

**# NK...**  
**@ transplantation rénale**

17 mars 2018

Tristan LEGRIS

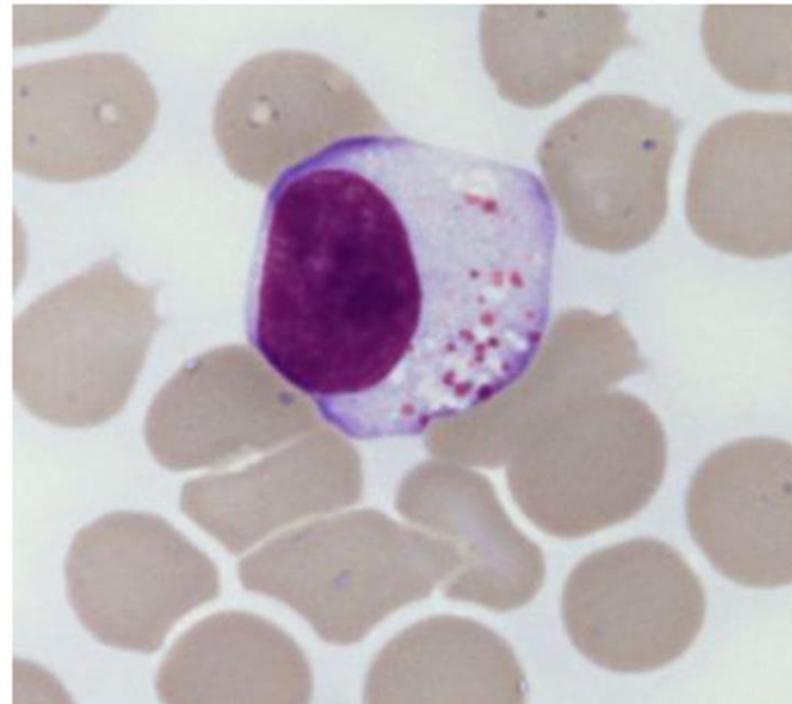
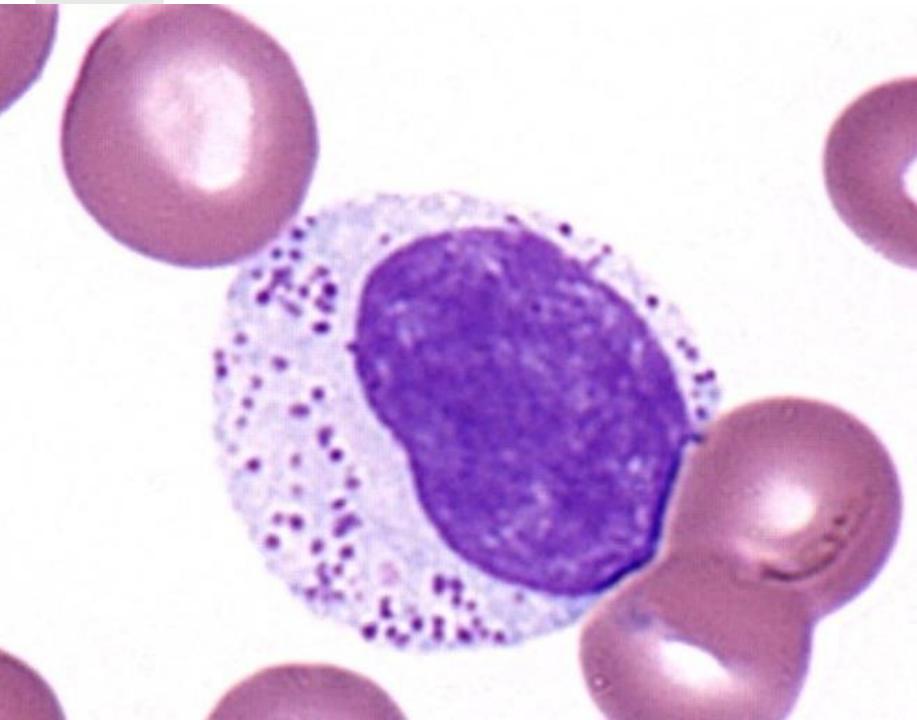
Hôpital Conception, Marseille

# # Natural Killer cells @ transplantation rénale

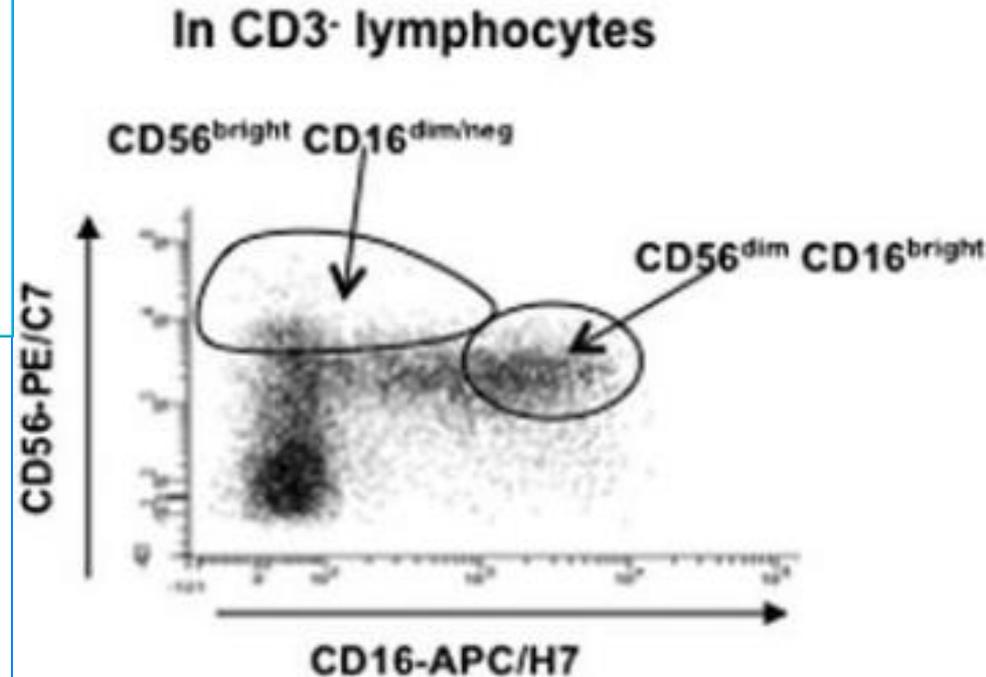
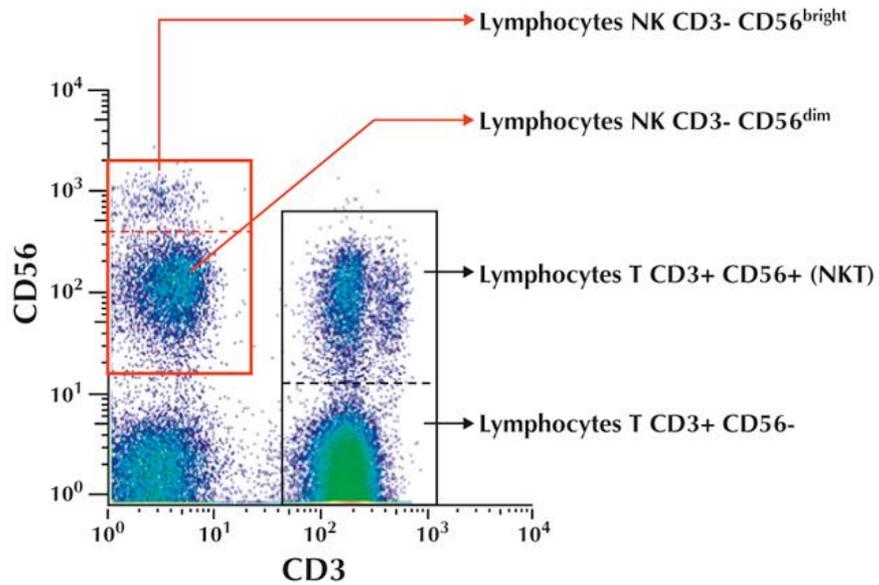
- ① #NK: rappels/généralités
- ② # Interactions KIR/HLA en greffe rénale?
- ③ #NK : 1<sup>ère</sup> ligne de défense?
  - ① CMV / BKv
  - ② Cancers
- ④ #NK et rejets médiés par anticorps?

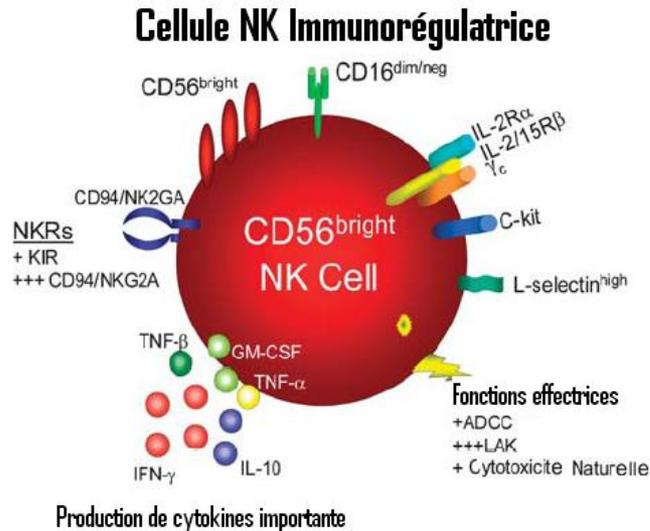
## Rappels/généralités

- 5 à 20% des lymphocytes du sang périphérique
- Grands lymphocytes, granules cytoplasmiques
- Turnover de 2 semaines
- Localisation : sang, moelle, foie, rate

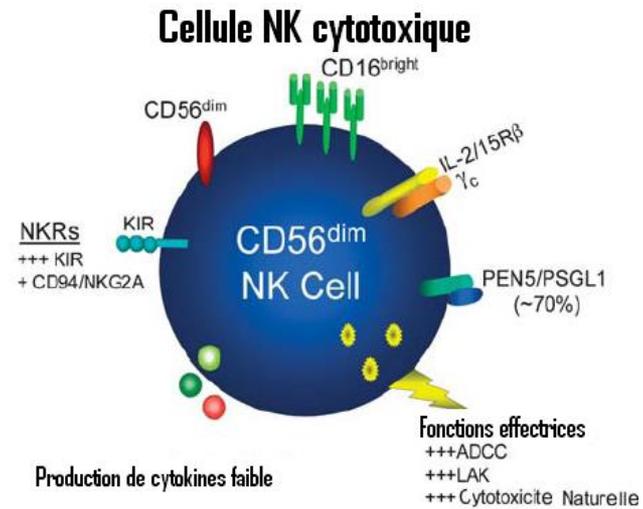


# Rappels/généralités





**CD3<sup>-</sup> CD56<sup>bright</sup> CD16<sup>dim</sup>**  
**≈ 10%**



**CD3<sup>-</sup> CD56<sup>dim</sup> CD16<sup>bright</sup>**  
**≈ 90%**

- Longtemps considérées comme cellules de l'immunité « innée »
  - Pas de stimulation antigénique par CPA
  - Pas de nécessité de coopération LT CD4
- Production de cytokines/interactions avec Ig/interactions avec DC
- Grand répertoire de récepteurs activateurs/inhibiteurs

# Cellule NK

Int  
rec

Activating receptors,  
adhesion or costimulation  
molecules

## KIR-L HLA-C, B and A

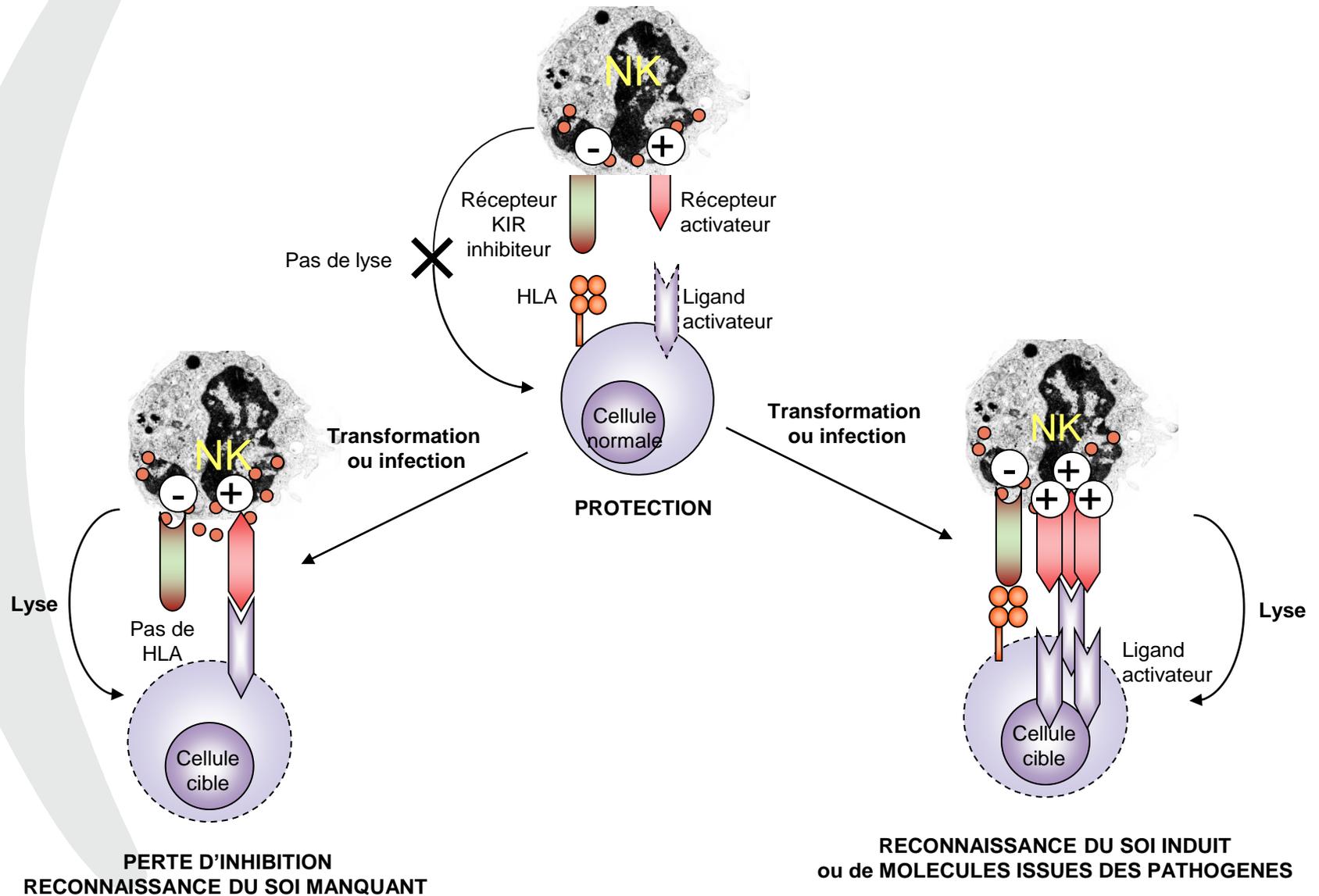
LILRB1 (CD85j, ILT2)	HLA class I
CD244 (2B4)	CD48
CS1 (CRACC, CD319)	CS1 (CRACC, CD319)
$\alpha_4\beta_1$ integrin	VCAM-1 (CD106)
$\beta_2$ integrins (CD11a-CD18, CD11b-CD18, CD11c-CD18)	ICAM-1 (CD54) ICAM-2 (CD102) CD23 iC3b...
CD226 (DNAM-1)	CD112 (Nectin-2), CD155 (Necl5)
CRTAM	Necl2

## CD16 Immunoglobulin G

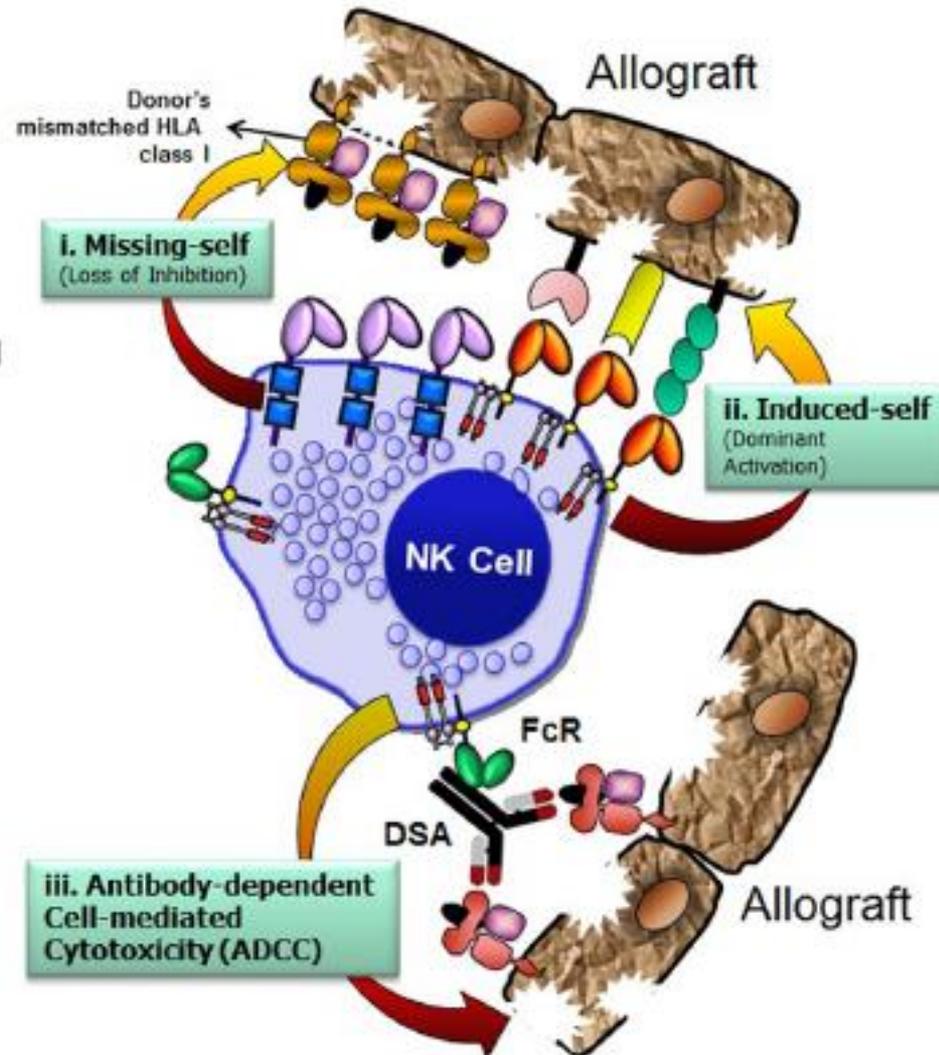
KIR-S	HLA-C, ?
CD94-NKG2C	HLA-E
CD94-NKG2E	HLA-E
NKG2D	ULBP (RAET), MICA, MICB
NTB-A	NTB-A
PEN-5	L-selectin
CD96 (Tactile)	CD155 (Necl5)
NKp80	AICL
CD100	CD72
NKp30	pp65, BAT-3, ?
NKp44	Viral hemagglutinins, ?
CEACAM1 (CD66)	CEACAM1 (CD66)
CD160 (BY55)	HLA-C

