K-VIROGREF

9EME JOURNÉE NATIONALE DU RÉSEAU

ANCERS RARES POST TRANSPLANTATION

Immunothérapie adoptive anti-infectieuse dans un contexte d'immunodépression

L'utilisation en clinique des Lymphocytes T anti-viraux

Danièle BENSOUSSAN,
CHRU de Nancy,
Intégrateur Biothérapie Bioproduction MTInov,
Unité CNRS 7365 IMoPA
Groupe MTI-PP SFGM-TC

CHRU NANCY

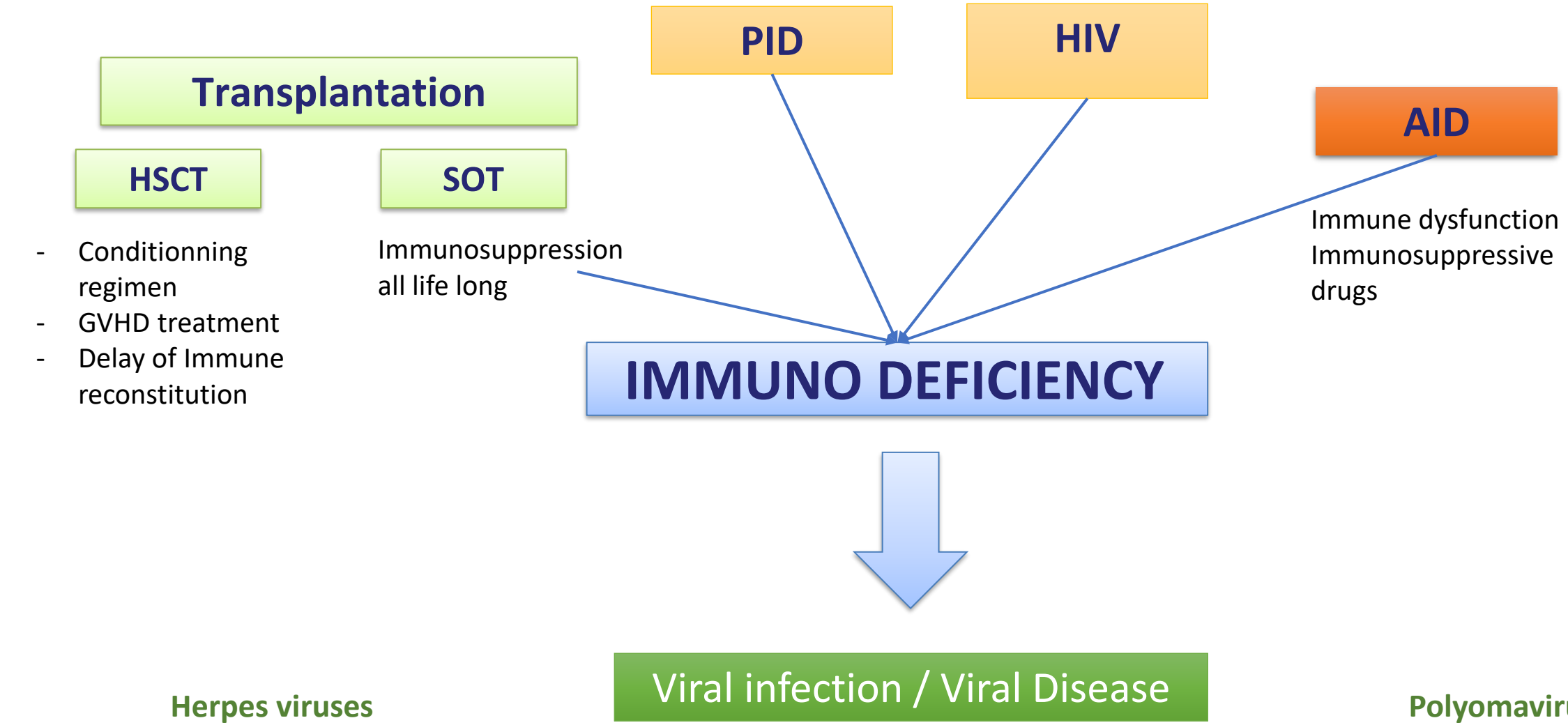
MTINOV LARGE SCALE HUMAN CELL PRODUCTION

UNIVERSITÉ DE LORRAINE

IMoPA

SFGM TC Société Francophone de GREFFE DE MOELLE et de Thérapie Cellulaire

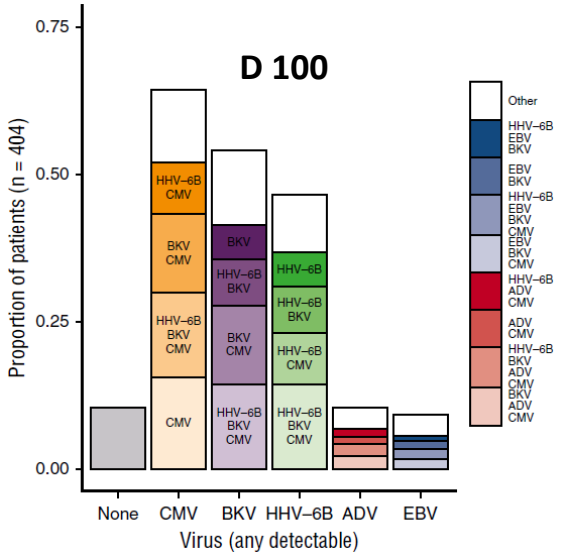
VST : in various contexts of immunodeficiency

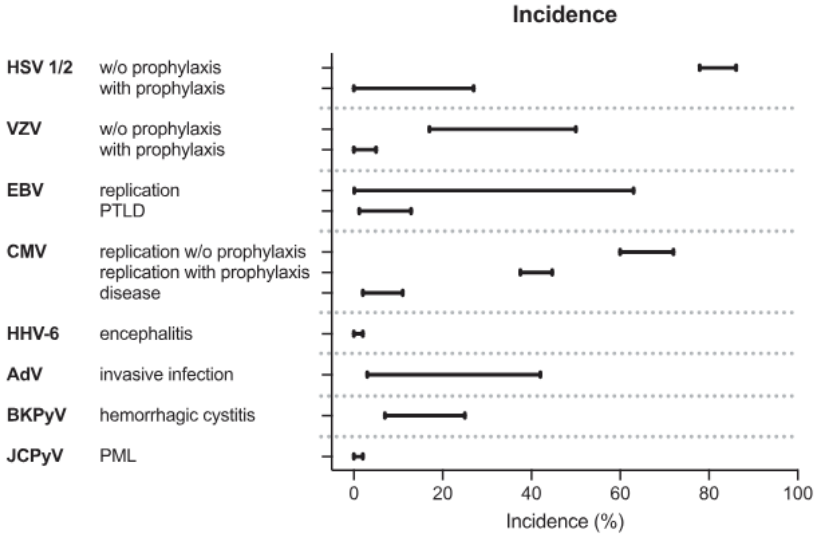


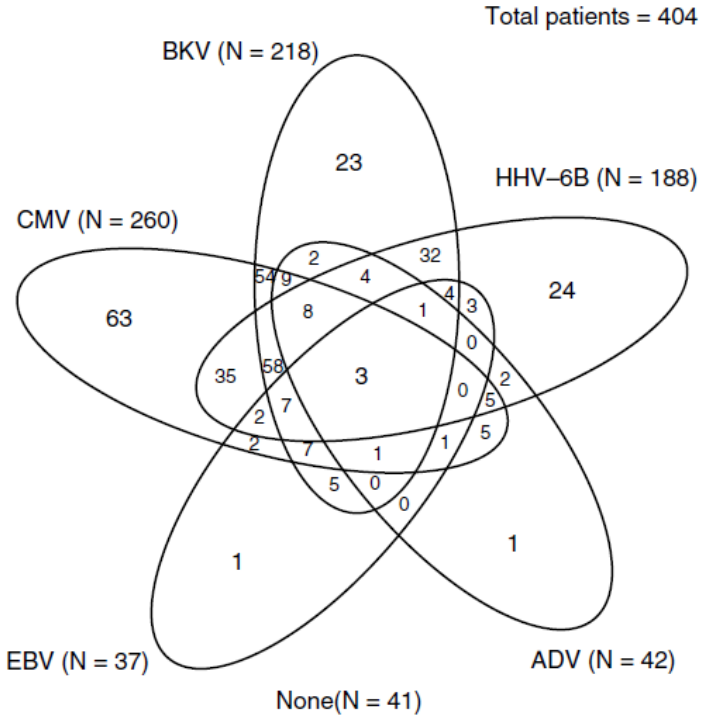
Viral Infections in Immunocompromised Patients

