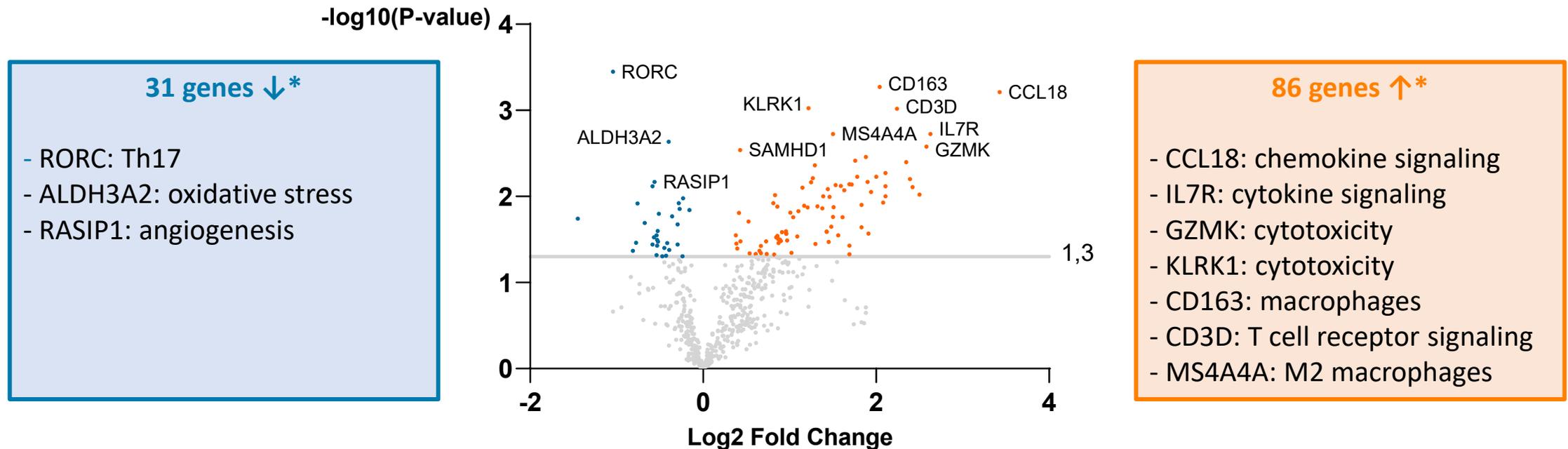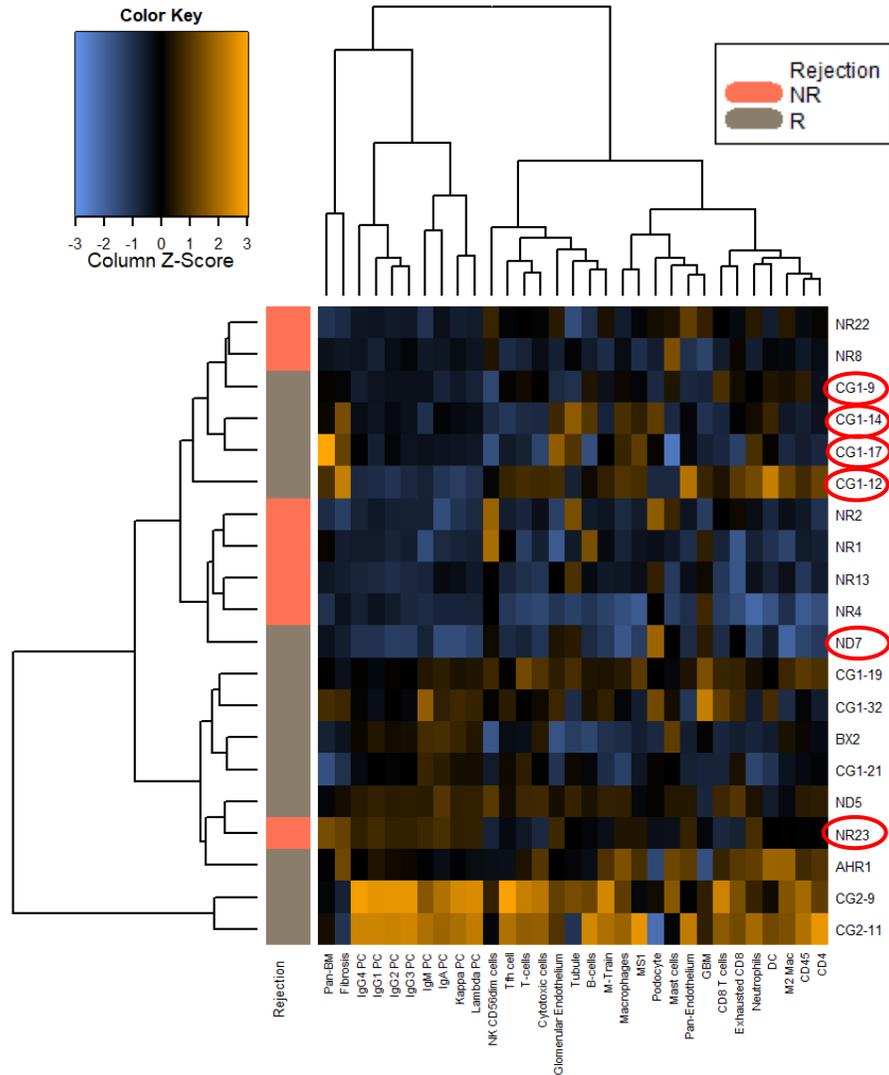No rejection