# Cellule cible

# Equilibre dynamique inhibition/activation

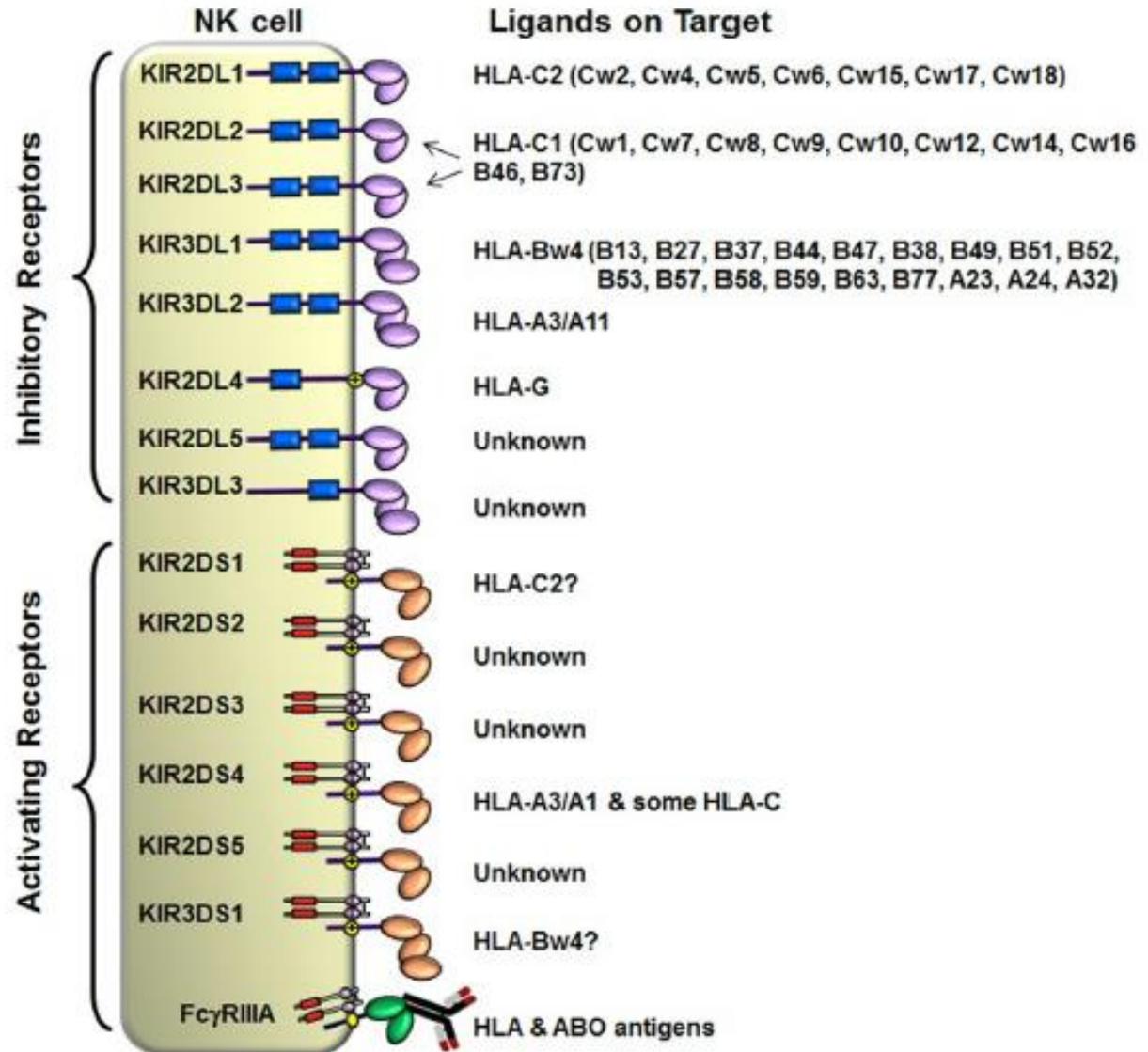


# Alloréactivité NK en transplantation?



# Rôle des KIR en greffe rénale?

# Rôle des KIR/HLA ?



## Mismatches KIR/KIR ligands (=HLA classe I)

	KIR-ligand mismatch <sup>1</sup>		
	Recipient		Donor HLA
	KIR	HLA	
KIR2DL1	KIR2DL1+	C2+	C2-
KIR2DL2/3	KIR2DL2+ or KIR2DL3+	C1+	C1-
KIR3DL1	KIR3DL1+	Bw4+	Bw4-
KIR3DL2	KIR3DL2+	A3+ or A11+	A3- and A11-
KIR2DS1	KIR2DS1+	C2-	C2+

## Mismatches KIR/HLA et rejets aigus

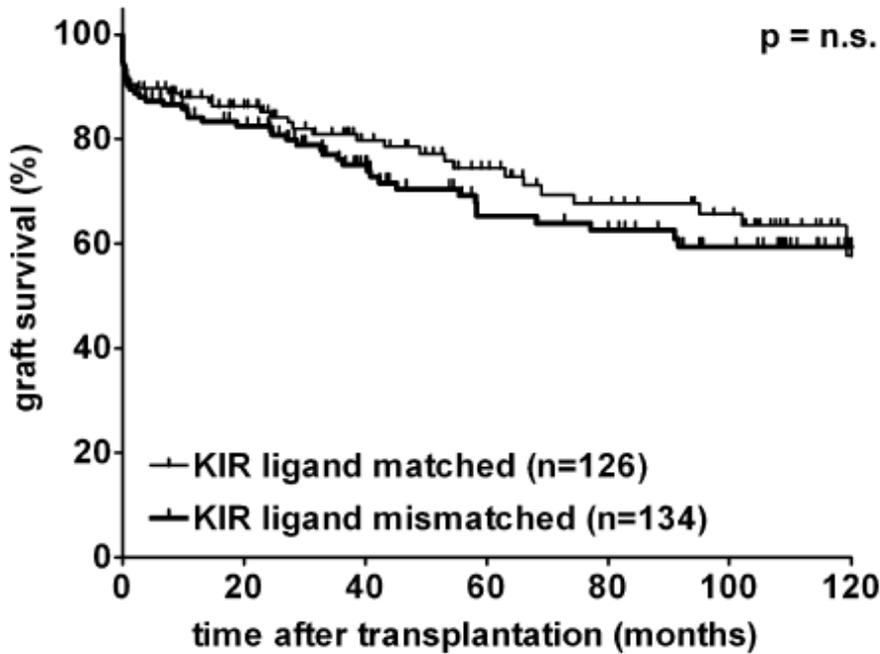
- 105 patients avec rejets cellulaires versus 119 stables
- génotypage HLA A, B et C chez donneur et receveurs
- génotypage KIR chez receveurs
- pour chaque KIR → comparaison avec HLA

KIR	HLA ligand	Acute rejection (n=105)			Controls (n=119)			P values
		Mismatch	Match	KIR-/HLA±	Mismatch	Match	KIR-/HLA±	
KIR2DL1	HLA-C/2	48 (45.7%)	53 (50.5%)	4 (3.8%)	46 (38.7%)	73 (61.3%)	0 (0%)	0.038
KIR2DL2/KIR2DS2	HLA-C/1	3 (2.9%)	33 (31.4%)	69 (65.7%)	9 (7.6%)	52 (43.7%)	58 (48.7%)	0.004
KIR2DL3	HLA-C/1	7 (6.7%)	89 (84.8%)	9 (8.6%)	25 (21.0%)	86 (72.3%)	8 (6.7%)	0.014
KIR3DL1	Bw4	38 (36.2%)	62 (59.0%)	5 (4.8%)	40 (33.6%)	76 (63.9%)	3 (2.5%)	NS
KIR3DL2	HLA-A3, A11	69 (65.7%)	34 (32.4%)	2 (1.9%)	72 (60.5%)	39 (32.8%)	8 (6.7%)	NS
KIR2DS1	HLA-C/2	24 (22.9%)	21 (20.0%)	60 (57.1%)	21 (17.6%)	27 (22.7%)	71 (59.7%)	NS
KIR2DS3	HLA-C/1	1 (1.0%)	28 (26.7%)	76 (72.4%)	5 (4.2%)	37 (31.1%)	77 (64.7%)	NS

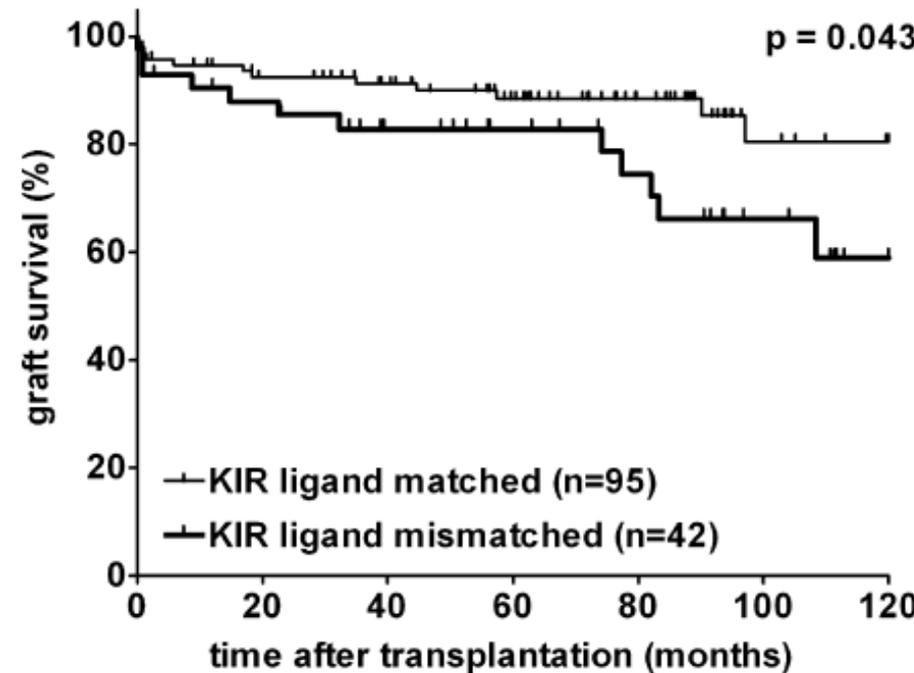
## Mismatches KIR/HLA et pronostic

- 397 transplantés à Leiden et Rotterdam, 1990→2004
- 137 compatibles en HLA A et B et 260 incompatibles
- Génotypage KIR chez receveurs

HLA-A,-B incompatible



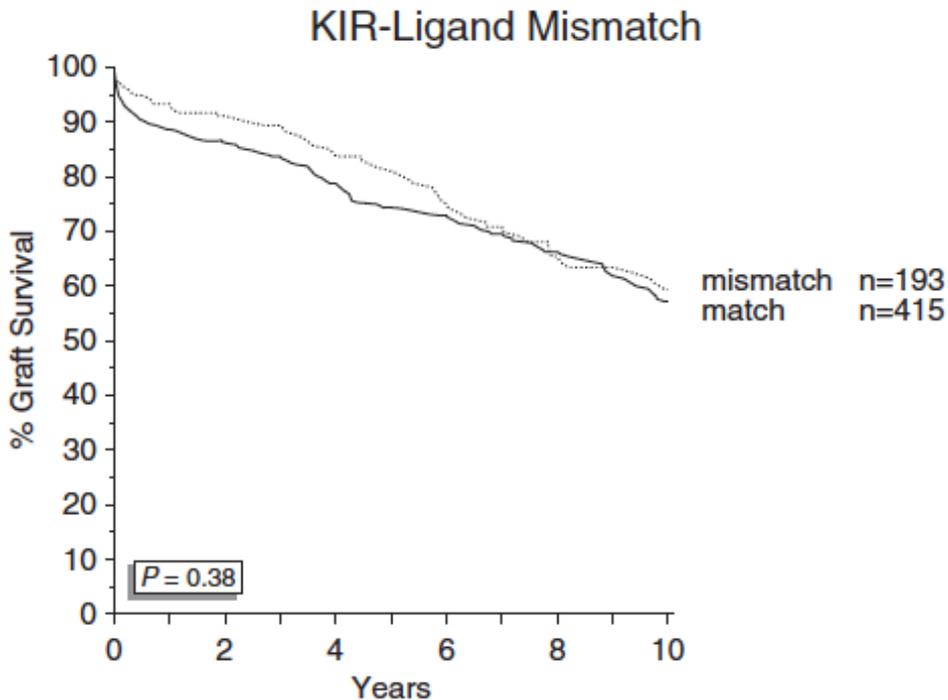
HLA-A,-B compatible



- Idem en analyse multivariée

## Mismatches KIR/HLA et pronostic

- Etude du CTS: 608 greffés HLA A-B-DR compatibles, 1988→2009
- Typages Cw (donneurs/receveurs) et KIR chez les receveurs



**Aucun effet, en multivariée, de:**

- mismatches KIR
- nombre de KIRi/KIRa
- haplotype KIR

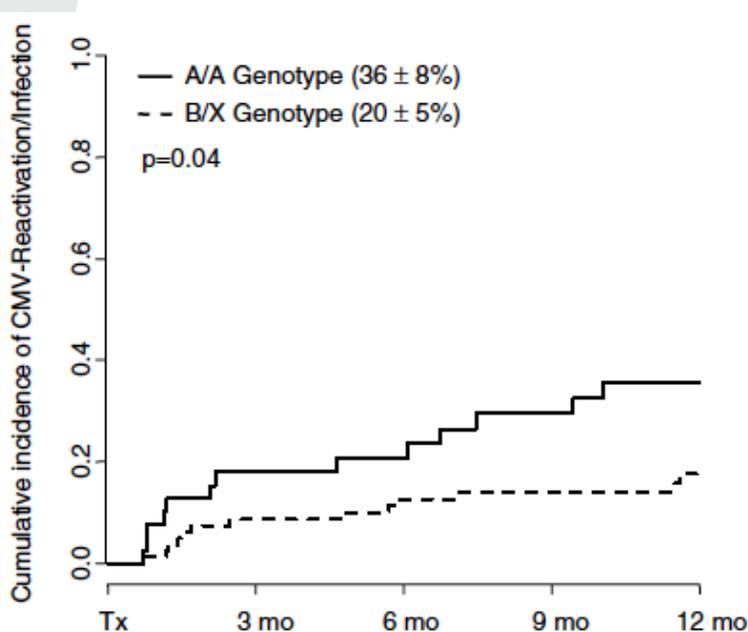
## KIR mismatches et alloimmunité:

- Rôle à priori assez limité
- Impact du mismatch KIR en cas de greffe bien matchée en HLA?
- Rôle dans certains rejets aigus vasculaires ?
  - inflammation de la microcirculation
  - sans DSA
  - équipe d'O. Thaunat

# NK, virus/cancers et greffe rénale?

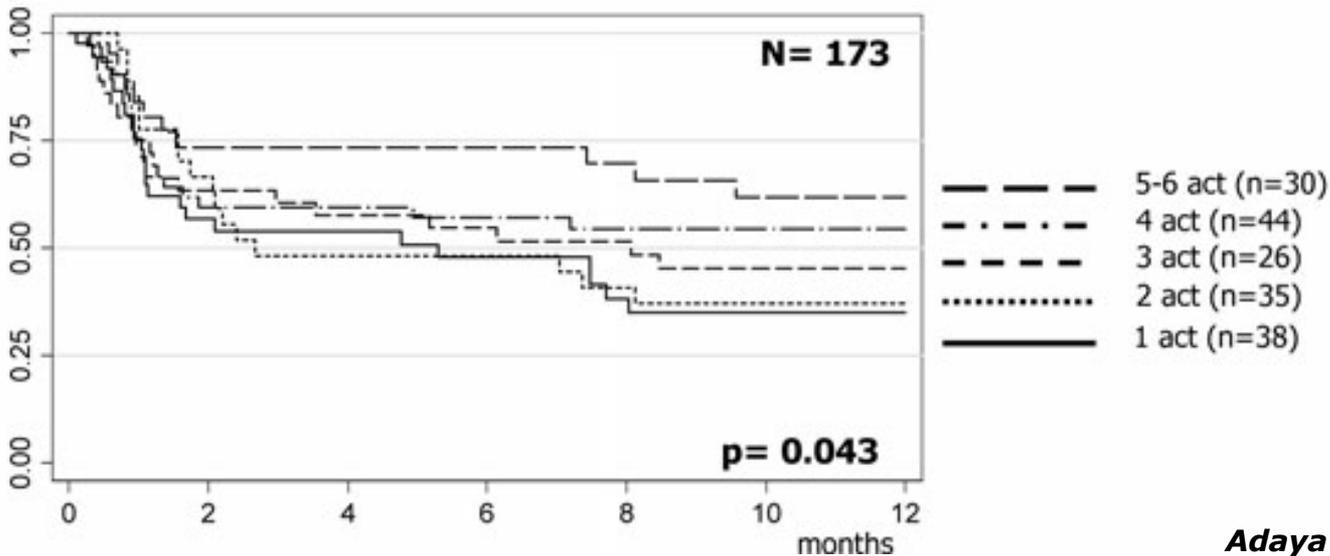
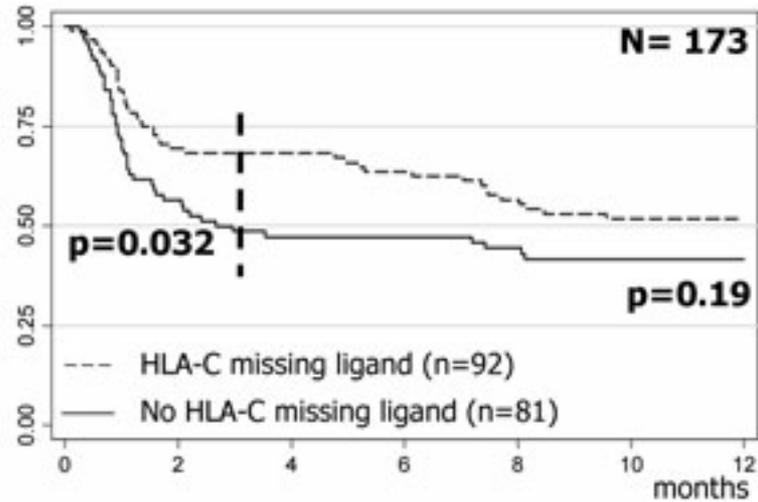
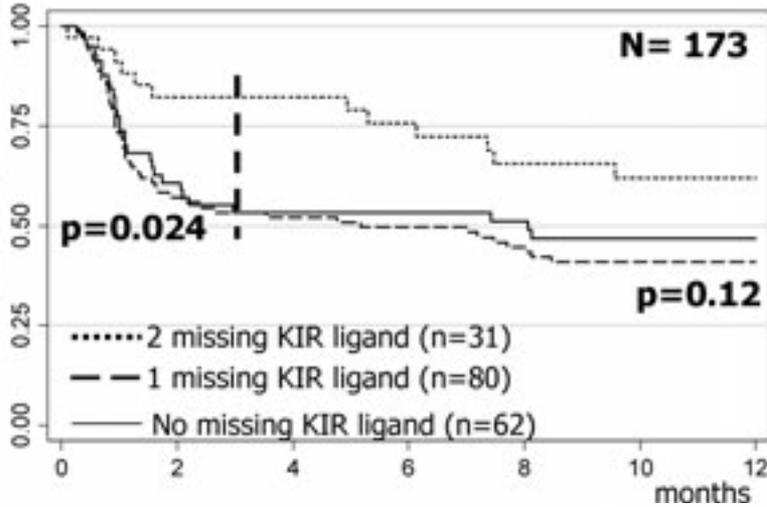
## Rôle des KIR dans l'infection à CMV?