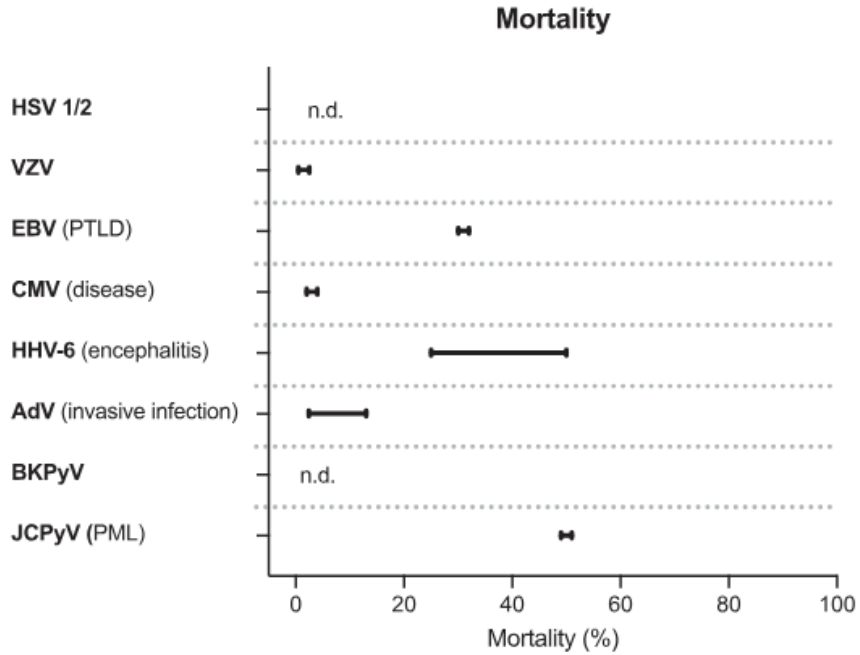
HSCT

Infections associated deaths : 24% in allo-HSCT







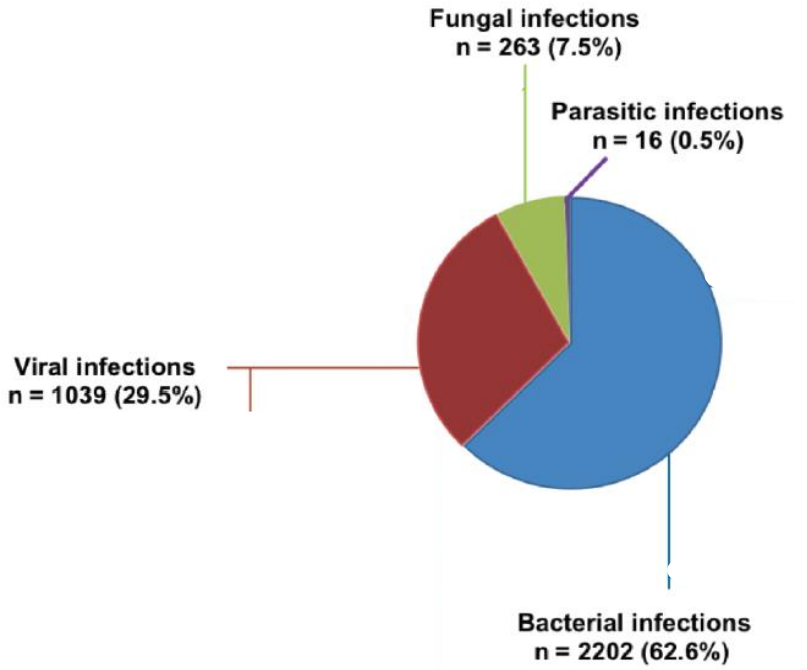


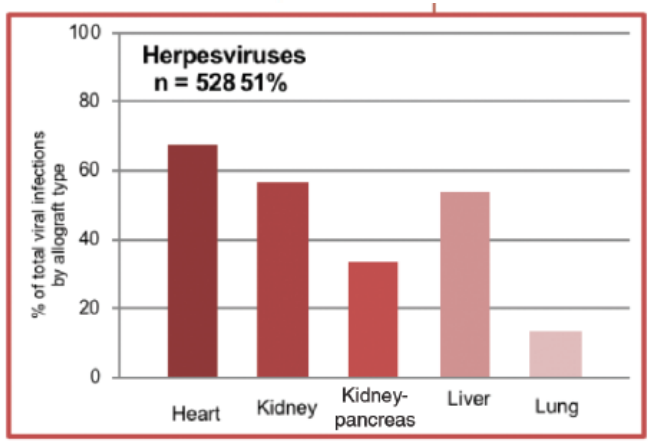
Hill J, Blood 2017; Styczynski J, 2020; Walti et al, Curr Opin Infect Dis, 2022;

Viral Infections in Immunocompromised Patients

SOT

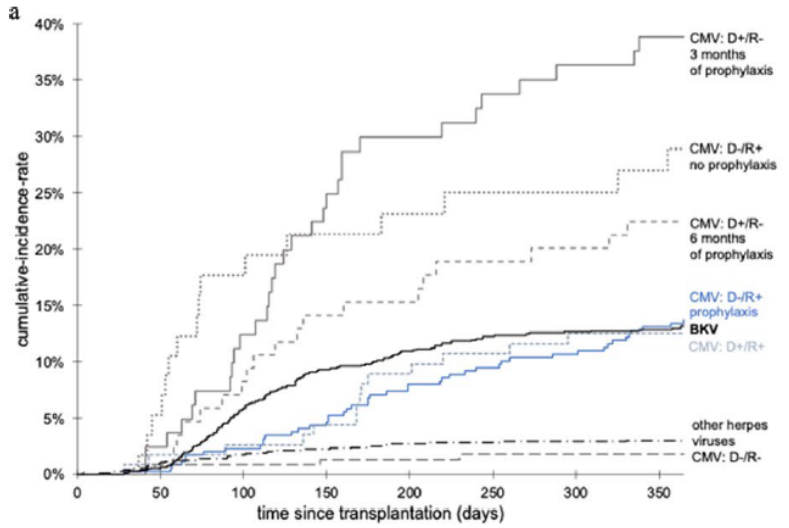
Infections associated deaths : 17% in SOT

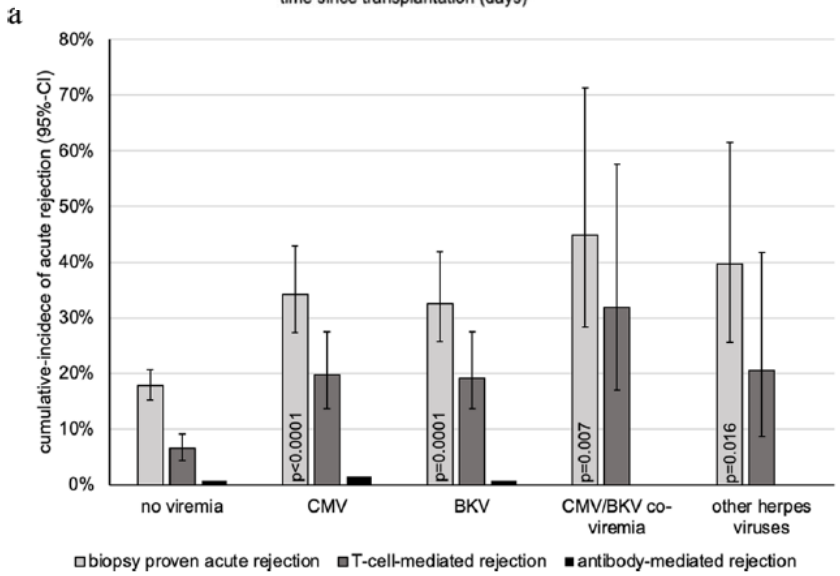




Virus	Incidence Germany
Herpes V CMV	**16,7%** 14,3%
PyV BKV	**13,2%** 13,2%

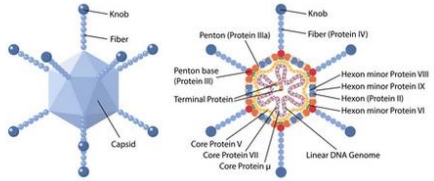
1035 Kidney transplant recipients from 5 centers (2014–2021)
German Center for Infection Research

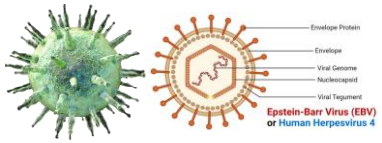


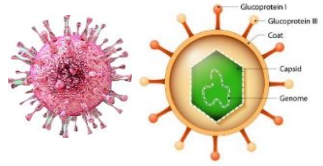


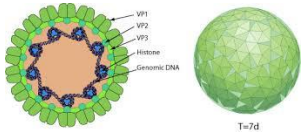
Van Delden, CID, 2020; Kotton et al, Am J of Transpl, 2017; Soeborg et al, 2022; Sommerer et al. BMC Nephrology, 2025

Viral Infections in Immunocompromised Patients









	Adenovirus	Epstein-Barr virus	Cytomegalovirus	Polyomavirus
Type of virus	DNA ds	Gamma herpes virus	Beta herpes virus	DNA ds
Prevalence post-HSCT : - **Replication/Infection** - **Disease**	 23% children / 3% adults 7% children / 1% adults	 68% 1,2-11,2%	 > 30% 2-5%	 BKV : 54%/JCV : 0,5 to 2% BKV HC : 30 to 75%
Prevalence post-SOT: - **Replication** - **Disease**		 1-32%	 14,3%	 BKV : 13,2%, JCV : 0,1%
Symptoms	Fever, Keratoconjunctivitis Respiratory infection Hepatitis, colitis Hemorrhagic cystitis	Fever, PTLD : *post transplant lymphoproliferative disorder*	Fever, Pneumonia, Gastrointestinal infections (esophagitis, gastritis, colitis) Encephalitis, myelitis	BKV : - Nephropathy : 1-10% of Kidney transplant, Rejection - Hemorragic cystitis : >10% in HSCT
Standard 1st line treatments (pre-emptive)	Cidofovir, Brincidofovir	Rituximab	Prophylaxis in HSCT: Letermovir	BKV : Cidofovir, hyperhydratation, cystoscopy… JCV : Immune restoration, ICI
Adverse effects of treatments	Nephrotoxicity, hepatotoxicity, hematotoxicity	B lymphocyte immunosuppression Hematotoxicity	Nephrotoxicity, hematotoxicity	Nephrotoxicity, ICI : organ rejection, GVHD

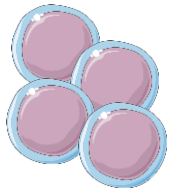
Viral Infection Treatment



Anti-viral drugs or mAb



Virus specific T cells (VSTs)