Rejection

Acute Rejection – Differently expressed genes

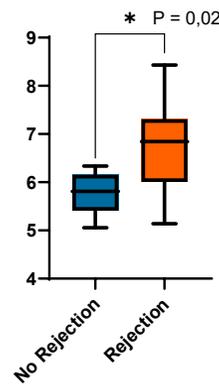**117** significantly differently expressed genes ➡ **31** underexpressed & **86** overexpressed
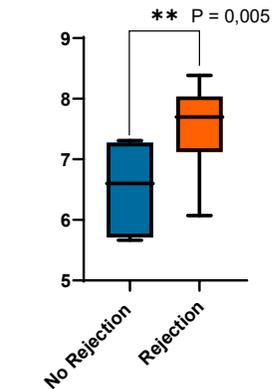
Rejection vs No Rejection (baseline)

-log10(P-value)

31 genes ↓ *

- RORC: Th17
- ALDH3A2: oxidative stress
- RASIP1: angiogenesis

RORC

ALDH3A2

RASIP1

KLRK1

CD163

CD3D

CCL18

SAMHD1

MS4A4A

IL7R

GZMK

1,3

Log2 Fold Change

86 genes ↑ *

- CCL18: chemokine signaling
- IL7R: cytokine signaling
- GZMK: cytotoxicity
- KLRK1: cytotoxicity
- CD163: macrophages
- CD3D: T cell receptor signaling
- MS4A4A: M2 macrophages

Acute Rejection – Cell type scores[1]

[1] cell type scores calculated based on the median expression of cell-specific genes

Acute Rejection – Pathway signature scores[1]