- Haplotype A: 1 seul KIRa exprimé (AA, n=40)
- Haplotype B: 5 KIRa exprimés (AB ou BB, n=82)



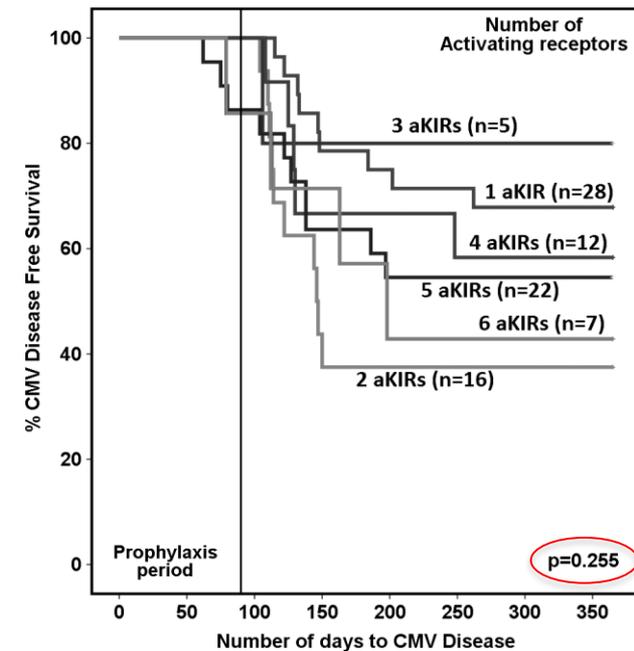
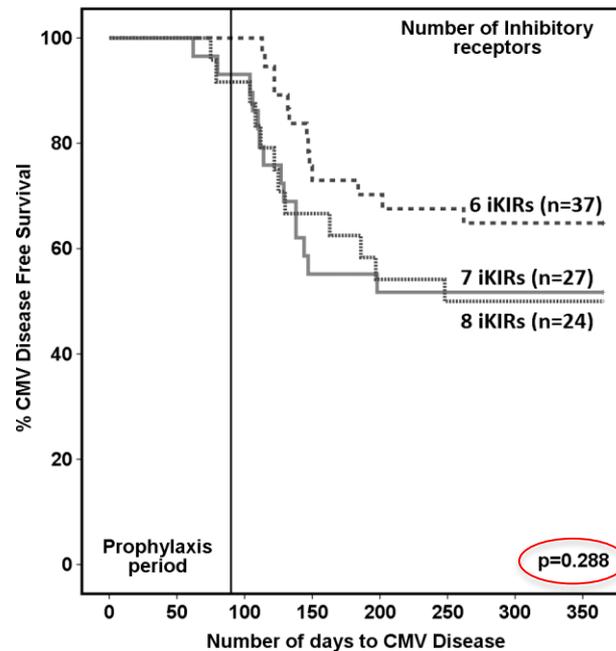
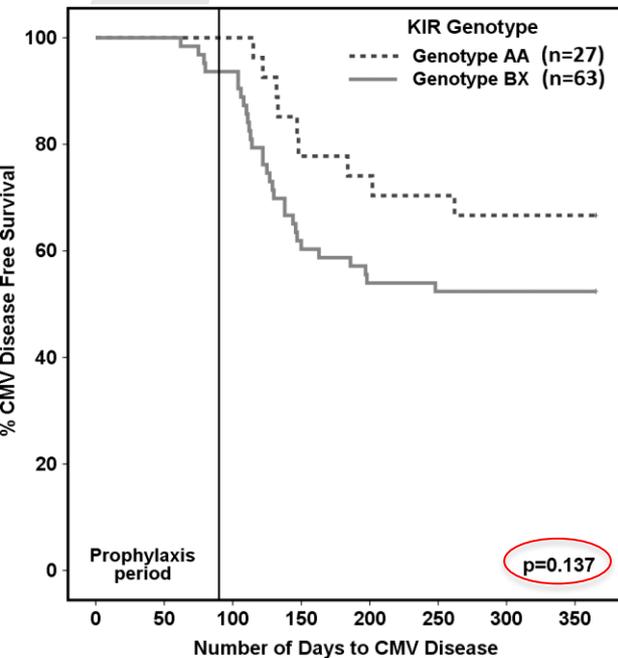
Risk factor	Cytomegalovirus		
	RR	95% CI	p
<b>Serology</b>			
Don pos/Rec pos	1.00	–	–
Don neg/Rec neg	0.12	0.02–0.97	0.04
Don neg/Rec pos	0.51	0.13–1.89	0.31
Don pos/Rec neg	5.19	1.87–14.4	0.002
<b>Valgancyclovir</b>			
During treatment	0.11	0.03–0.43	0.002
<b>Immunosuppression</b>			
Rapamycin versus prednisone	0.45	0.20–1.04	0.06
<b>Induction</b>			
Prednisone containing	n.s.		
<b>KIR Genotype</b>			
A/A	1.0	–	–
B/X	0.34	0.15–0.76	0.009
<b>Rejection</b>			
–Requiring therapy	n.s.		

# Rôle des KIR dans l'infection à CMV?



# Rôle des KIR dans l'infection à CMV?

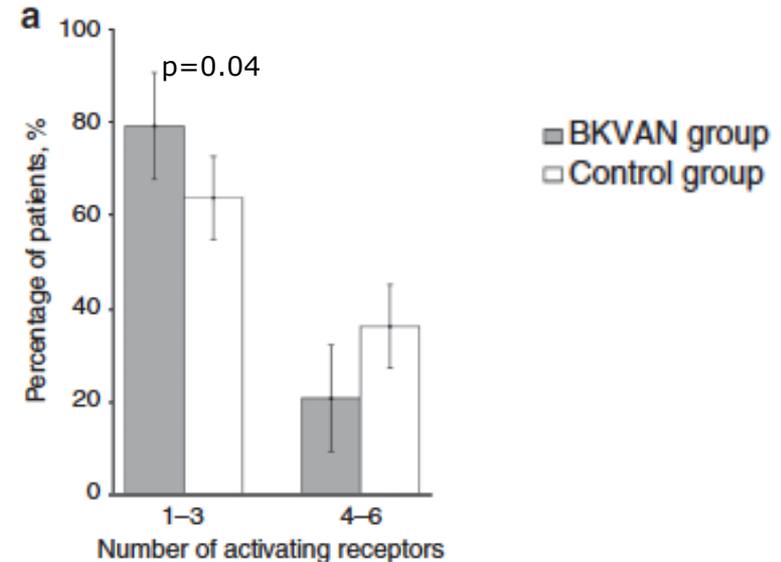
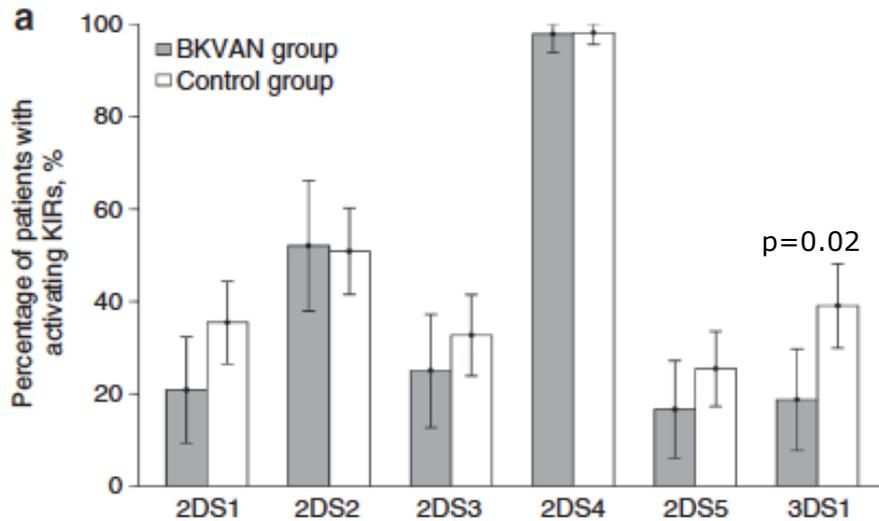
- Génotype complet KIRa et KIRi chez 90 receveurs D+/R-
- Redboud (Hollande) 2002→2011



- Pas d'influence des mismatches KIR/HLA

# Rôle des KIR dans l'infection à BKv?

- 48 patients avec néphropathie à BKv vs 110 non-infectés
- Charité, Berlin

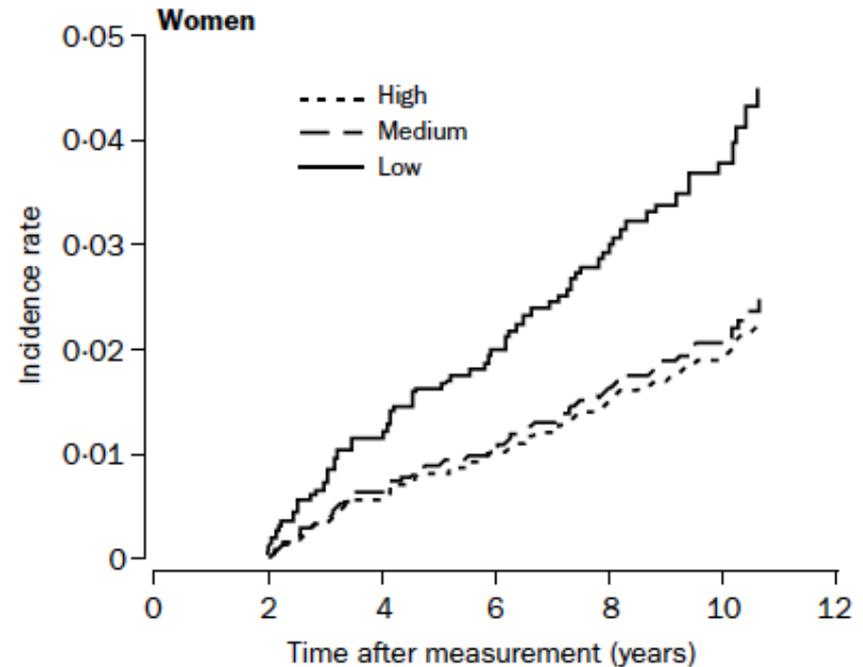
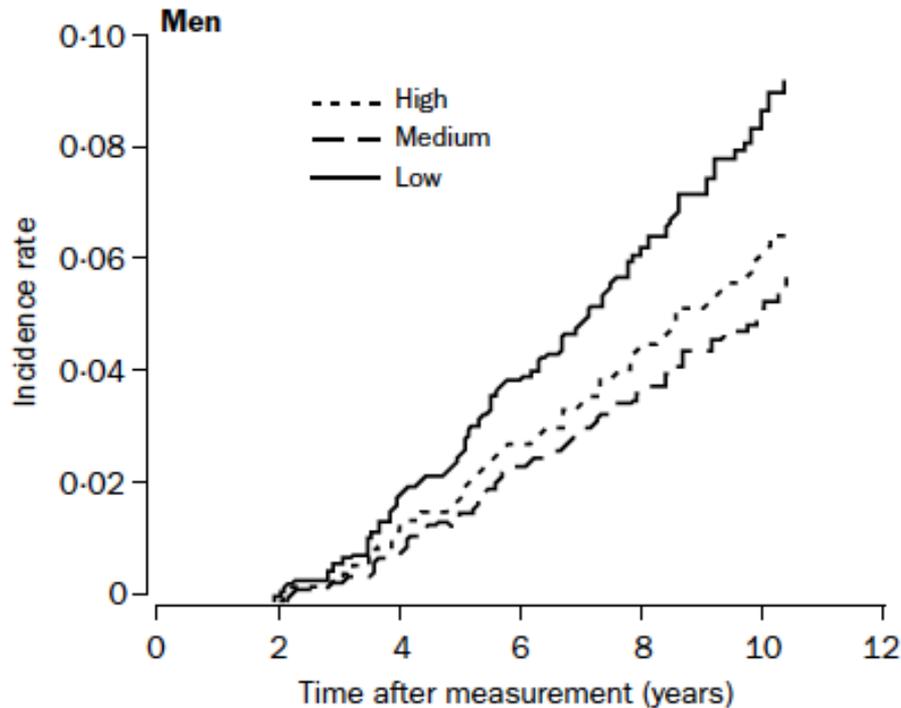


## Rôle des KIR dans l'infection à BKv?

Patients	Total	U <sup>-</sup> P <sup>-</sup>	U <sup>+</sup> P <sup>-</sup>	U <sup>+</sup> P <sup>+</sup>
Number	103	60	21	22
Donor HLA-C*07 negative	65 (63%)	41 (68%)	12 (57%)	12 (54%)
KIR haplotype AA	28 (27%)	18 (30%)	5 (24%)	5 (23%)
KIR haplotypes AB+BB	75 (73%)	42 (70%)	16 (76%)	17 (77%)
Number of recipient KIR activators				
≤3	62 (60%)	37 (62%)	11 (52%)	14 (64%)
>3	41 (40%)	23 (38%)	10 (48%)	8 (36%)
R: KIR2DS1 <sup>+</sup> C1 <sup>+</sup> D: C2 <sup>+</sup>	20 (19.5%)	7 (12%)	7 (33%)	6*** (27%)
R: KIR2DS2 <sup>+</sup> C2 <sup>+</sup> D: C1 <sup>+</sup>	33 (32%)	14 (23%)	12 (57%)	7*** (32%)
R: KIR3DS1 <sup>+</sup> Bw6 <sup>+</sup> D: Bw4 <sup>+</sup>	22 (100%)	10 (45%)	5 (23%)	7 (32%)
Missing (C1/C2/Bw4)				
0	62 (60%)	36 (60%)	13 (62%)	13 (59%)
1	34 (33%)	20 (33%)	5 (24%)	9 (41%)
2	7 (7%)	4 (7%)	3 (14%)	0

## NK et cancers: population générale

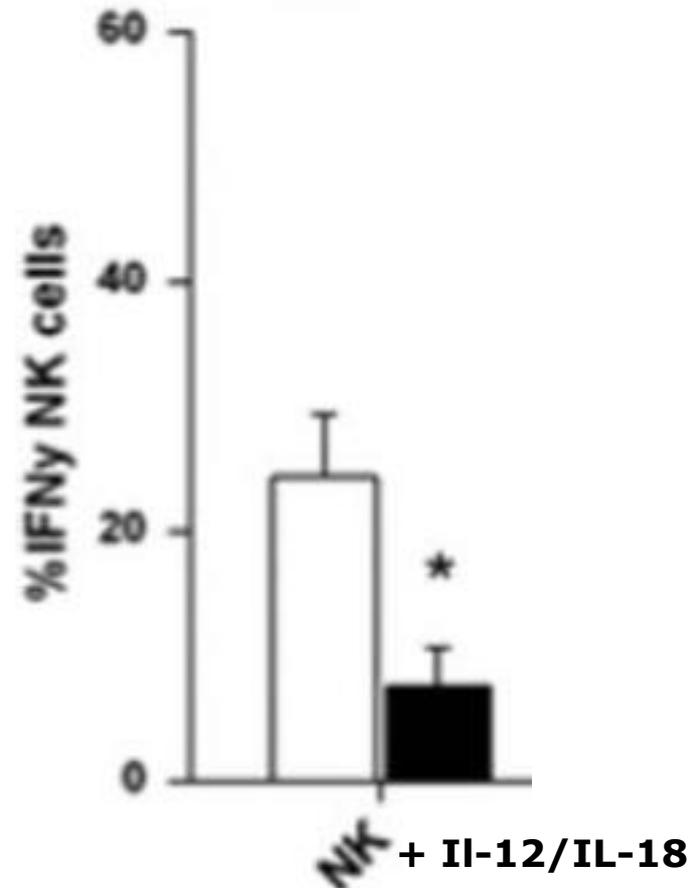
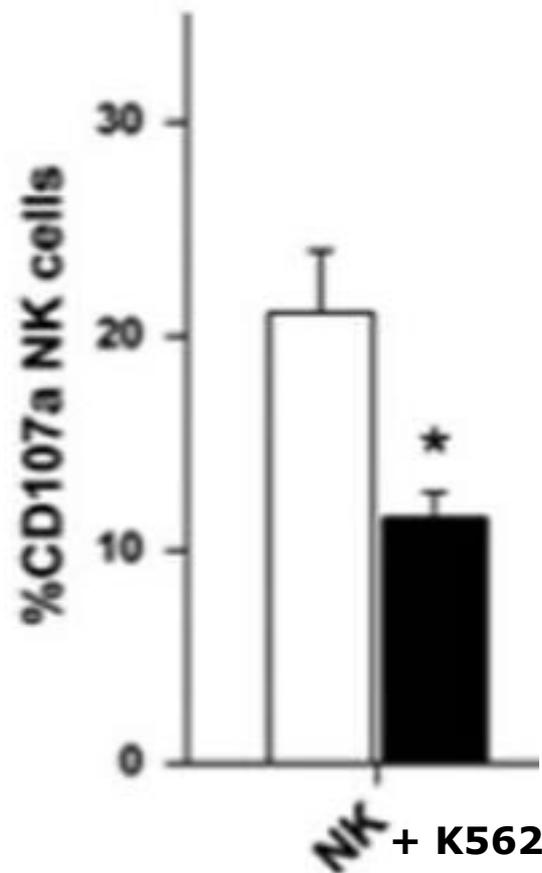
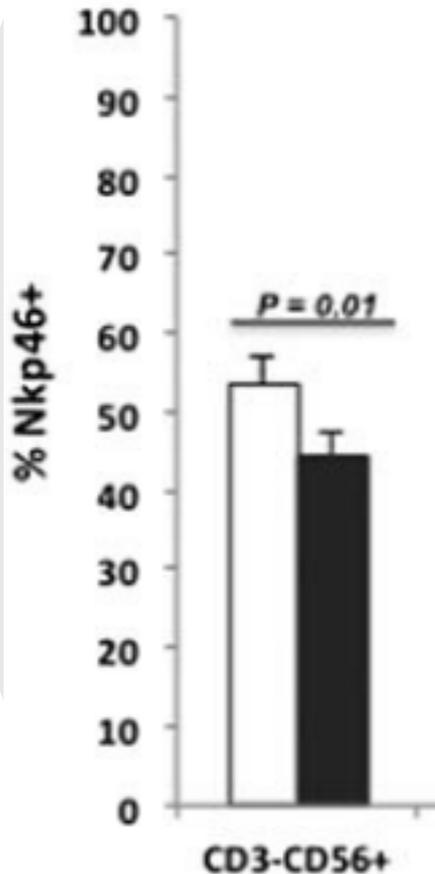
- Etude de cohorte prospective au Japon entre 1986 et 1990 (n=3625; > 40 ans)
- Test de cytotoxicité à l'inclusion
- Suivi de 11 ans en moyenne