Efficiency: 70 to 80% of the patients

Do not prevent from further reactivations

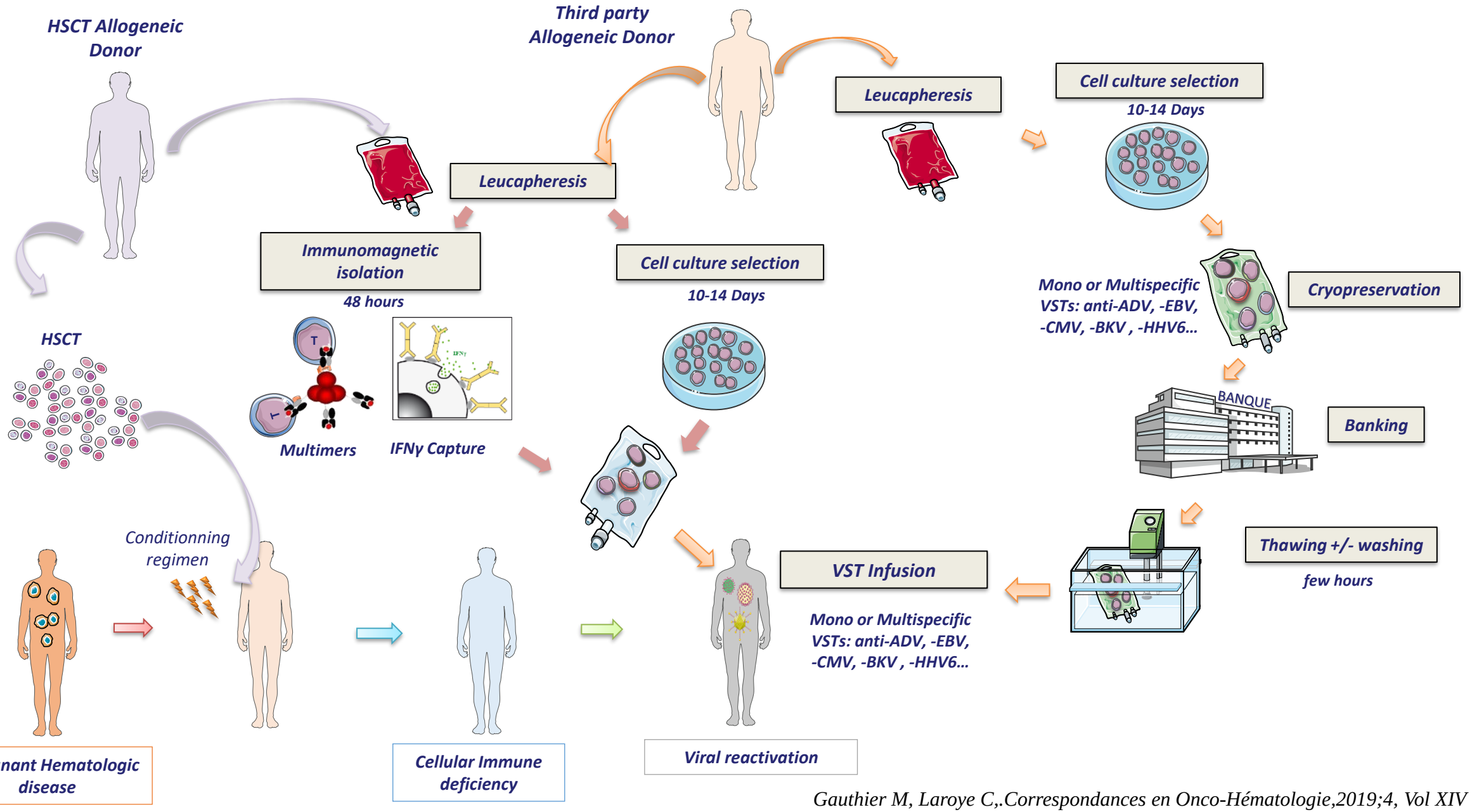
Toxicity

Restore immune system

Prevent from further viral reactivation

GVHD ?

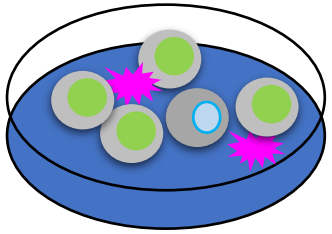
Viral Specific T Cell Isolation Methods



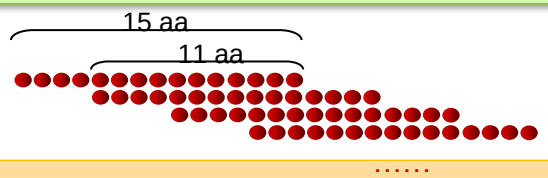
Gauthier M, Laroye C,.Correspondances en Onco-Hématologie,2019;4, Vol XIV

Anti-Viral T Cell Generation:

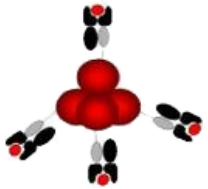
Main advantages and drawbacks of each method



Cell Banking / Off-the-shelf VSTs
Low frequency VSTs
No HLA restriction
Multivirus-VSTs



Time consuming (10 to 14 days)
Technically demanding: limits broad clinical implementation



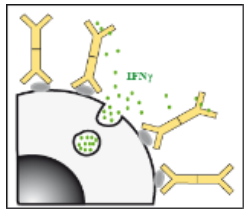
Fast and easy method

Broad clinical implementation

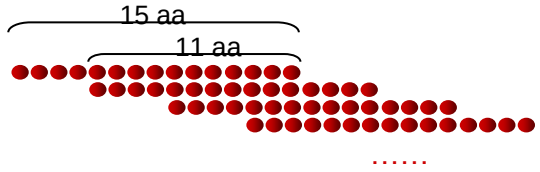
HLA restriction (library, mainly HLA class I)
Impossible for Low frequency circulating VSTs (ADV, BKV, etc..)







Fast and easy method
Broad clinical implementation
No HLA restriction, polyclonal
CD4 and CD8 + cells



Limited to VST secreting IFNγ
Low number of VSTs infused

Gauthier M, Laroye C,.Correspondances en Onco-Hématologie,2019;4, Vol XIV

Safety data on VSTs in allo-HSCT context

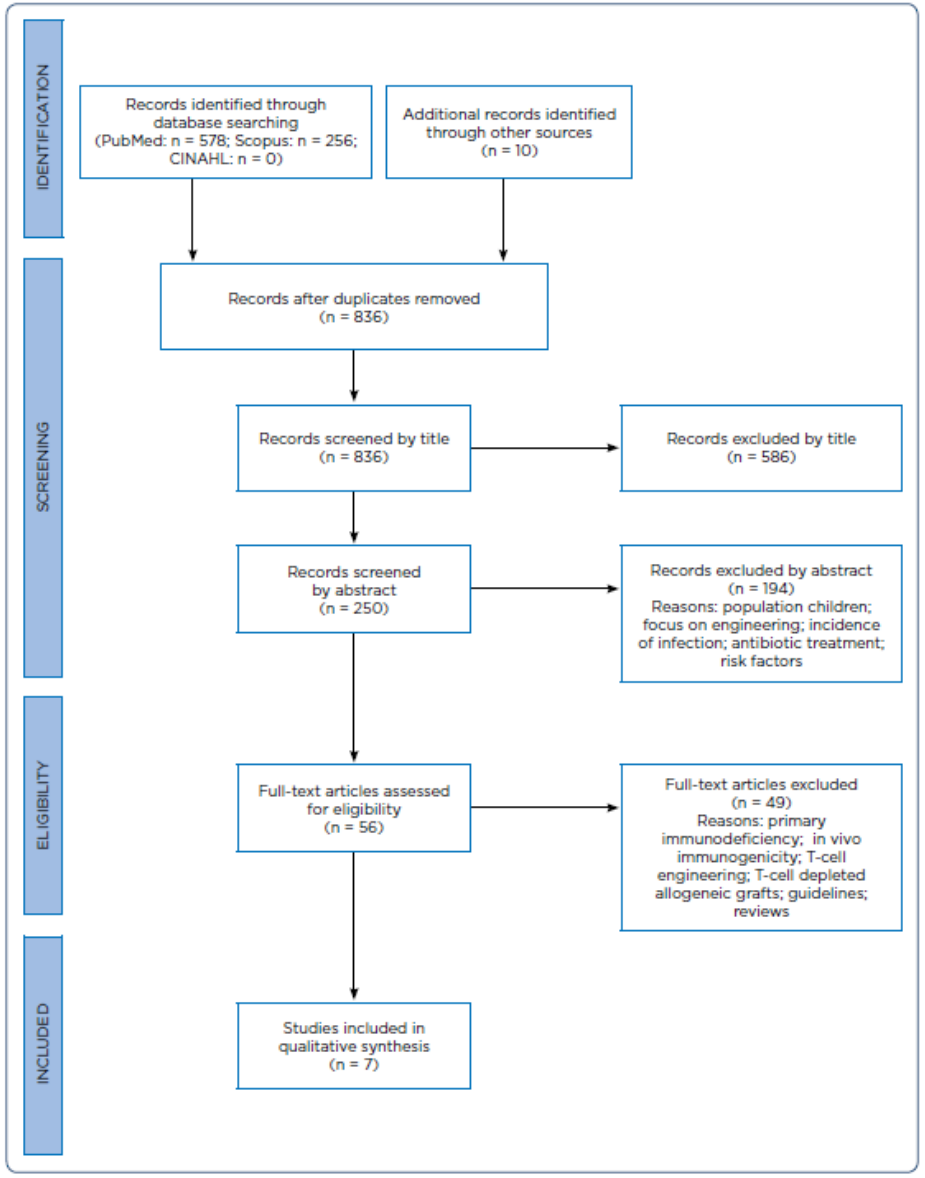


Table 2. Reported Adverse Effects of Virus-Specific T-Cell Therapy

Author (year) and country	Acute GVHD	Chronic GVHD	Recurrent GVHD	De novo GVHD	Cytokine release syndrome	Infusion toxicity	Other adverse events
Feucht (2015) Germany	-	-	-	2 grade I 4 grade II–III	-	-	None
Koehne (2015) United States	-	-	None	-	None; 2 retinitis (UR)	None	None
Leen (2013) United States	6 grade I 1 grade II 1 grade III	-	1 skin	2 grade I skin	-	None	2 TA-TMA (sirolimus) 1 graft failure (UR)
Neuenhahn (2017) Germany	1 grade II–III GI	1 (r/t reduction of immunosuppression)	-	-	-	1 fever (UR)	1 cystitis
Papadopoulou (2014) United States	-	-	None	1 grade II skin	None	None	2 TA-TMA
Qian (2017) France	-	-	3	None	-	-	None
Tzannou (2017) United States	-	-	1 grade III GI 3 grade I–II skin	2 grade I skin	None	1 fever (UR)	1 hydronephrosis

Note. GVHD = graft-vs.-host disease; - = not addressed in the study; none = addressed in study and no occurrences; UR = unrelated; TA-TMA = transplant-associated thrombotic microangiopathy; GI = gastrointestinal; r/t = related to.