Correlation histology & cell type scores

Graft survival

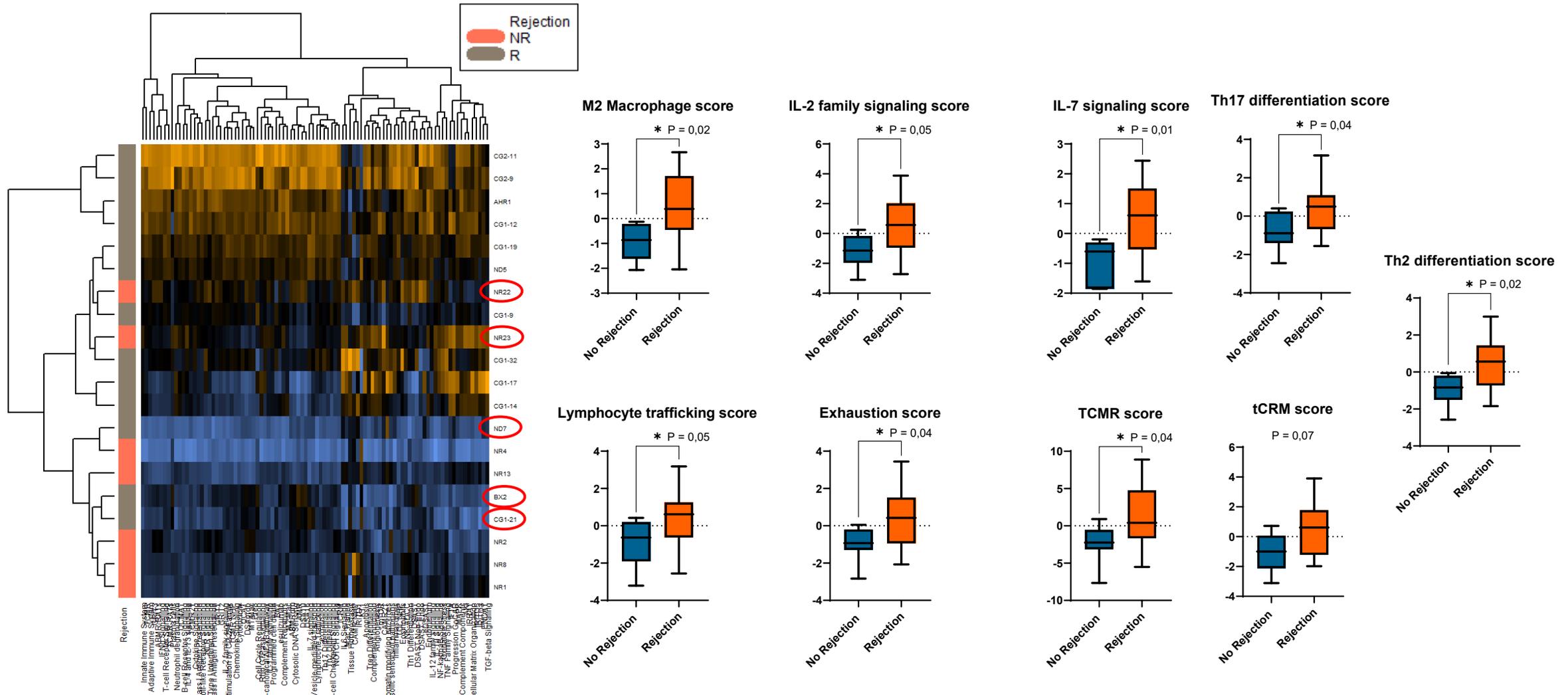|	Overall (n=20)	No rejection (n= 7)	Rejection (n= 13)	P value
Pancreas graft survival (n, %): Failed	5 (25%)	0 (0,0%)	5 (38,5%)	0,117
Functioning	15 (75%)	7 (100%)	8 (61,5%)	

Pancreas graft survival

Log-Rank P = 0,1172
HR = 0,2 (95% CI: 0,03 - 1,5)

Graft survival – Differently expressed genes

88 signficantly differently expressed genes ➡ **45** underexpressed & **43** overexpressed

Graft failure vs Graft survival (baseline)

45 genes ↓*

- MARCH8: NF-kappaB signaling
- AKR1C3: KT1, Oxidative stress
- DDX50: NF-kappa-B & IRF3 Signaling
- XBP1: Eosinophils, Oxidative stress
- HDAC3: Chromatin modifying enzymes,NOTCH signaling

-log10(P-value)

AKR1C3   MARCH8
DDX50
EZH2
XBP1   PTX3
RPL19   HDAC3   TFRC   S100A9
S100A8
1,3

Log2 Fold Change

43 genes ↑*

- EZH2: Chromatin modifying enzymes, Oxidative stress
- PTX3: M2 transition
- S100A9: M2 transition
- TFRC: M2 transition
- S100A8: M2 transition

M2

Conclusions

Transcriptomic analysis revealed distinct differences between the Rejection and No Rejection group

1. **Cell-type score comparisons** demonstrated significant differences in **dendritic cell** and **T-cell-related scores** between the rejection and no-rejection groups

2. **Pathway signature analysis** indicated significantly higher scores for **M2 macrophages**, **IL-7** and **IL-2** signaling, **Th17** and **Th2** differentiation, and **TCMR-related pathways** in the rejection group

3. Recipients with **graft failure** exhibited a significantly **higher expression** of **M2** macrophage-associated genes

4. A deeper understanding of the **molecular drivers** of acute pancreas transplant rejection could enhance **diagnostic accuracy** and support the development of a **pancreas-specific gene panel**, with potential therapeutic implications

Acknowledgements

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