- Après ajustement sur l'âge, BMI, habitus, alimentation

## NK et cancers: population greffée rénale

- 42 greffés avec cancer (surtout peau) et 41 contrôles
- Pas de différence dans les nombres totaux de NK périphériques

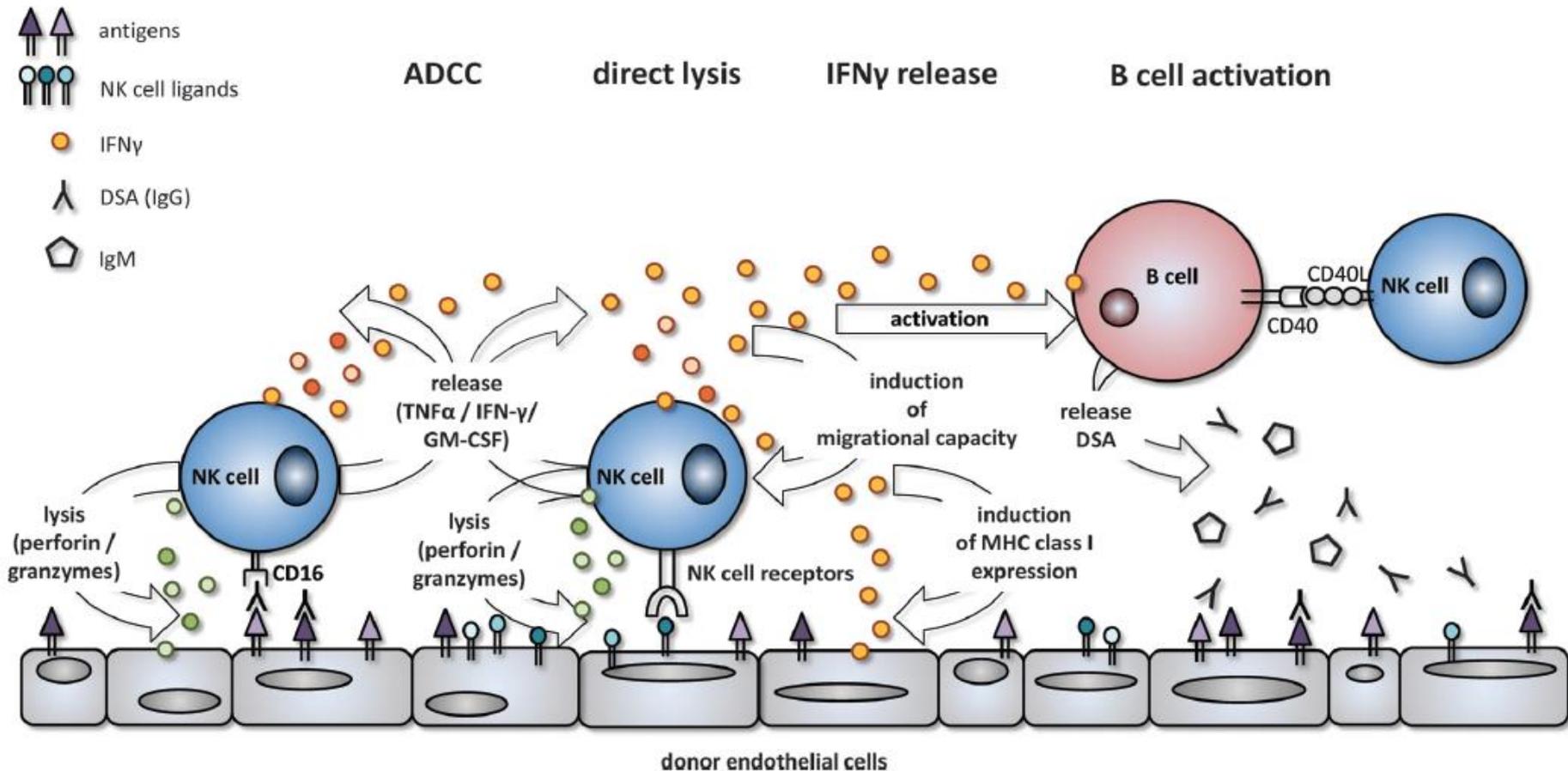


# Rôles des NK dans les AMR?

# NK et rejets médiés par anticorps (AMR)

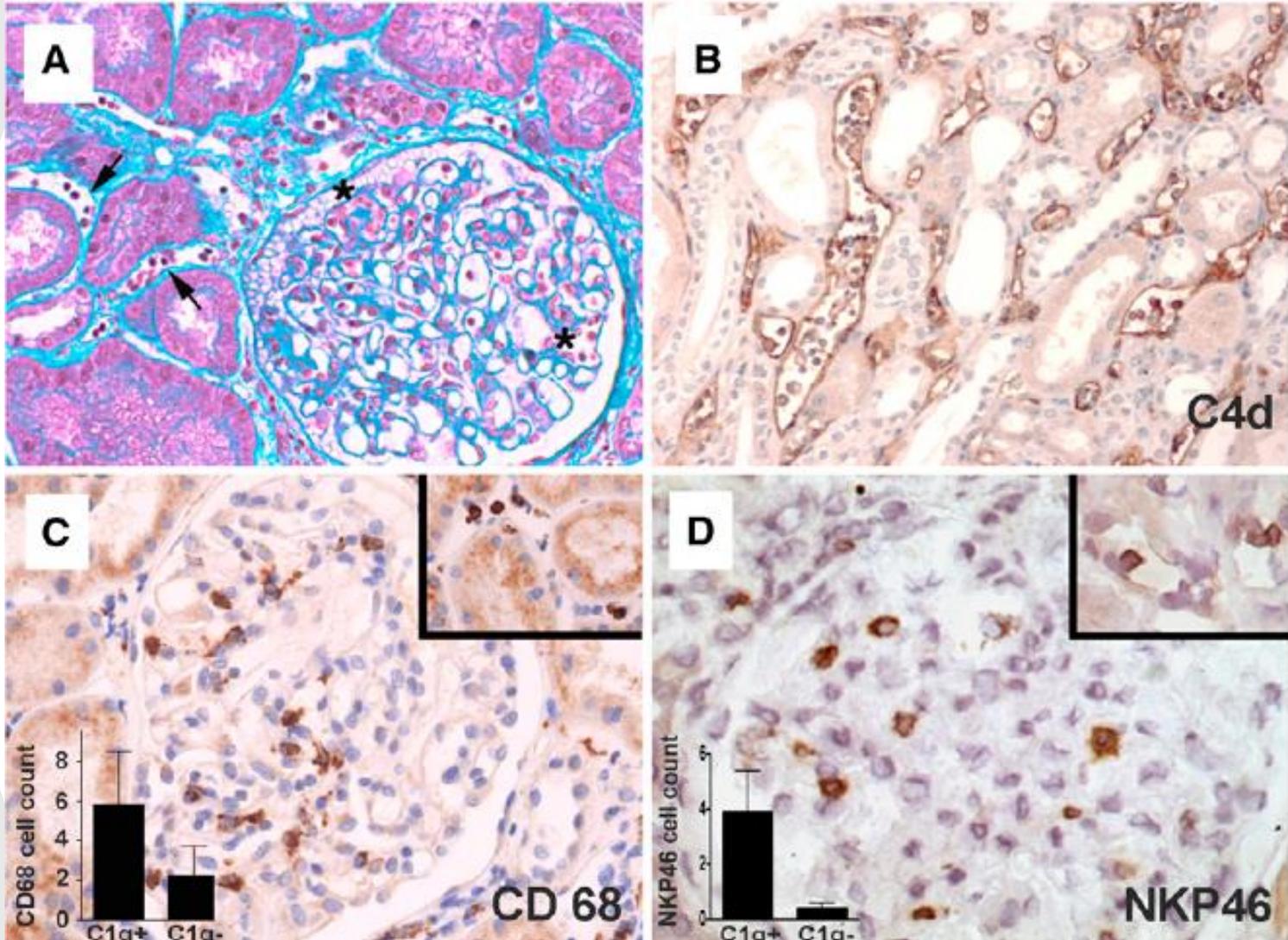
- 50-60% des AMR sont C4d négatifs

*Aubert O et al, JASN, 2017*

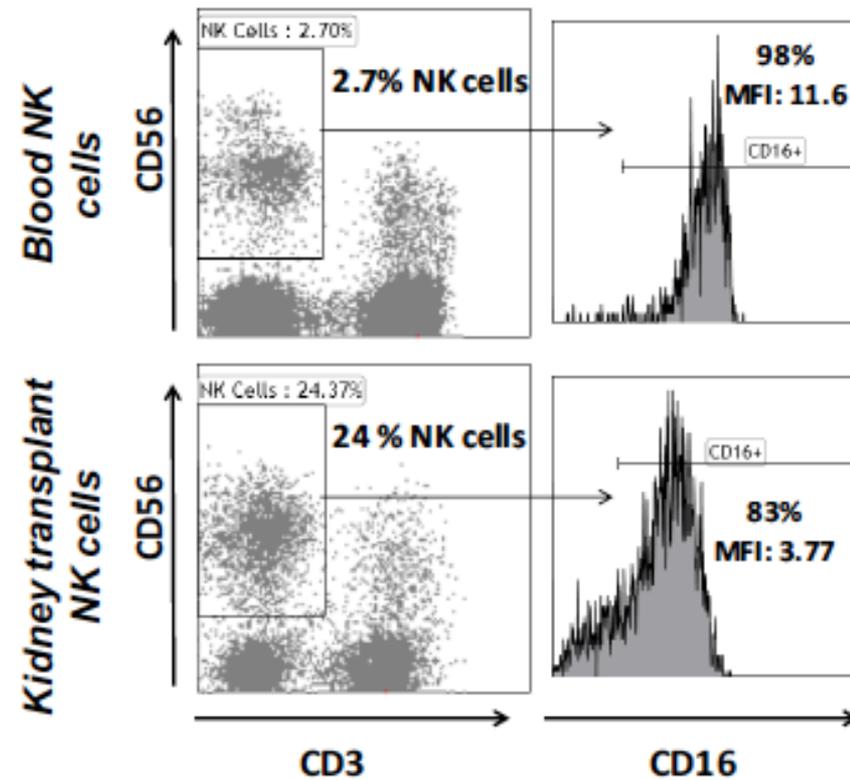
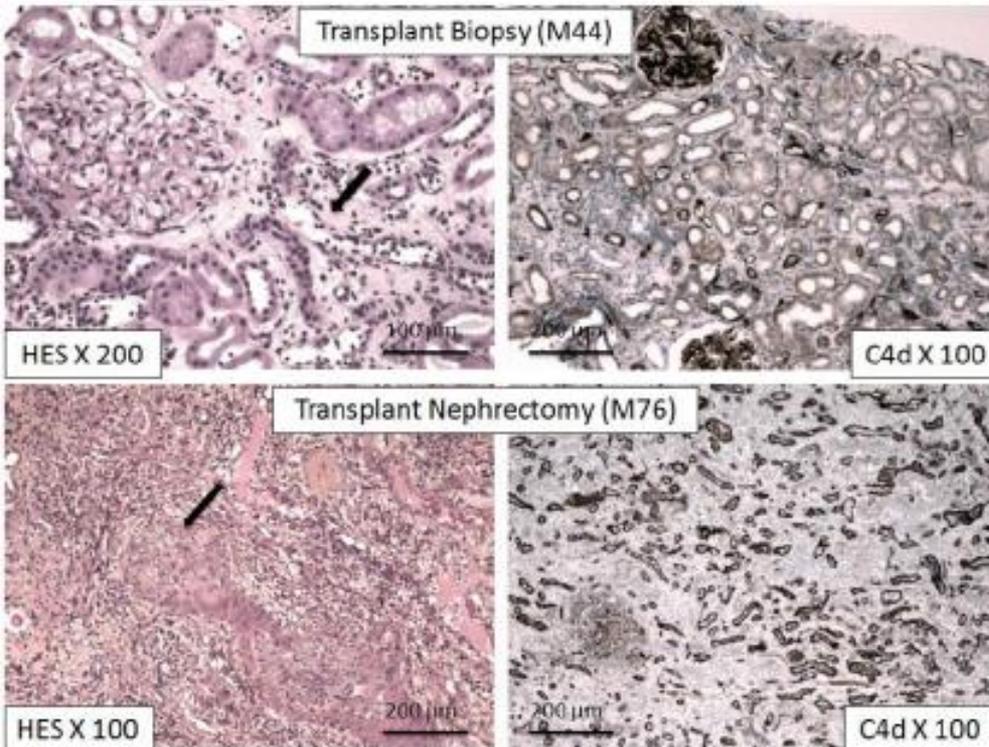


*Resch T et al, Transplantation, 2015*

# NK et AMR: quid des biopsies?

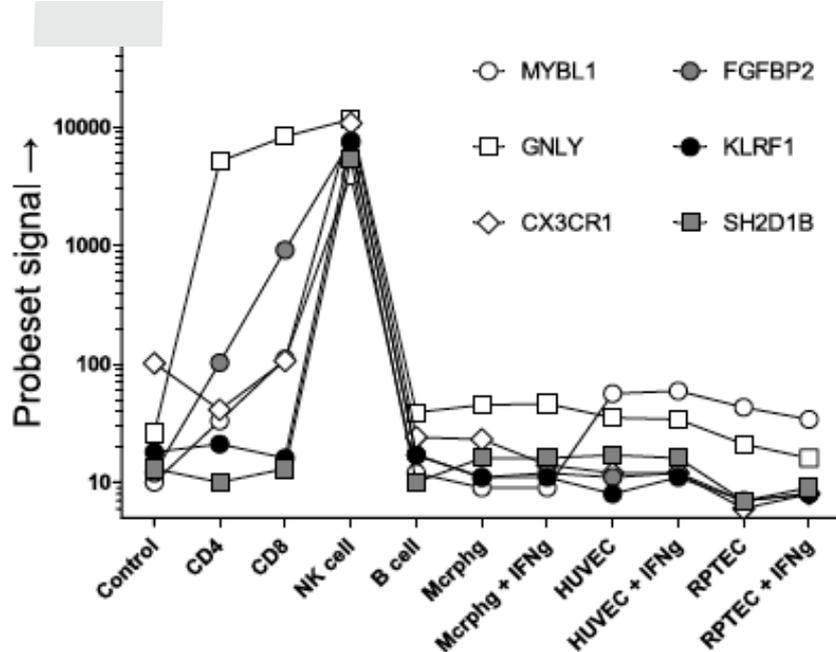
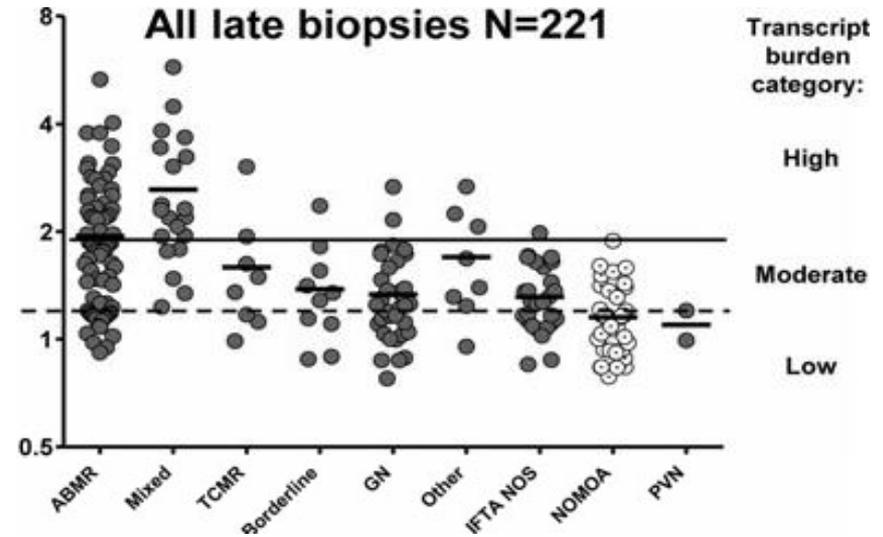
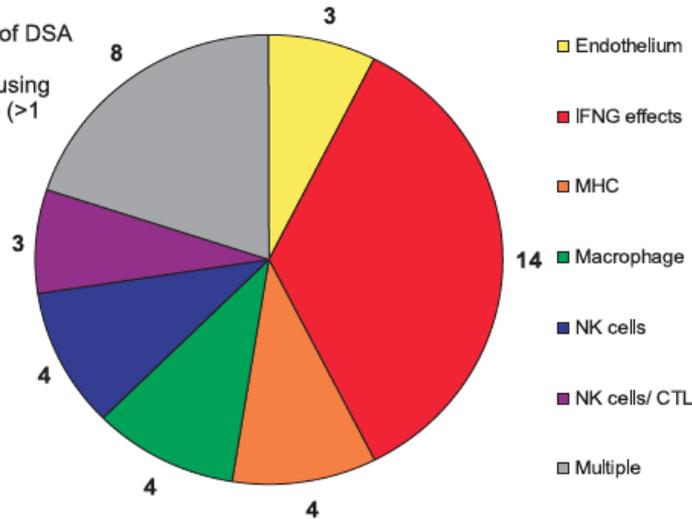