Ccls : Little to no severe side effects associated with VST therapy

Simmons et al, 2019

IFNg Based Immunomagnetic selection:
A french experience

IFN-γ Based Immunomagnetic Selection

ADV/CMV/EBV + Donor: HSCT donor/ Haplo-identical

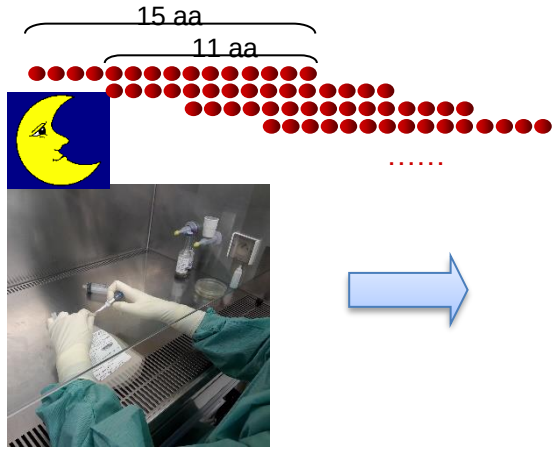


PBMC collection

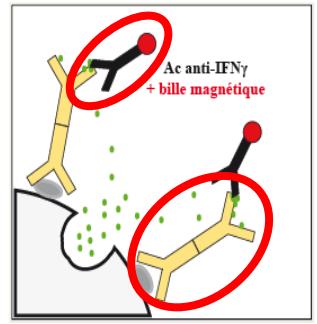




QC: Reception
- Numeration
- Flow cytometry
- Microbiology



Stimulation 6 hs, 37C
PepTivator



Cytokine Capture System
(Miltenyi Biotec)

QC: before selection
- Numeration
- Flow cytometry





Positive Fraction
VST enriched

QC: after selection
- Numeration
- Flow cytometry
- Microbiology
- Expansion for Functionnal tests





ADV/CMV /EBV + Patient





or

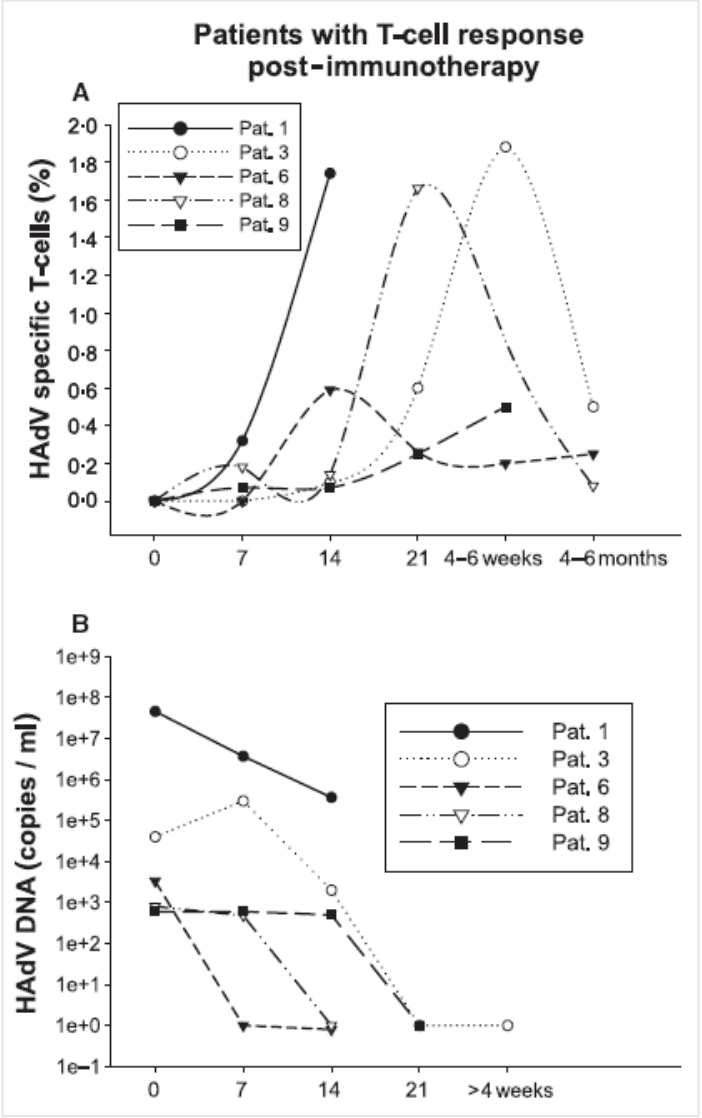


Conditionning

First Publication

Feuchtinger et al, 2006

	Patient no.								
	1	2	3	4	5	6	7	8	9
T-cell transfer									
Day post-SCT	+378	+152	+77	+97	+84	+53	+40	+62	+64
$CD3^+$ cells/kg	5000	20 000	6000	50 000	25 000	3000	12 700	1200	3300
Acute clinical side effects*	No	No	No	No	No	No	No	No	No
Deterioration in laboratory results	No	CRP, bilirubin†	No	No	CRP‡	No	No	No	No
GvHD postimmunotherapy	Skin GvHD II post-T-cell transfer	No	No	No	No	No	No	No	GvHD improved from grades IV to II
In vivo expansion of specific T cells postimmunotherapy	Yes	No	Yes	Not evaluable	Not evaluable	Yes	Not evaluable	Yes	Yes
Clinical course postimmunotherapy	Multiorgan failure, lung, heart, kidney, liver, bone marrow	Generalised lethal HAdV-infection	Clearance of HAdV	Death because of NHL relapse	Death because of HAdV-infection	Clearance of HAdV	Died 1 d later because of pre-existing myocardial infarction	Clearance of HAdV	Clearance of HAdV
Additional antiviral treatment postimmunotherapy	Ganciclovir, cidofovir	Ganciclovir, cidofovir	Ribavirin, cidofovir	Ribavirin, cidofovir	Ribavirin, cidofovir	Ribavirin		Ribavirin	Cidofovir
HAdV infection 7 d postimmunotherapy									
Change of HAdV copies/ml blood	↓1 log	↓×0·16	↑1 log	↑2 log	↑×4·28	Clearance of HAdV		↑0·4 log	Neg
Change of HAdV copies/ml stool	HAdV PCR positive	↑2 log	↓×0·75	No change	No change	HAdV PCR positive		↓4 log	↓× 0·8
Other body fluids	Urine, throat swab, stool positive	CSF pos, urine pos 3×10^5	Urine pos 3×10^4			No			No
HAdV infection 14–20 d postimmunotherapy									
Change of HAdV copies/ml blood	↓2 log	↑×3	↓2 log	↑3 log§	Not evaluable	Clearance of HAdV		Clearance of HAdV	Neg
Other body fluids	Urine, throat swab, stool positive	Stool, CSF, urine positive 3×10^7	Stool positive	Stool positive		Stool neg.		Stool pos 3×10^7	Clearance of HAdV in stool
HAdV infection 30 d postimmunotherapy									
Course of HAdV infection	Death because of HAdV-infection	Not evaluable	Clearance of HAdV	Not evaluable	Not evaluable	Clearance of HAdV	Not evaluable	Clearance in blood, stool pos 3×10^5	Clearance of HAdV



Qian et al. Journal of Hematology & Oncology (2017) 10:102
DOI 10.1186/s13045-017-0469-0

Journal of Hematology & Oncology

RESEARCH Open Access

Curative or pre-emptive adenovirus-specific T cell transfer from matched unrelated or third party haploidentical donors after HSCT, including UCB transplantations: a successful phase I/II multicenter clinical trial

Chongsheng Qian[1,2†], Arnaud Campidelli[1†], Yingying Wang[1,2], Huili Cai[3], Véronique Venard[4], Hélène Jeulin[4], Jean Hugues Dalle[5], Cécile Pochon[13], Maud D'aveni[13], Benedicte Bruno[6], Catherine Paillard[7], Stéphane Vigouroux[8], Charlotte Jubert[9], Patrice Ceballos[10], Aude Marie-Cardine[11], Claire Galambrun[12], Clément Cholle[14], Isabelle Clerc Urmes[15], Nadine Petitpain[16], Marcelo De Carvalho Bittencourt[3], Véronique Decot[1,2], Loïc Reppel[1,2], Alexandra Salmon[13], Laurence Clement[13] and Danièle Bensoussan[1,2,14*]

Nancy Clinical Trial Results

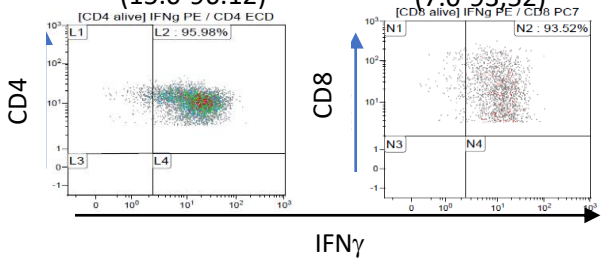
Main objective: Toxicity: absence of GVHD *de novo* or reactivation at M1
Secondary objectives: Efficacy: Viral load decrease and Specific IR increase.