# NK et AMR: quid des biopsies?

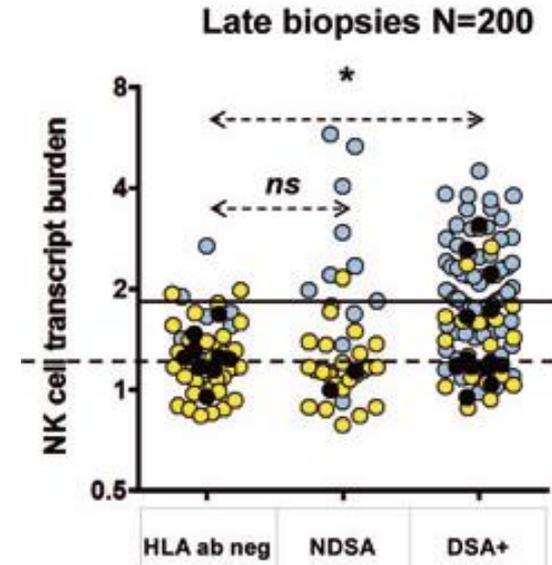


# NK et AMR: arguments transcriptomiques

Annotation of DSA associated transcripts using only 89 late (>1 year post-transplant) biopsies



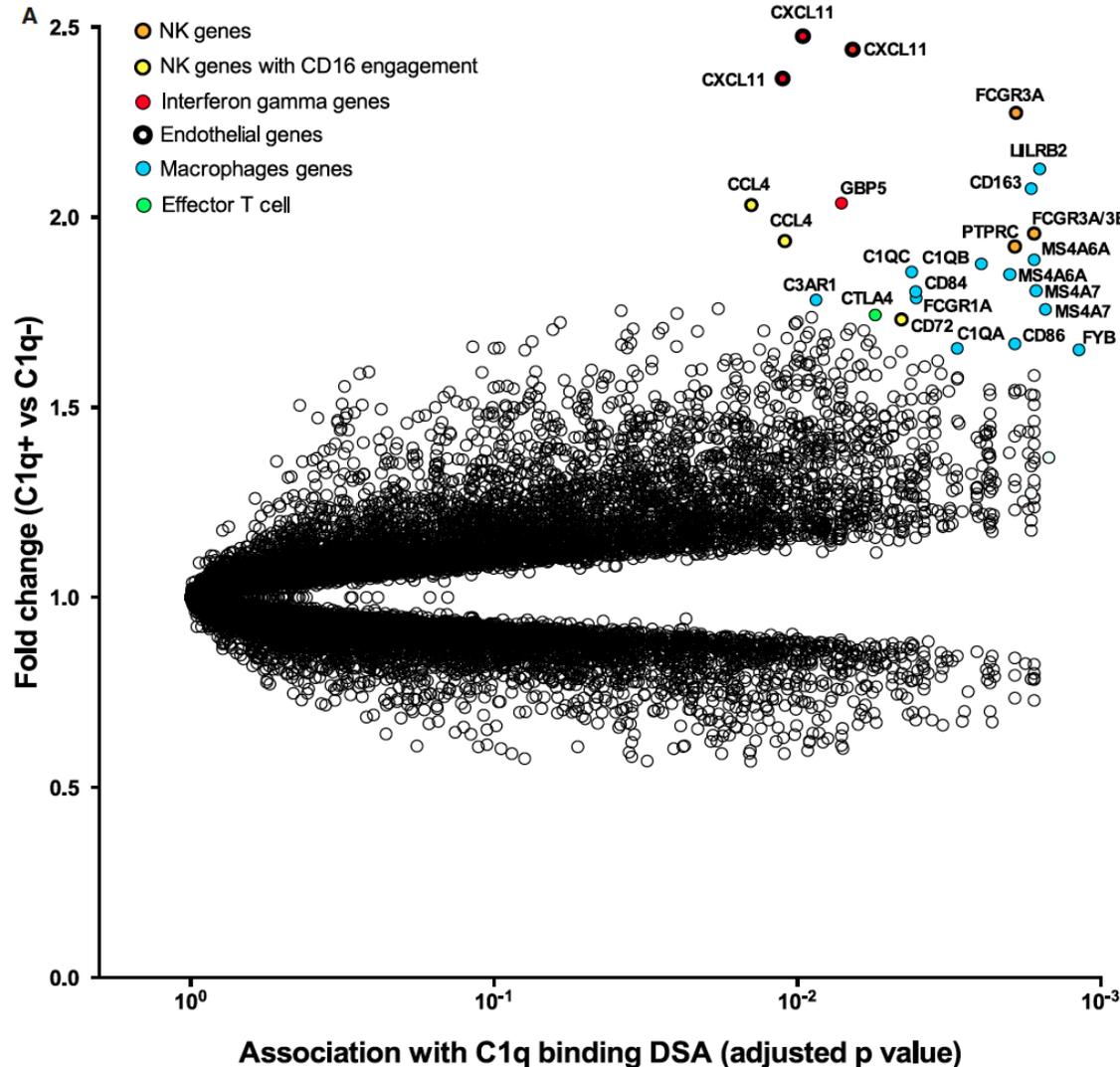
Hidalgo LG et al, AJT, 2010



Hidalgo LG et al, AJT, 2012



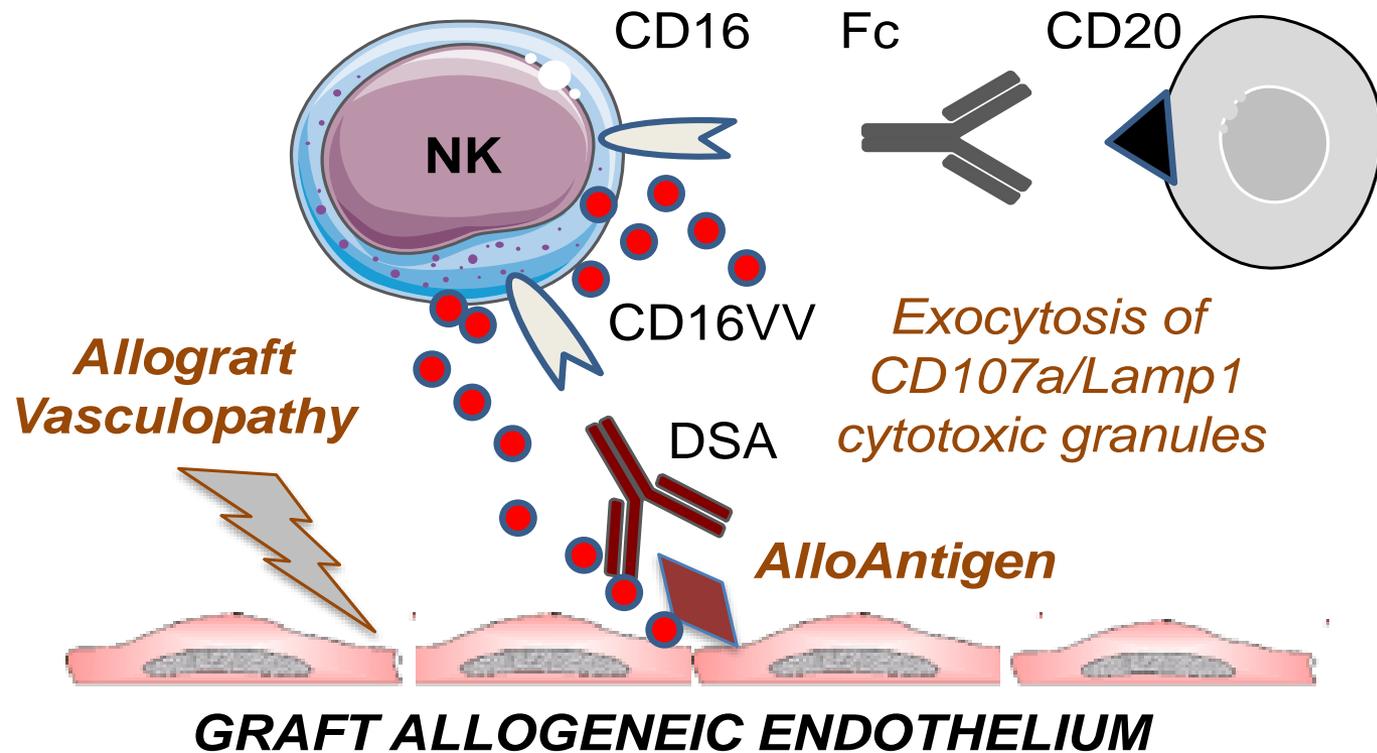
# NK et AMR: arguments transcriptomiques



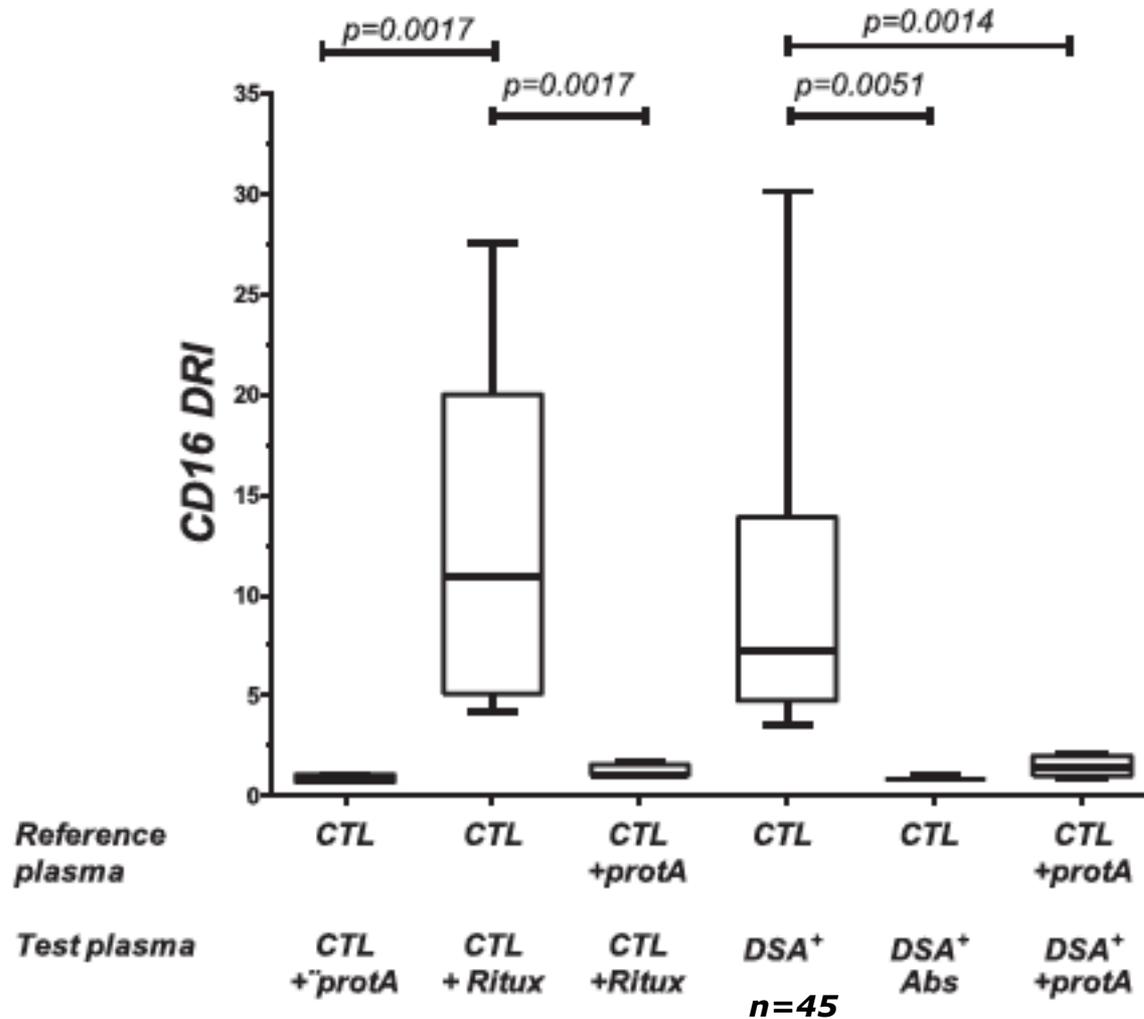
Transcrits associés aux DSA  
fixant le C1q  
157 patients < 1 an de greffe

# NK et DSA: importance du FcR CD16

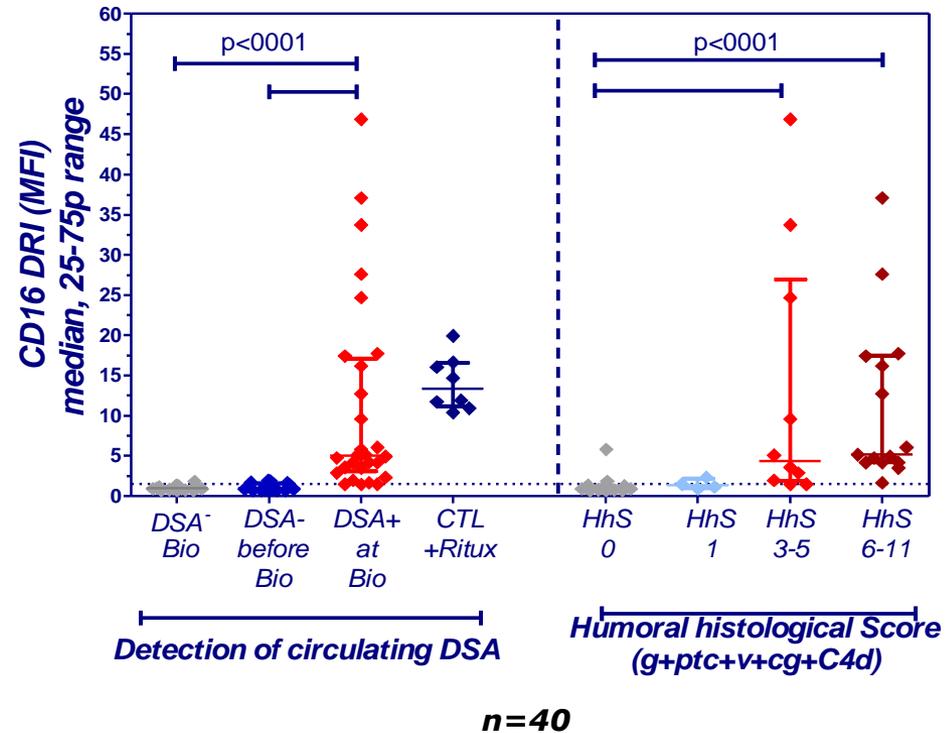
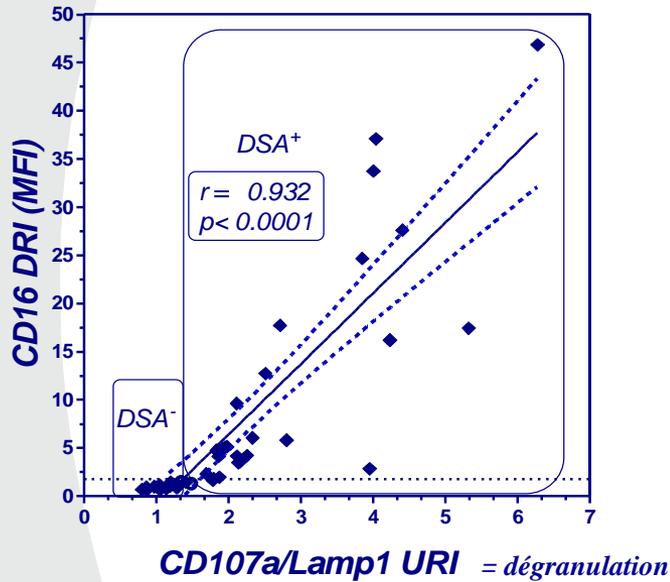
## NK cell humoral activation Test (NK-CHAT)