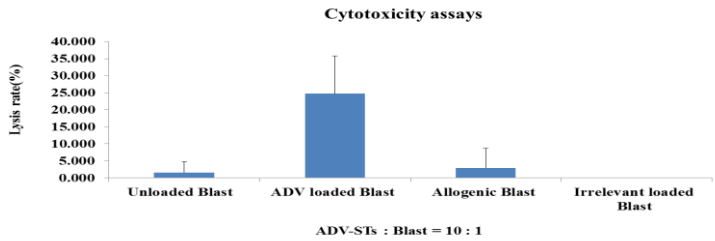
Patients: Inclusion: n=14 (ADV disease : 11, ADV infection : 3)
Treated: n=11 : 6 Haplo-VST, 5 MUD VST

Infused cells

72.81 % (15.0-96.12)

54.09 % (7.0-93,52)





Mean infused cell dose : CD3IFNg+/kg : 5,83 +/-8,23 10^3

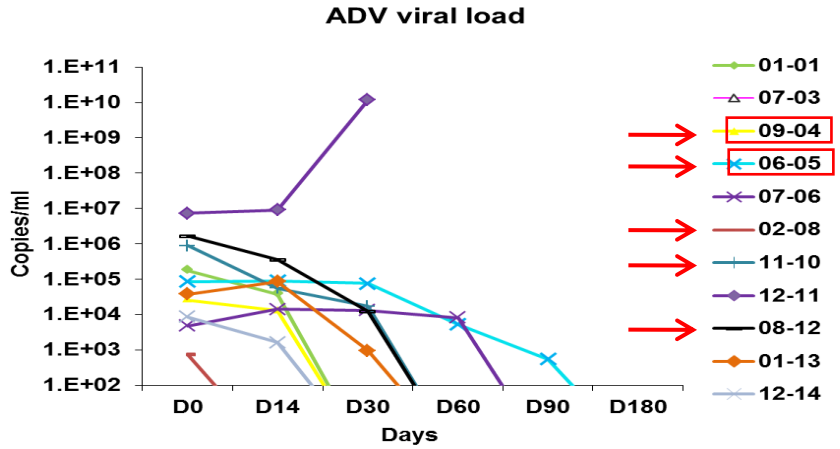
Safety/Toxicity

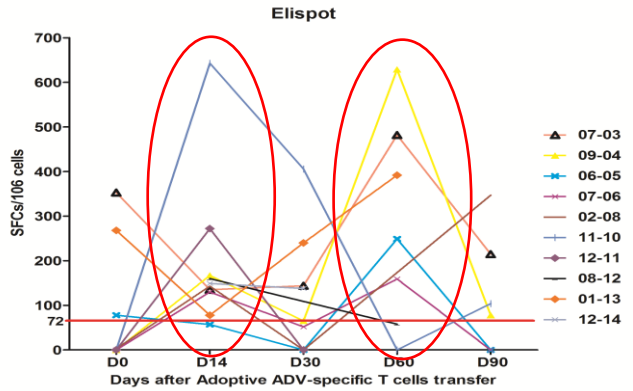
Early safety: chills n=1

***De novo* GVHD at M1: n=0**

GVHD reactivation at M1: n=3
- 1 aGVHD grade I, resolutive
- 1 aGVHD grade III, non resolutive
- 1 extensive cGVHD

Efficacy





Nancy Clinical Trial Conclusion

Overall survival: 7/11 (Follow-up of 18 months - up to 42 months)

Adenovirus infection and disease: No reactivation after clearance

- **Fast**
- **Safe: 3/11 GVHD reactivation: VST or IS decrease?**
- **Efficient: 10/11 patients CR + Immune reconstitution**

  (2 patients: ADV-VST alone as a pre-emptive strategy)
- **No difference between ADV-VST from original HSCT donors and Haploidentical third party donors**

1st report of ADV-VSTs generated by IFN-γ based immunomagnetic selection from Haploidentical third party donors for a series of UCB transplanted patients: clearance of ADV viral load in 5/6 patients

French ATMP Group (2016-2024)

ANSM
- ATMP Approval
  - ADV-VST (MTIPP-009)
  - CMV-VST (MTIPP-010)
  - EBV-VST (MTIPP-011)
- Non Opposition: PyV-VST

SFGM-TC
- ATMP group
- Pdt : Pr JH Dalle



Pharmacovigilance Nancy Hospital (Dr Petitpain/Dr Prontskus)
Prospective collection of safety and efficacy data
- ANSM annual report

UTCT Nancy Hospital
- VST production
- VST transfer to clinical departments for infusion

210
Referral procedures
Sent to ATMP group

→ **34 procedures considered non relevant/opinion reserved**

166
Favorable opinions

→ **94 patients non treated (or EBVallo or TRACE)**

72
Patients treated with VSTs

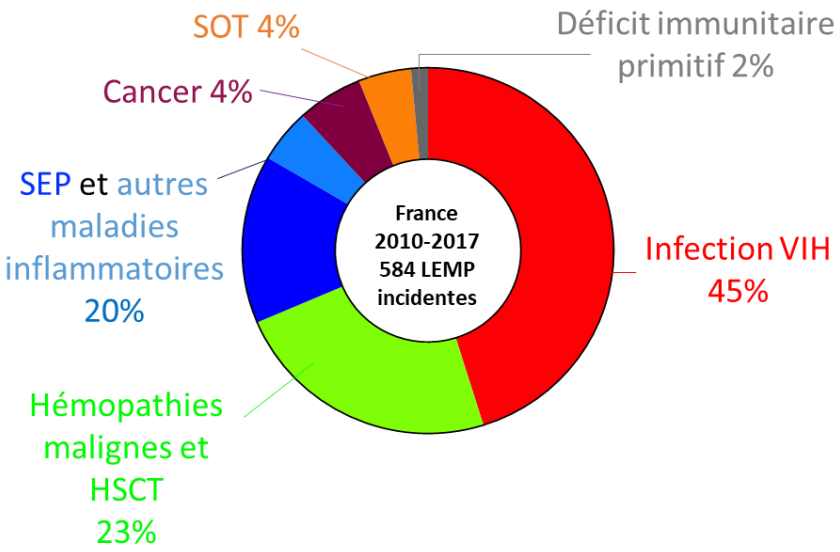
- 33 ADV
- 12 CMV
- 11 EBV
- 2 ADV+ CMV
- 1 ADV+ BKV
- 3 BKV
- 10 JCV

Follow-up / Pharmacovigilance

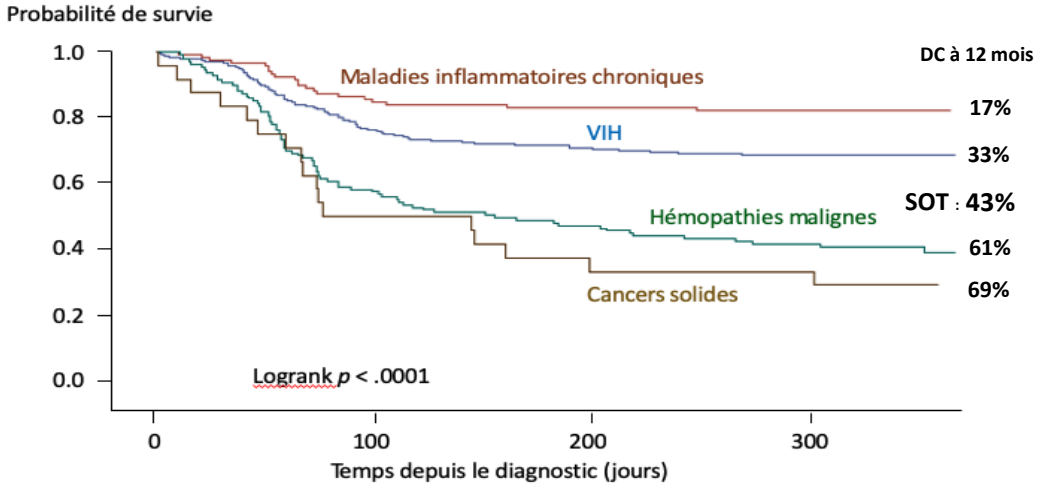
Data Base B2DInov en cours de paramétrage