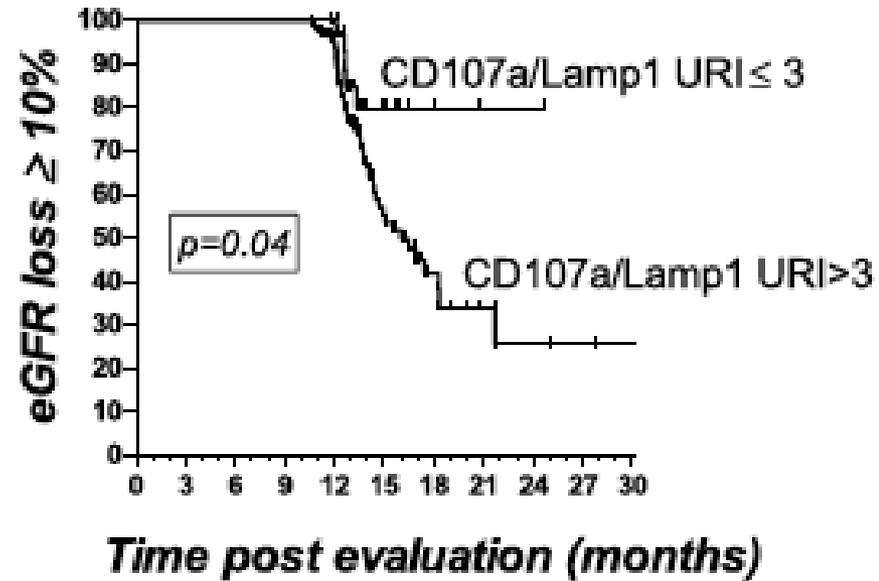
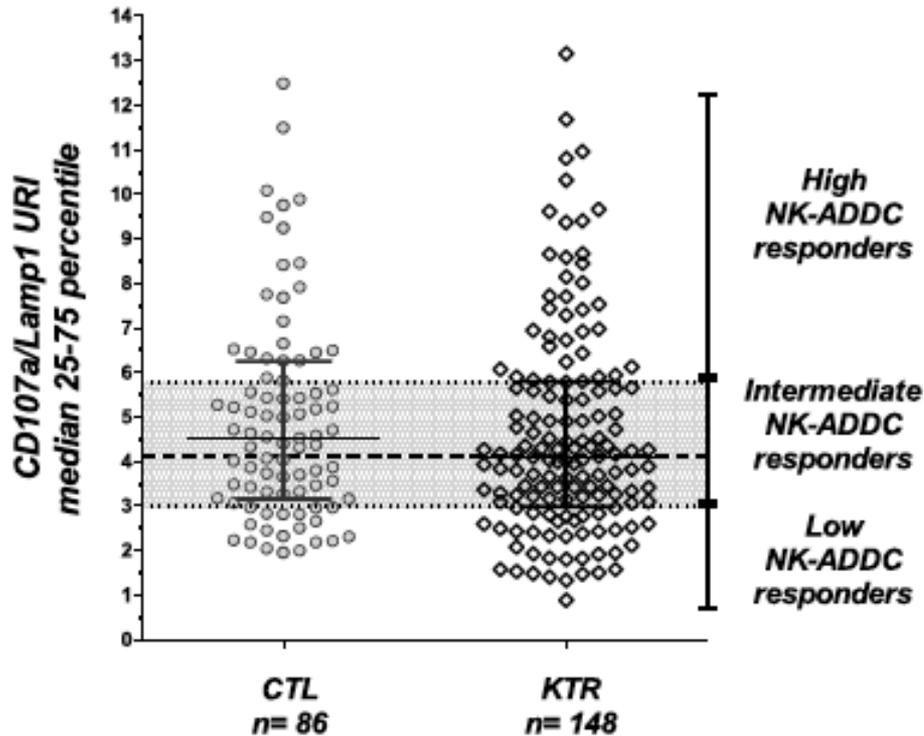
# NK et DSA: importance du FcR CD16



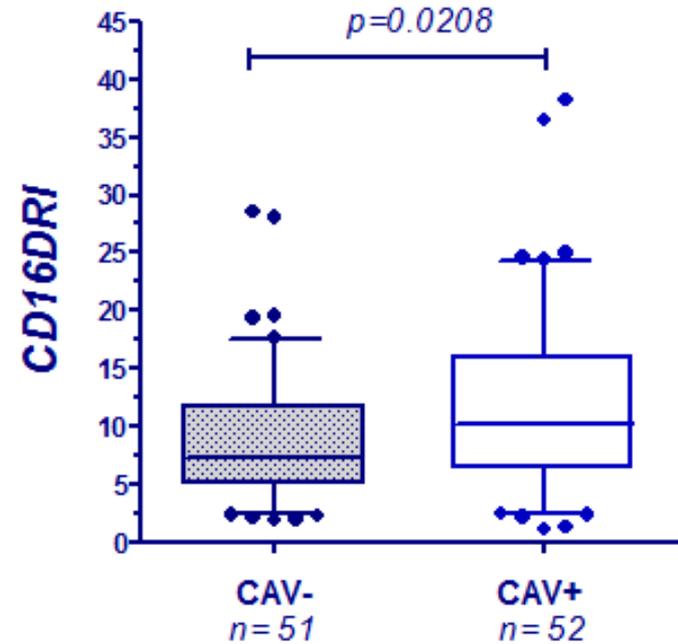
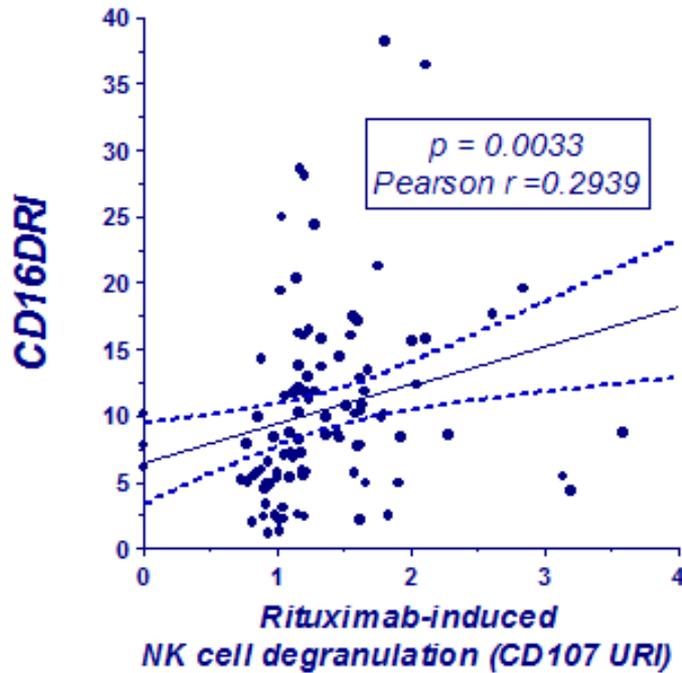
# NK et DSA: importance du FcR CD16



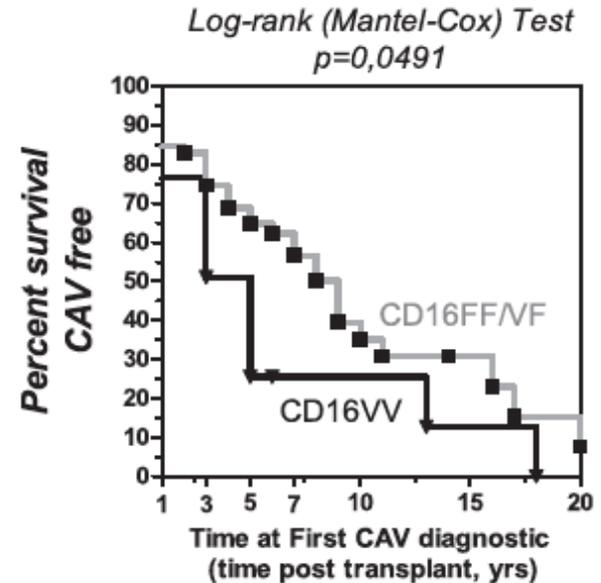
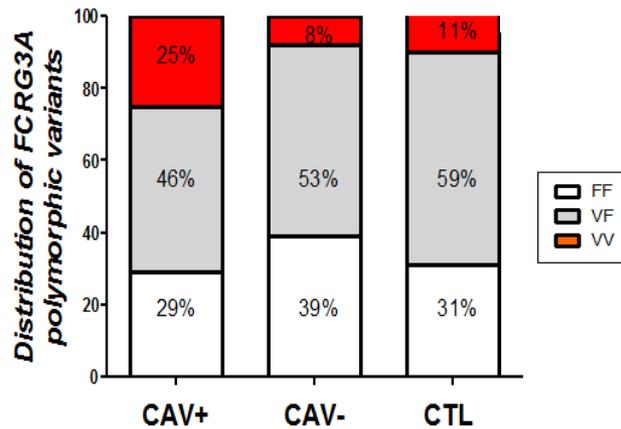
# CD16 et fonction du greffon



# CD16 et greffe cardiaque

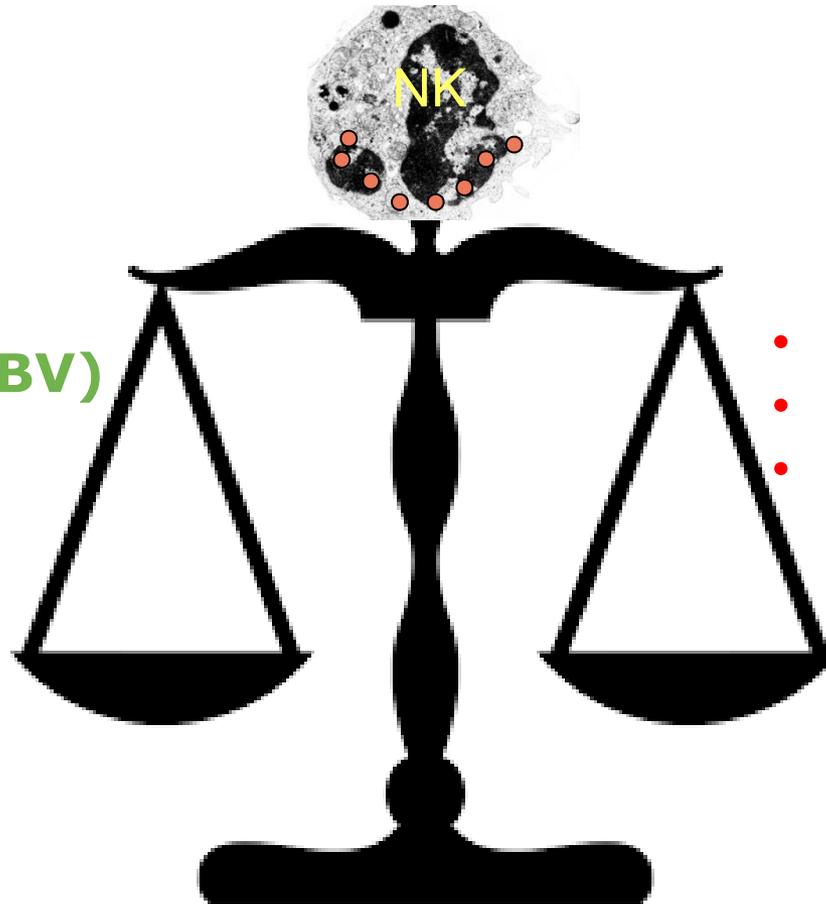


# Importance du polymorphisme de CD16



Multivariate predictors of cardiac allograft vasculopathy	Odds ratio	$p$	95% CI
CD16VV	7.45	0.034	1.163 – 47.784
CKD at time of enrollment (eGFR<60 ml/min/1.73 m <sup>2</sup> )	7.25	0.030	1.146 – 15.729
Donor age at transplant	1.08	0.004	1.026 – 1.144
Years since transplantation	1.34	0.009	1.077 – 1.665
Post-transplant bacterial infection	3.2	0.037	1.070 – 9.031
Recipient BMI at time of enrollment	1.13	0.049	1.000 – 1.287
Post-transplant CMV infection	2.76	0.182	0.625 – 12.262
Immunosuppressive regimen (ABC)	0.83	0.736	0.294 – 2.373
Recipient age at time of enrollment	0.96	0.166	0.907 – 1.017

# Conclusions



- Virus (CMV, EBV)
- Cancers
- GvL

- **AMR tardifs**
- **CD16++**
- **Rejets vasculaires**  
**DSA- (KIR)?**

## Enjeux:

- **Impact pronostic du polymorphisme CD16 en greffe rénale?**
- **Mieux comprendre le rôle des IS sur le NK**
- **Immunothérapie ciblée des NK (CD16) dans les AMR?**



**INSERM U1076**

**Chloé Dumoulin,  
Stéphane Robert  
Françoise Dignat George  
Florence Sabatier  
P. Paul**

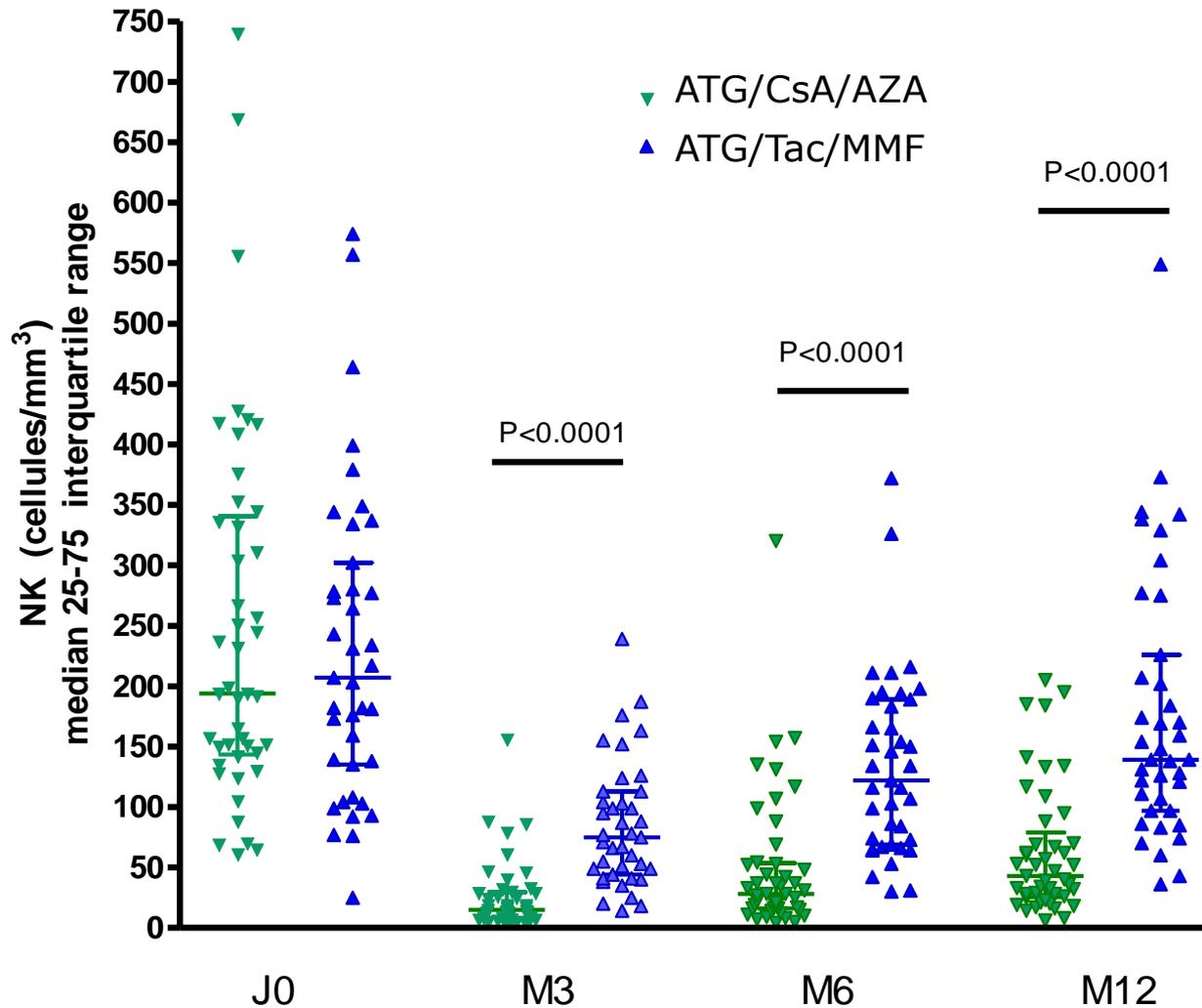


**Néphrologie, Conception  
Valérie Moal  
Henri Vacher-Coponat  
Stéphane Burtey  
Philippe Brunet**

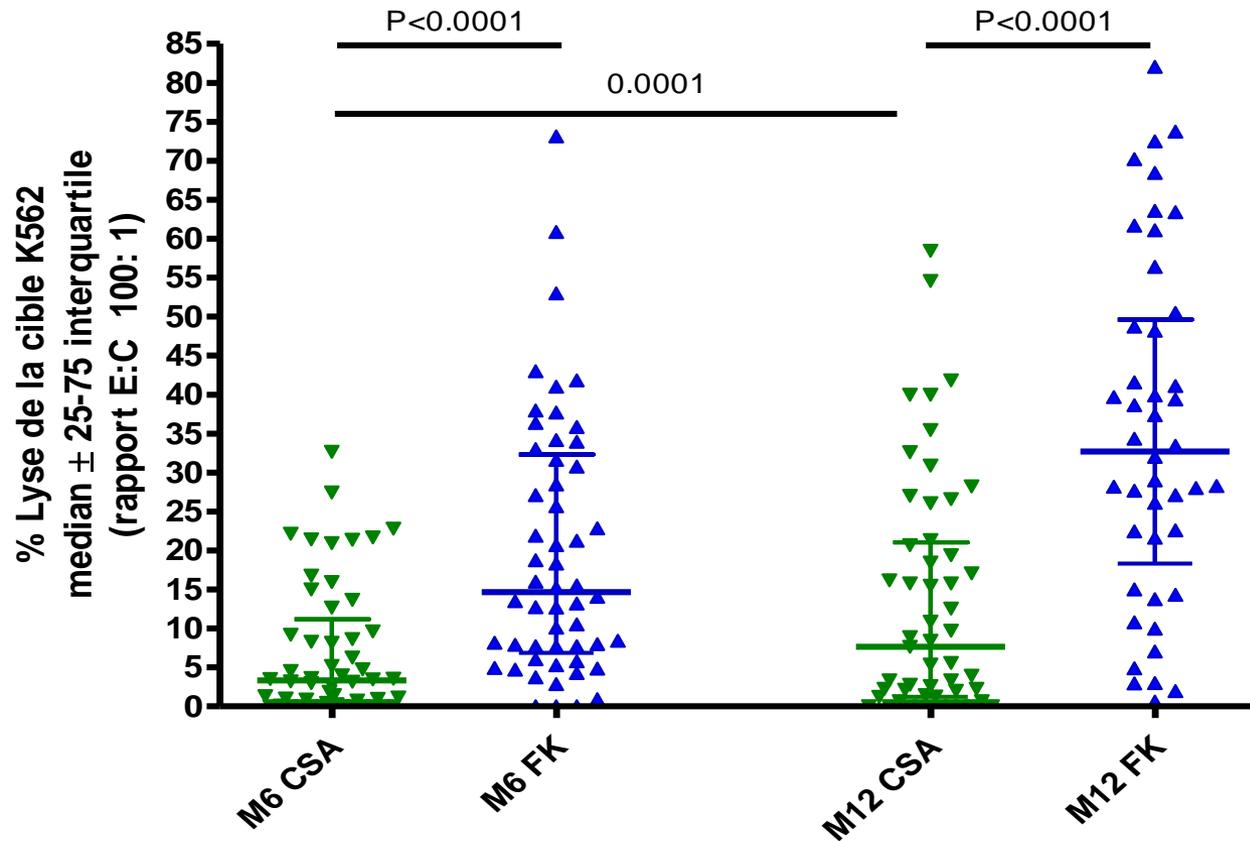
**Labo Hématologie, Hop. Conception  
L Arnaud  
L Lyonnet  
P Paul  
F Dignat George**