JCV infections in Immunocompromised Patients

Contextes d'immunodépression



Probabilité de survie selon contexte



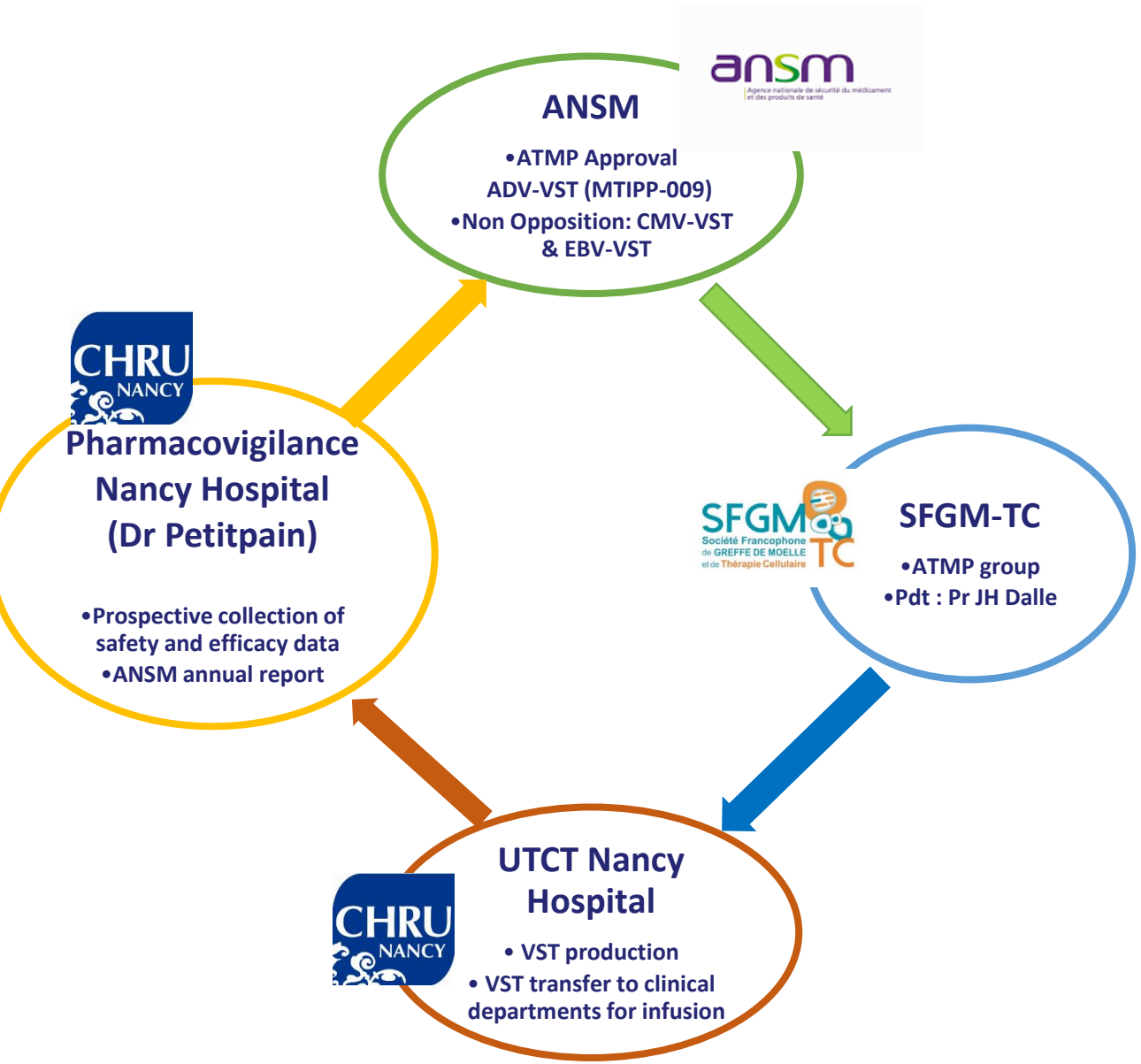
- SNDS, France, 2010-2017:
  - Rare: Incidence 0,11 /100 000 PA [IC95% 0,10-0,12] (60-90/yrs)
  - Grave: Mortalité toute cause à 12 mois: 38% [IC95% 34-42]

RCP « Infections du SNC par le virus JC »
n= 106 patients au 09/05/2025

- RCP « réactive » depuis 10/2023, création à la demande de l'ANSM
  - Infectiologie : Pr. V. MARTINEZ (Paris), Pr. G. MARTIN-BLONDEL (Toulouse)
  - Neurologie : Pr. B. STANKOFF (Paris), Dr. J. GASNAULT (Paris), Pr. N. WEISS (Paris)
  - Hématologie : Pr. D. ROOS-WEIL (Paris)
  - Immunologie : Dr. Y. TAOUFIK (Paris), Dr E. TREINER (Toulouse)
  - Thérapie cellulaire : Pr. D. BENSOUSSAN (Nancy) ➔ *Lien groupe MTI-PP SFGM-TC*
  - Virologie : Dr. S. LHOMME (Toulouse)
  - Neuroradiologie : Pr. F. BONNEVILLE (Toulouse), Dr N. SHOR (Paris)
- Associée à un registre prospectif (THEOREM)
- rcp-jc@chu-toulouse.fr

Joly M, Brain, 2023; à partir d'une diapositive de G Martin-Blondel

Real Life Study (2016-2021)





80
Referral procedures
Sent to ATMP group

30 procedures considered non relevant

50
Favorable opinions

21 patients non treated

29
Patients treated with VSTs

13 ADV | 7 CMV | 8 EBV | 1 ADV+ CMV

Follow-up / Pharmacovigilance

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Material and Methods

- **Inclusion Criteria**



  Allo-HSCT patients

  Viral replication and /or Tissue infection with ADV, CMV and/or EBV resistant to 2 weeks of anti-viral treatment

- **Exclusion Criteria**



  Uncontrolled aGVHD>II or cGVHD>II

  Corticosteroids > 1mg/kg/j

- **Safety at 3 months:**

  Alloreactivity,

  Graft failure or graft dysfunction

  Infections

  Serious adverse events collection

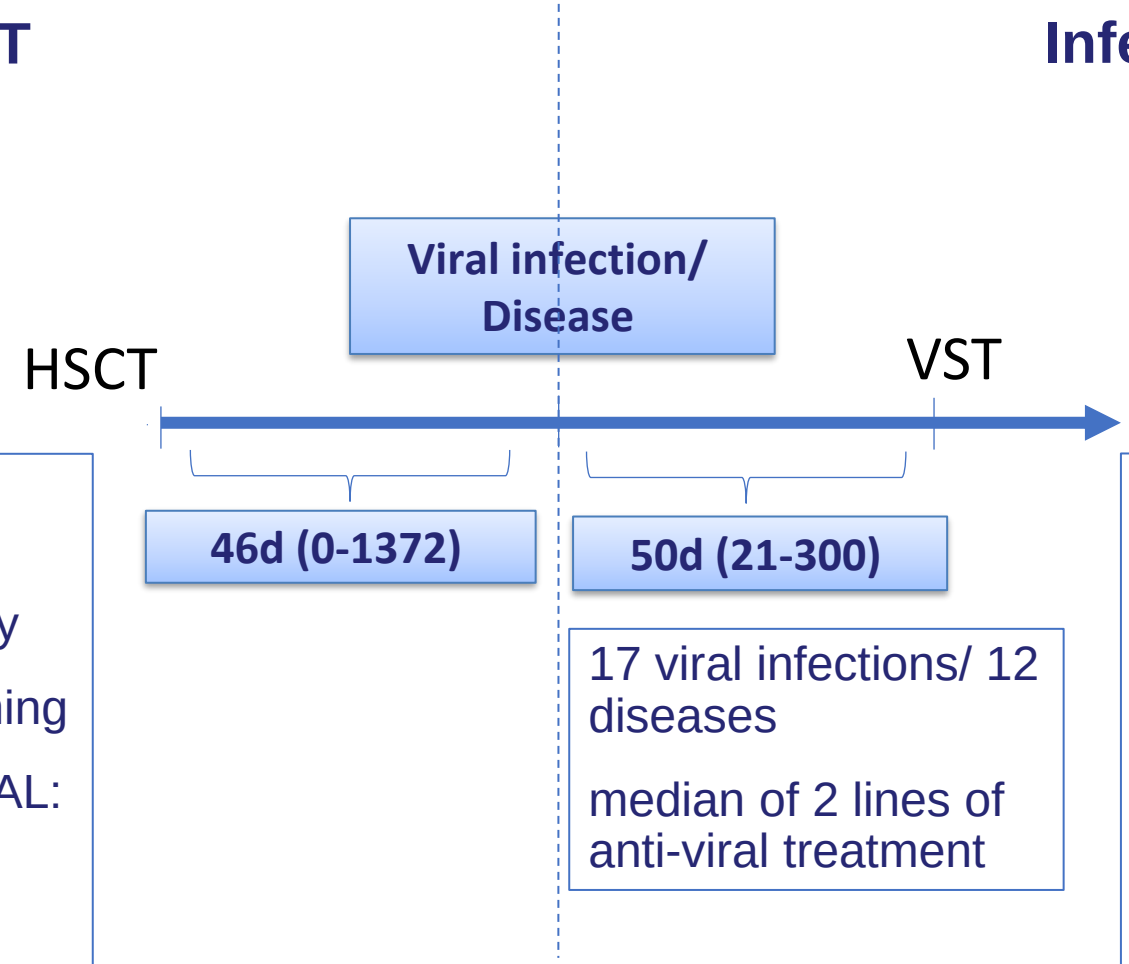
- **Efficacy at 3 months**

  CR: complete clearance of the virus

  PR: viral load decrease > 1 log

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Results: Patient characteristics



17 Children, 12 Adults

Non malignant (n=15) *vs* malignant (n=14) hemopathy

48% NMA or RIC conditionning

100% *in vivo* T depletion (SAL: 59%)

38% treated aGVHD at the time of VST infusion (n=11)

17 viral infections/ 12 diseases

median of 2 lines of anti-viral treatment

55% familial third party donor

5 patients: 2 infusions

Mean infused Dose:

1,43 +/- 3,06 10^4 CD3+ Tcells/kg.