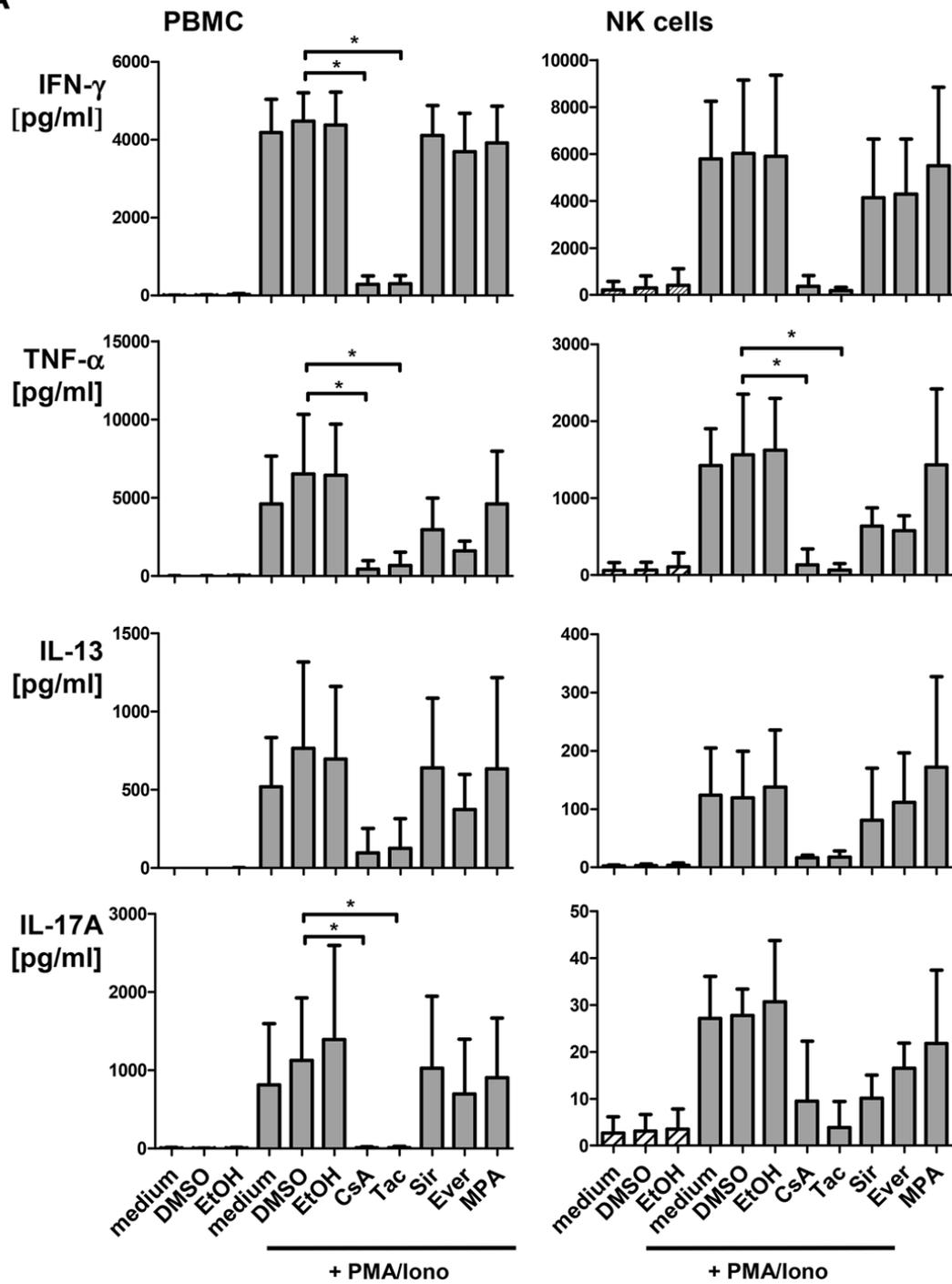
**Etablissement Français du Sang  
Laboratoire d'immunogénétique  
Christophe Picard  
Agnès Basire**

# Effets des immunosuppresseurs sur les NK



# Effets des immunosuppresseurs sur les NK

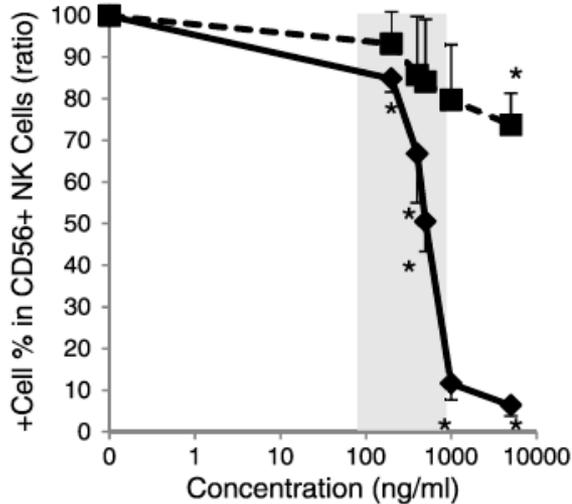




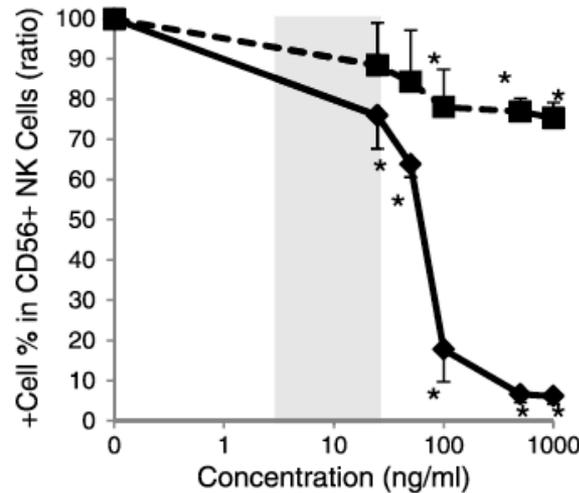
*Hoffmann U et al, PLOS one, 2015*  
*Neudoerfl C et al, Frontiers Immunol., 2013*

# Immunosuppresseurs et ADCC liés aux DSA

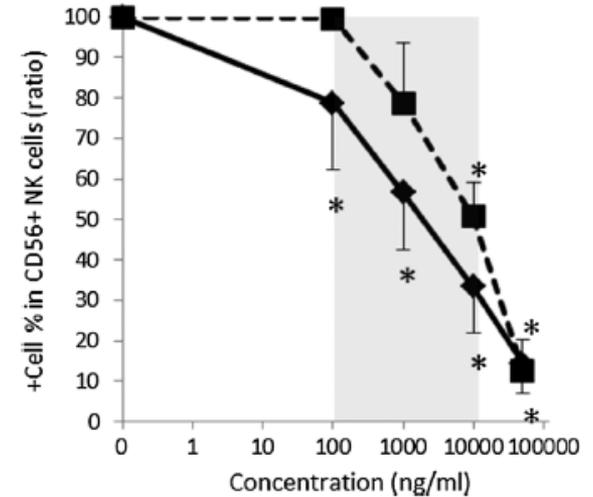
A. Cyclosporine A



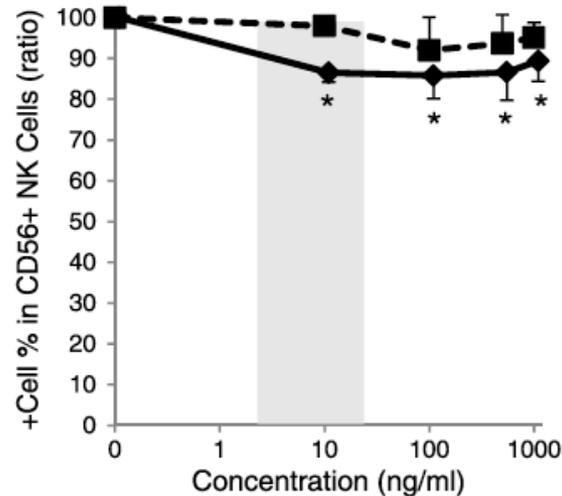
B. Tacrolimus



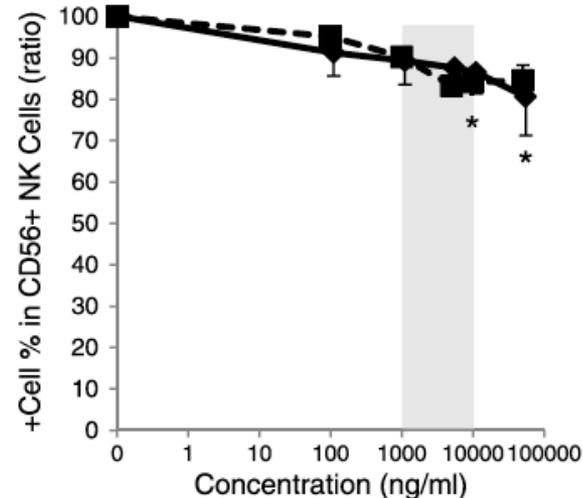
C. Prednisone



D. Sirolimus



E. Mycophenolate Mofetil

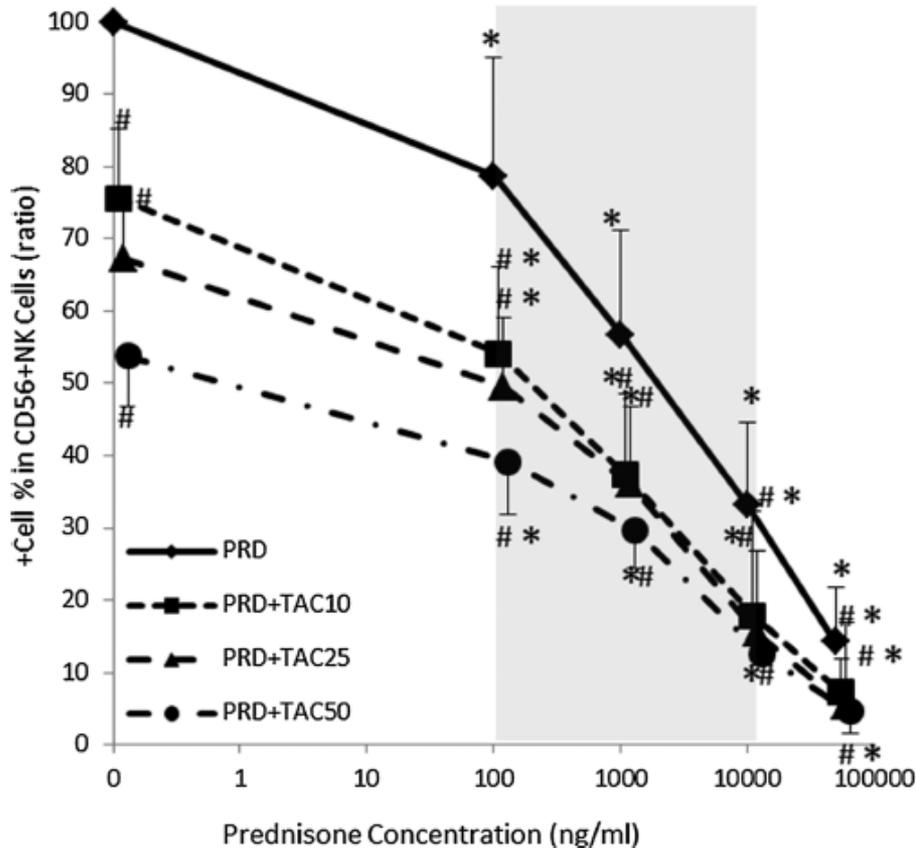


--- dégranulation

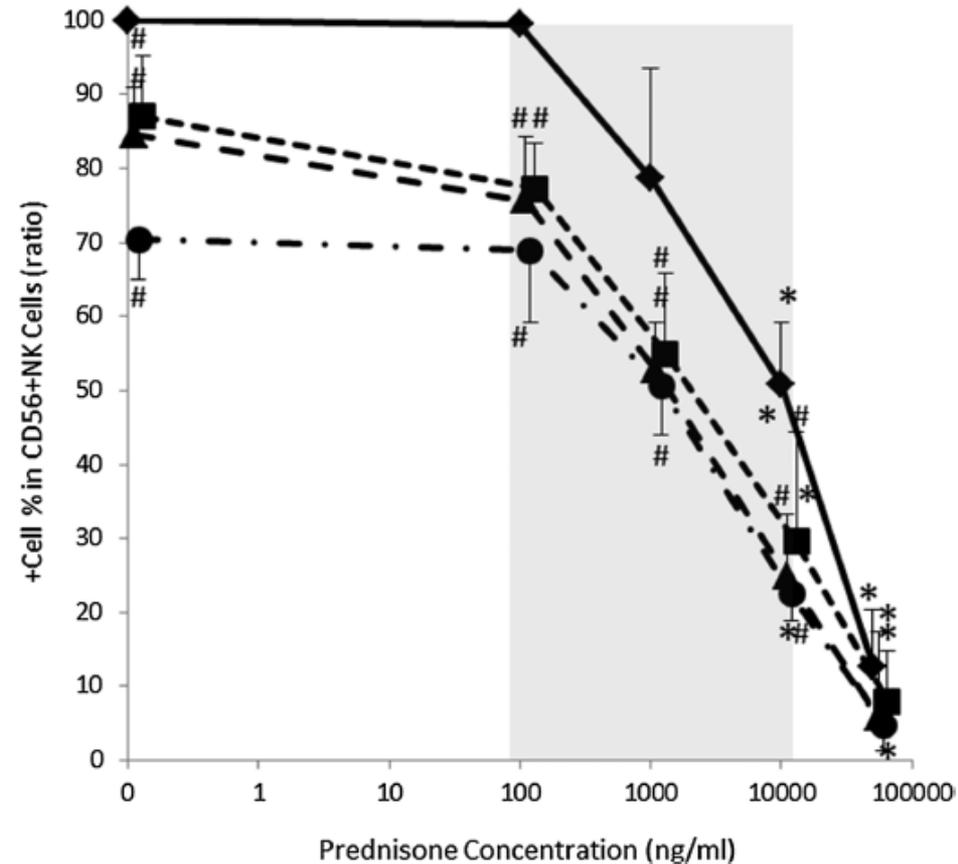
— IFN-gamma

# Immunosuppresseurs et ADCC liés aux DSA

**A. Tacrolimus + Prednisone: IFN $\gamma$**



**B. Tacrolimus + Prednisone: CD107a**

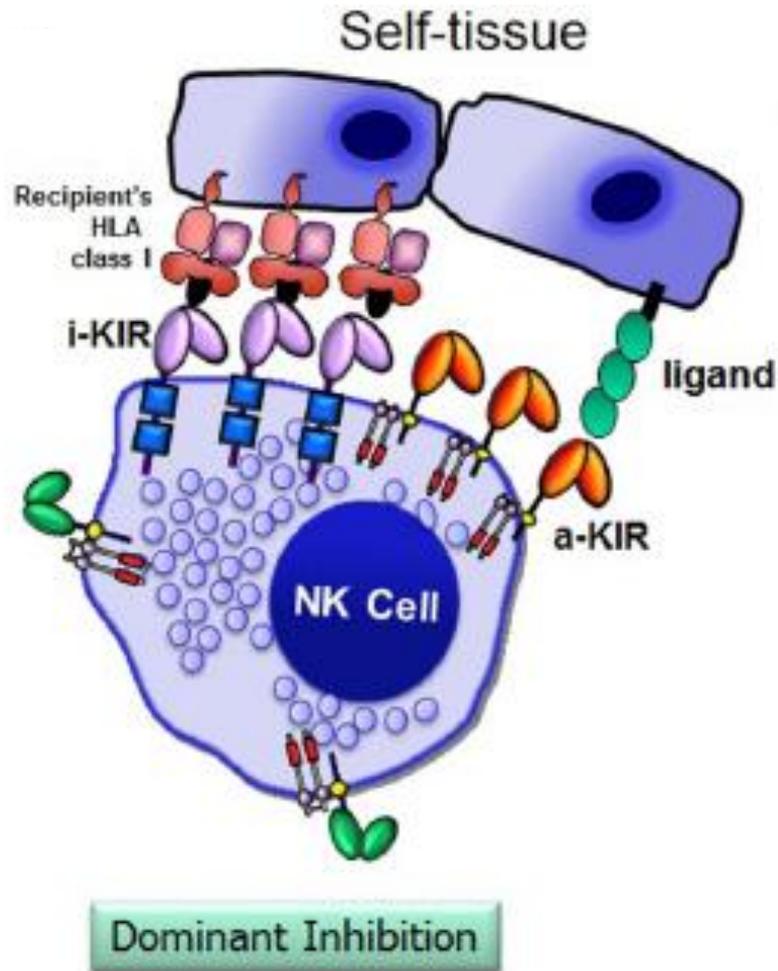




# Première partie

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# Alloréactivité NK en transplantation?



# Cellule NK

Inhibitory  
receptors

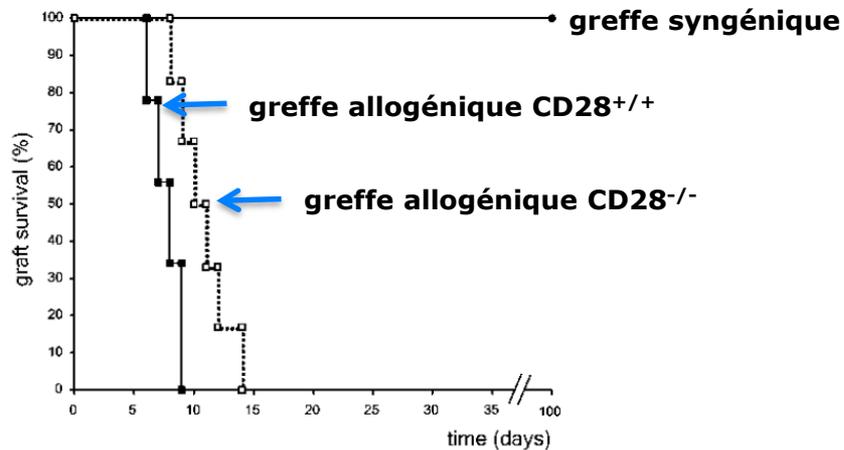
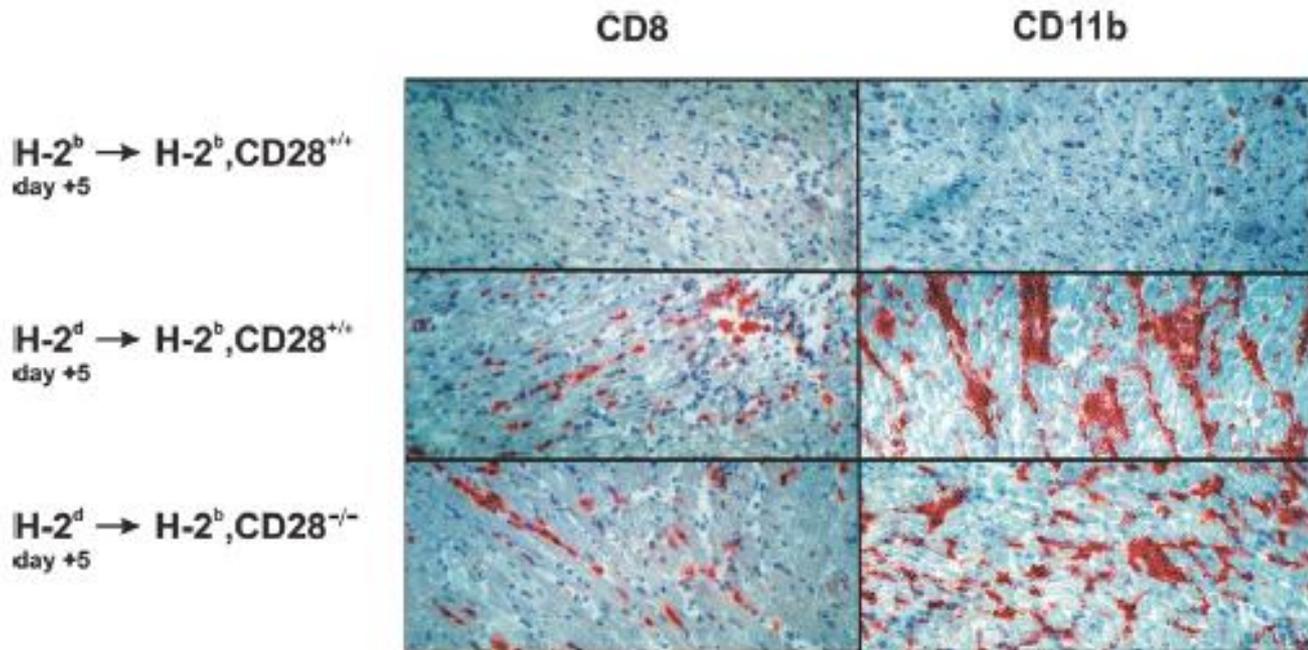
KIR-L	HLA-C, B and A
LAIR-1	Collagen
CD94-NKG2A	HLA-E
SIGLEC 3, 7, 9	Sialic acid
KLRG1	Cadherins
NKR-P1A	LLT-1
LILRB1 (CD85j, ILT2)	HLA class I

Activating receptors,  
adhesion or costimulation  
molecules

CD244 (2B4)	CD48
CS1 (CRACC, CD319)	CS1 (CRACC, CD319)
$\alpha_4\beta_1$ integrin	VCAM-1 (CD106)
$\beta_2$ integrins (CD11a-CD18, CD11b-CD18, CD11c-CD18)	ICAM-1 (CD54) ICAM-2 (CD102) CD23 iC3b...
CD226 (DNAM-1)	CD112 (Nectin-2), CD155 (Nect5)
CRTAM	Nect2
CD27	CD70
CD16	Immunoglobulin G
NKp46	Viral hemagglutinins, ?
KIR-S	HLA-C, ?
CD94-NKG2C	HLA-E
CD94-NKG2E	HLA-E
NKG2D	ULBP (RAET), MICA, MICB
NTB-A	NTB-A
PEN-5	L-selectin
CD96 (Tactile)	CD155 (Nect5)
NKp80	AICL
CD100	CD72
NKp30	pp65, BAT-3, ?
NKp44	Viral hemagglutinins, ?
CEACAM1 (CD66)	CEACAM1 (CD66)
CD160 (BY55)	HLA-C

# Cellule cible

# NK et modèles murins d'allogreffes



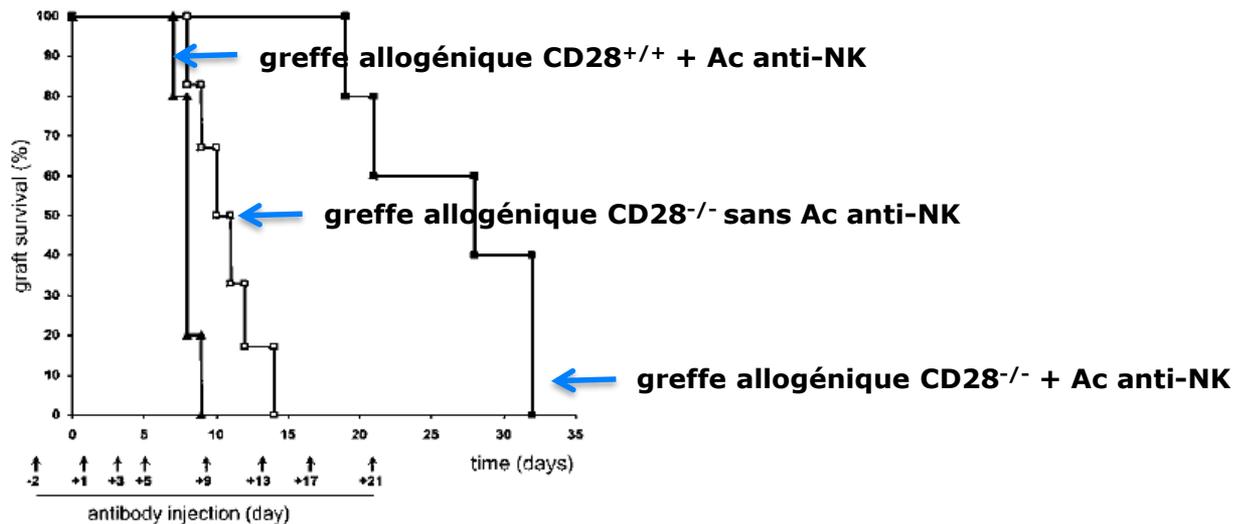
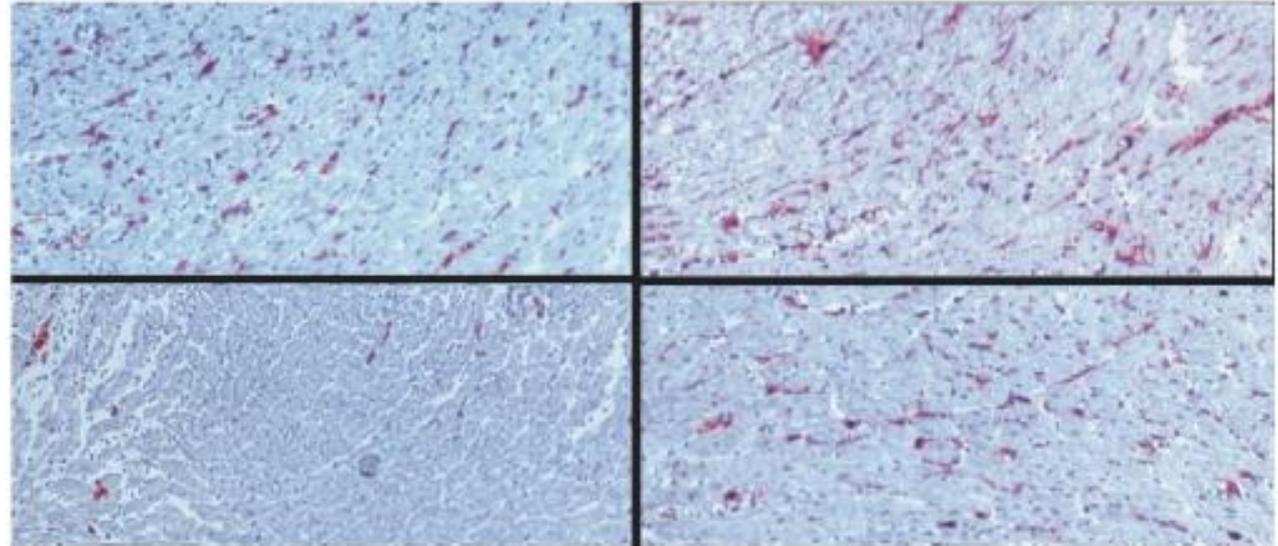
# NK et modèles murins d'allogreffes

CD8

CD11b

H-2<sup>d</sup> → H-2<sup>b</sup>, CD28<sup>+/+</sup>  
+ αNK1.1 mAb  
day +5

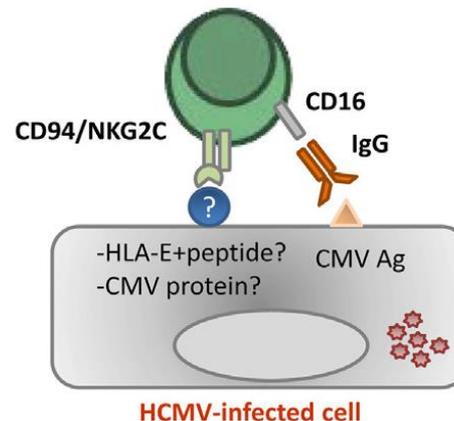
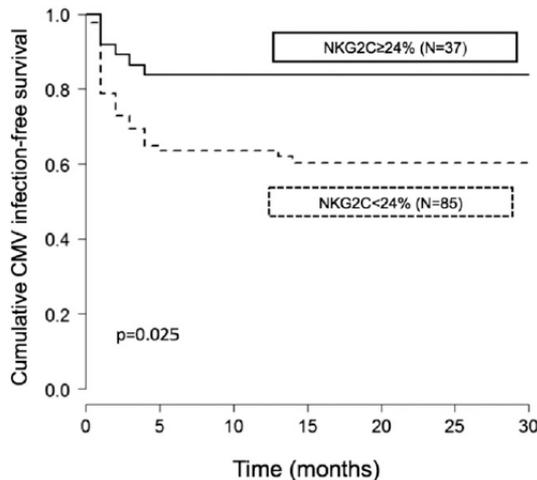
H-2<sup>d</sup> → H-2<sup>b</sup>, CD28<sup>-/-</sup>  
+ αNK1.1 mAb  
day +5



# Sous-population NK protectrice?

All Cases

	CMV Viremia (n = 39)	No CMV Viremia (n = 83)	p
% Total NK, mean ± SD	13.7 ± 7.1	13.8 ± 8.6	0.62
NK c./μl <sup>c</sup> , mean ± SD	174.0 ± 115.9	183.8 ± 162.4	0.60
% NKG2C <sup>+</sup> <sup>d</sup> , mean ± SD	15.1 ± 12.3	23.7 ± 19.7	0.017
NKG2C <sup>+</sup> c./μl, mean ± SD	28.0 ± 32.6	44.4 ± 62.3	0.11
% NKG2A <sup>+</sup> , mean ± SD	49.6 ± 16.2	46.2 ± 19.2	0.31
NKG2A <sup>+</sup> c./μl, mean ± SD	85.0 ± 65.3	73.4 ± 58.8	0.32
% KIR <sup>+</sup> , mean ± SD	56.5 ± 17.2	56.3 ± 16.3	0.89
KIR <sup>+</sup> c./μl, mean ± SD	99.1 ± 81	110.0 ± 105.0	0.98
% CD161 <sup>+</sup> , mean ± SD	52.7 ± 24.6	50.2 ± 23.5	0.57
CD161 <sup>+</sup> c./μl, mean ± SD	75.3 ± 51.5	85.1 ± 74.1	0.92



Redondo-Pachon D et al, *J. Immunol.*, 2016  
Lopez-Botet M et al, *Front. Immunol.*, 2017

## BKv et transcrits NK dans les biopsies

Pathogenesis-based transcripts	BKVN vs. normal biopsy and no viremia	Normal biopsy and BK viremia vs. normal biopsy and no viremia	BKVN vs. normal biopsy and BK viremia
KT	0.98	0.46	0.95
IRIT	0.04	0.96	0.001
GRIT	0.008	0.41	0.002
QCAT	0.001	0.34	0.002
CMAT	0.001	0.24	0.001
AMA	0.001	0.79	0.001
BAT	0.002	0.72	0.001
NKAT	0.002	0.88	0.001
ENDAT	0.25	0.88	0.09

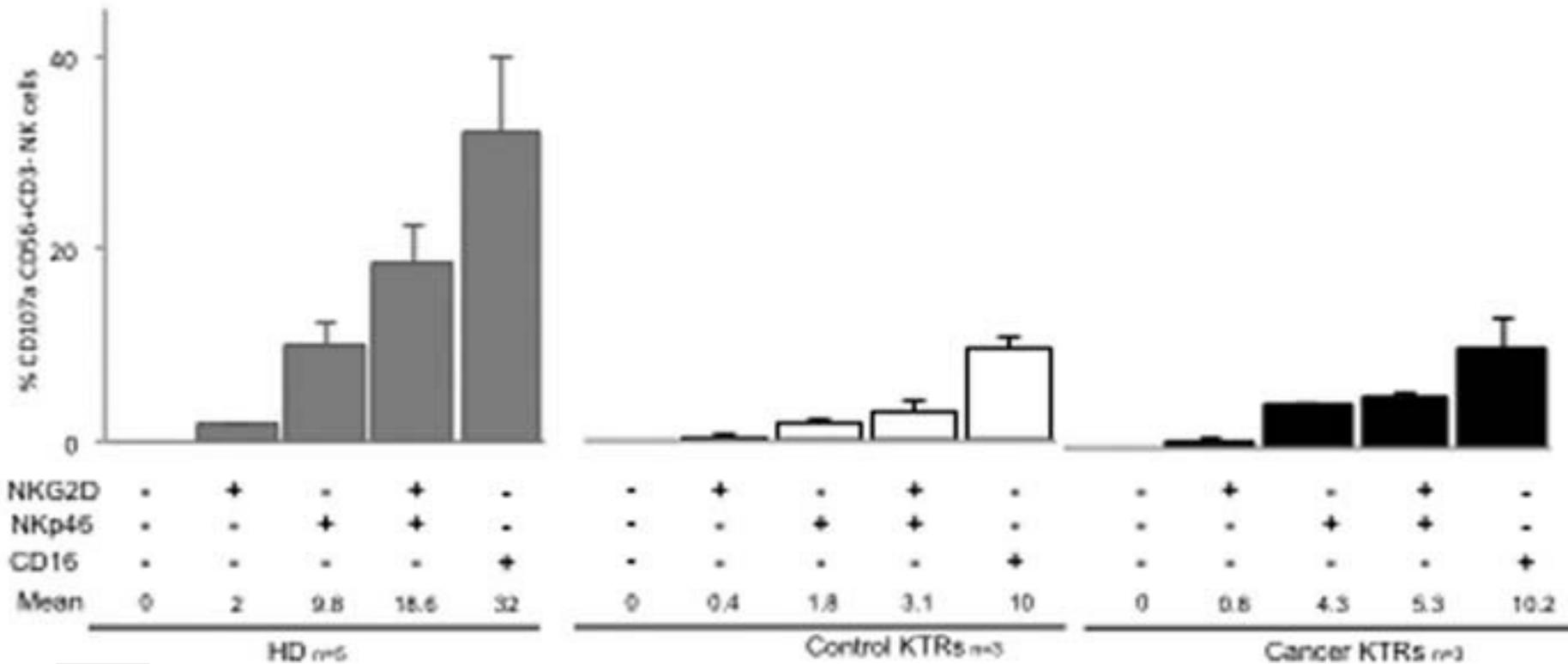
n=17 biopsies

*Lubetzky M et al, Transplantation, 2014*

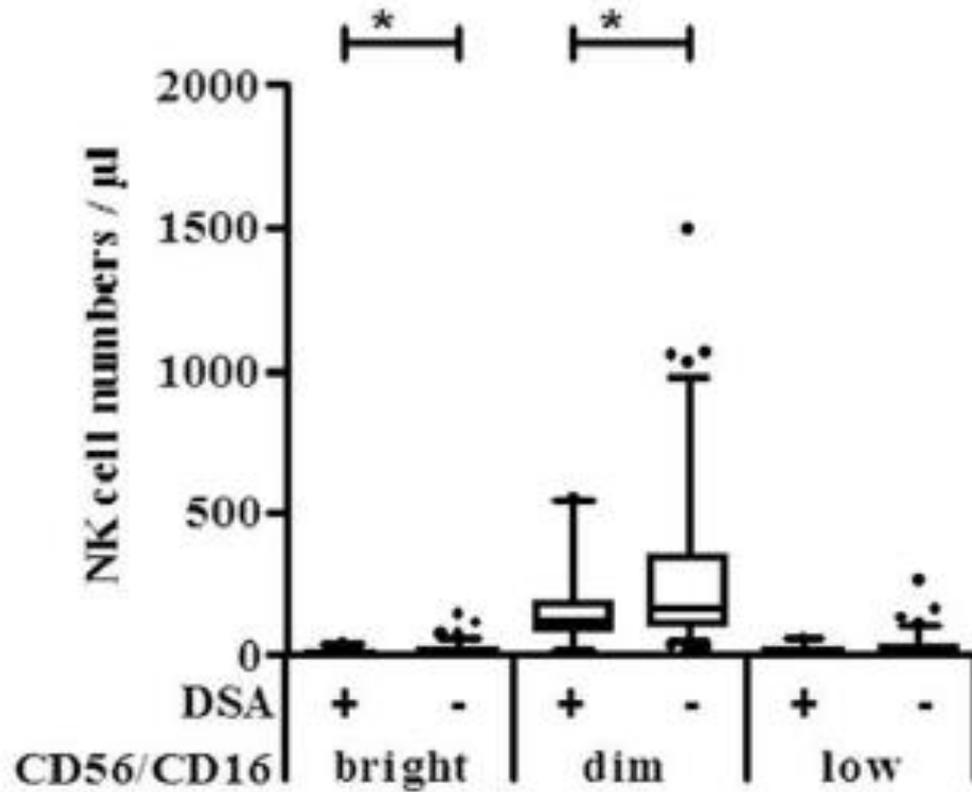
- Rôle des miRNA du BKv se liant à ULBP3, ligand de NKG2D, dans l'évasion immune du virus

*Bauman Y et al, Cell Host and Microbe, 2011*

# NK et cancers: population greffée rénale



## NK et DSA: CD16 *in vivo*



# CD16 et fonction du greffon