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Results: Safety and Efficacy

Safety/Causes of deaths

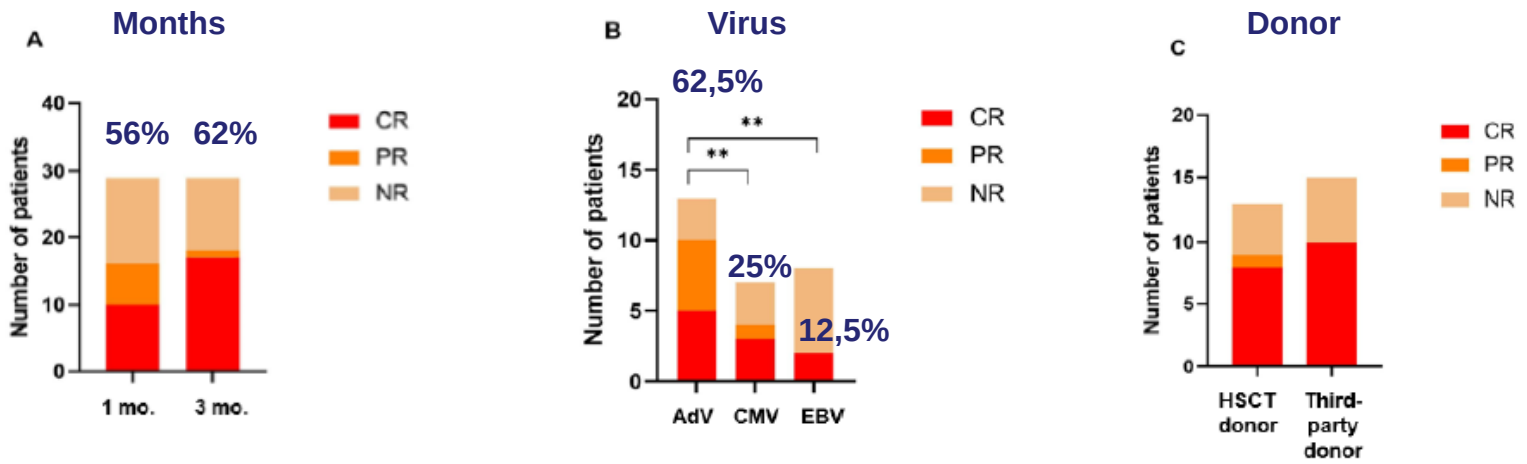
- **GVHD:**
  - 2 aGVHD II-IV reactivation
  - 1 unproven *de novo* cGVHD leading to death

  All of 3 leading to death

- **Infection deaths:**
  - 8 of original viral infection/disease
  - 1 septic shock
  - 1 BKV infection

- **TRM: 3**
- **Relapse: 1**

Efficacy



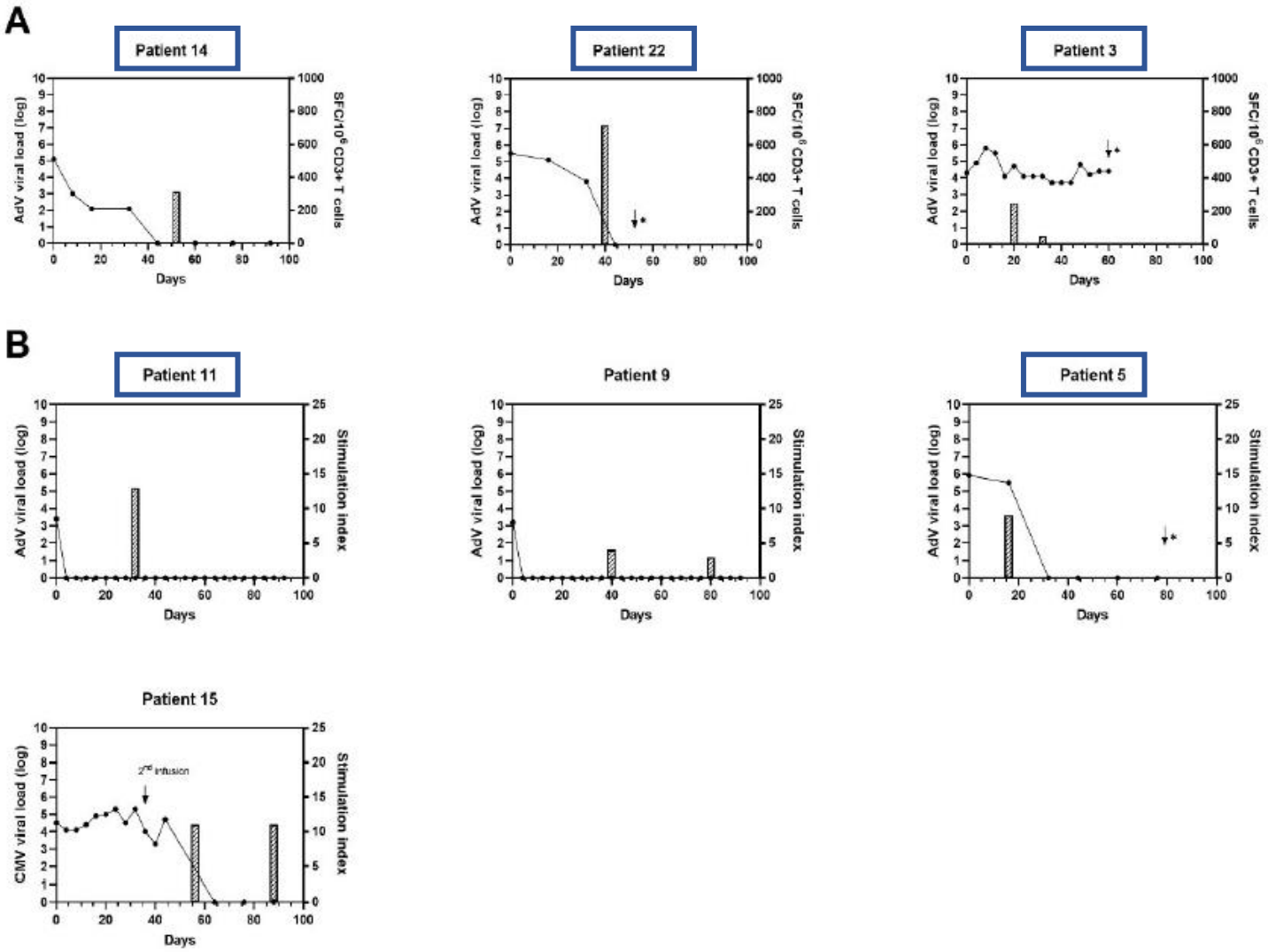
No influence on response rate at 1 month:

- Donor Type (p=0,415)
- *In vivo* T-cell depletion (p=1,000)
- Immunosuppression modulation (p=0,626)
- Continuation of corticosteroids <1mg/kg/d (p=0,677)
- Ruxolitinib at the time of VSTs (n=4)
- Curative anti-viral treatment post-VSTs (p=0,412)

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Results: Viral Immune reconstitution

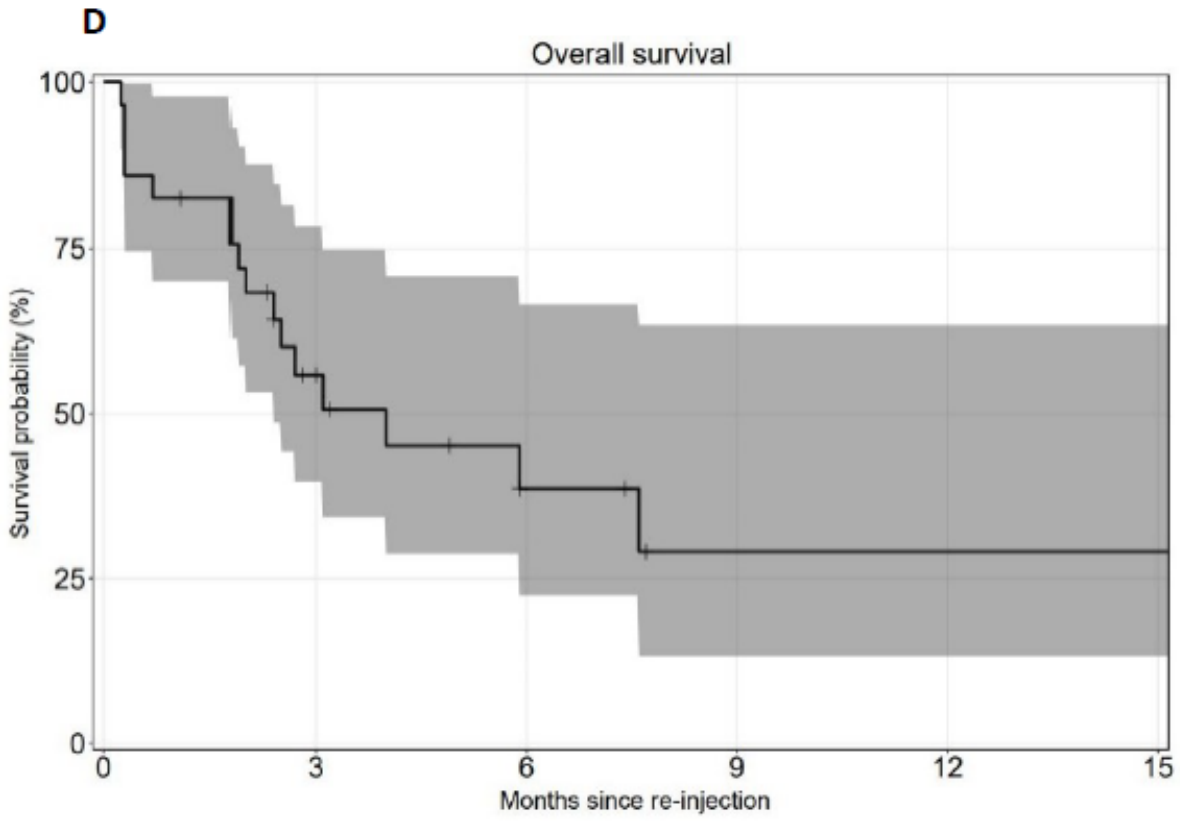
- Evaluated only in few centers
- Techniques :
  - ELIspot-IFN-γ
  - T proliferative assay
- Patients 14, 22, 3, 11, 5 received corticosteroids

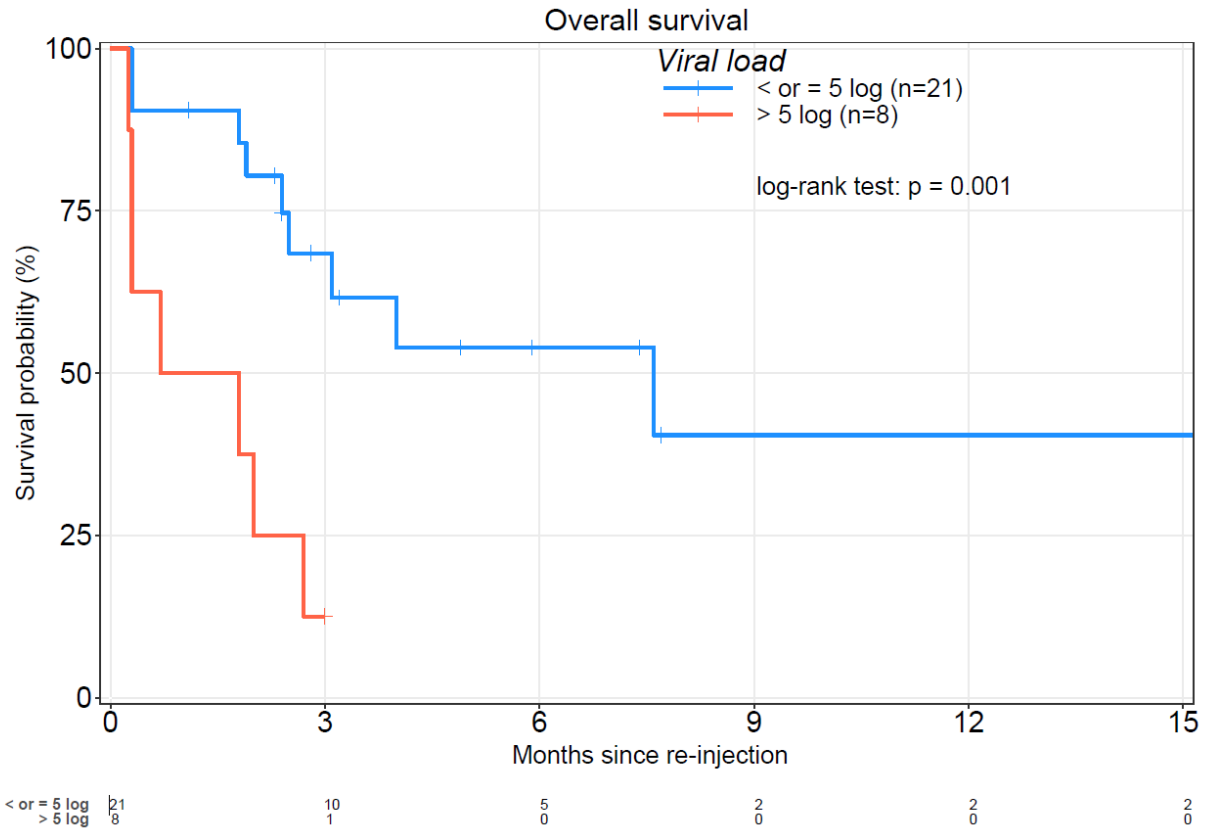


The arrow alone symbolizes the time for second VST infusion. The arrow accompanied by an asterisk signifies day of death.

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Results: Overall Survival





Survie Globale : 29%

Hazane Leroyer E et al.. Hemasphere. HemaSphere. 2024;8:e40

Real Life Study Conclusion

First large real life study with VST cells immunomagnetically isolated from third party donors

- **Safety: 3/29 GVHD reactivation (10%): VST or IS?**
- **Efficacy:** **- ORR 62% à 3 mois**

  - Significantly higher for ADV pats / CMV or EBV. Of note the median delay between infection and VST infusion was 39 vs 85 or 52 respectively

  - Immune reconstitution highlighted in 7 patients
- **Real life study vs Clinical Trial:**

  - Many lines of anti-viral treatment

  - Delay before initiating VST (/CT)

  → **Threshold at 5 log of Viral load: *the earliest, the best!***
- **Interesting findings:**

  - Repeated infusions: 5 patients, 3 with RC

  - Steroids: do not impair VST functionality but expansion (*in vitro*)

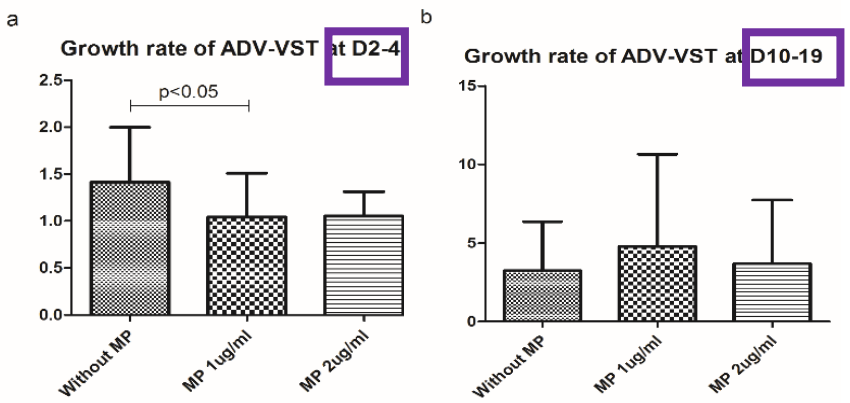
  - Ruxolitinib?

Campidelli A et al.. Cytotherapy. 2018; Pan et al. J Cell Mol Med. 2021; Rumi et al, Hematological Oncology. 2020)

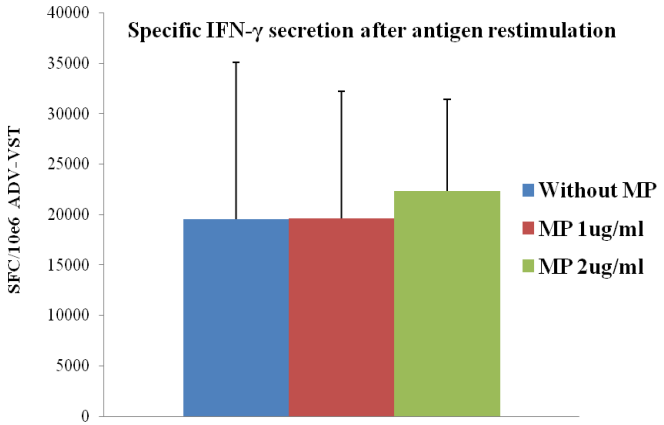
Impact of IS drug on VSTs?

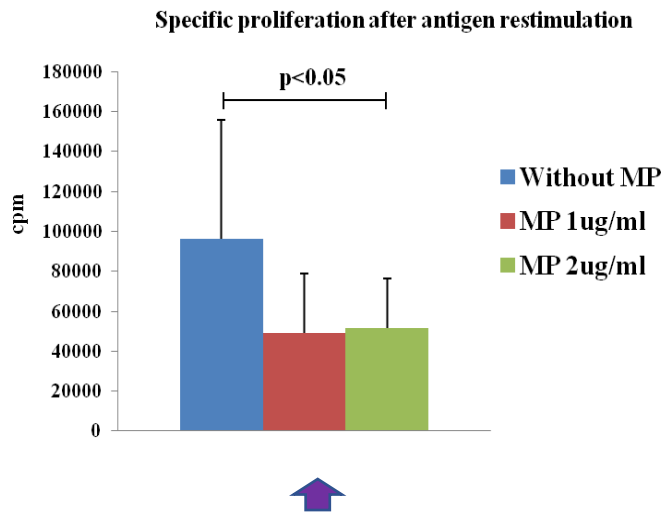
Méthylprednisolone

Expansion of ADV-VST: proliferative ratio *



* proliferative ratio: the cell number/ the last cell number





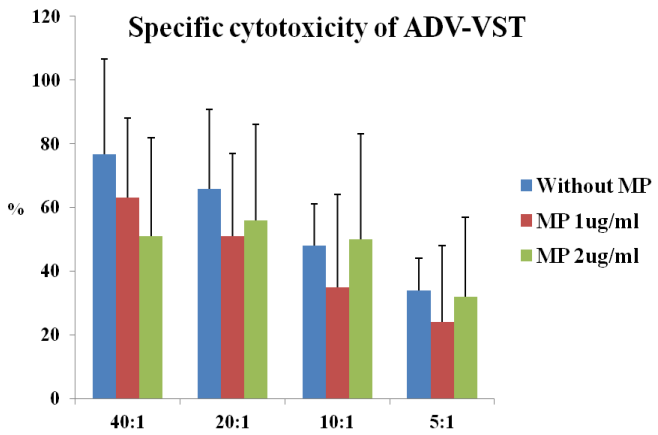
Mature subsets

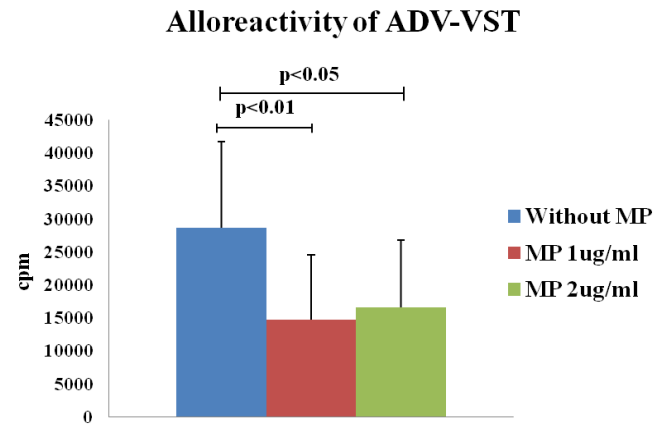
Immature subsets

Probably a main impact of MP on immature T cell subsets

Martinez-Caceres, *Clin Exp Immunol 2002*

What about other IS drugs like Ruxolutinib?





Campidelli et al, Cytotherapy, 2018

Perspectives

- **Companies developing VST (Ebvallo, P Fabre) AMM in Europe**
- **According to ISCT: controlled Clinical Trials required to allow the use of VST as:**
  - **Prophylaxis (Jiang et al, 2022)**
  - **First line therapy**

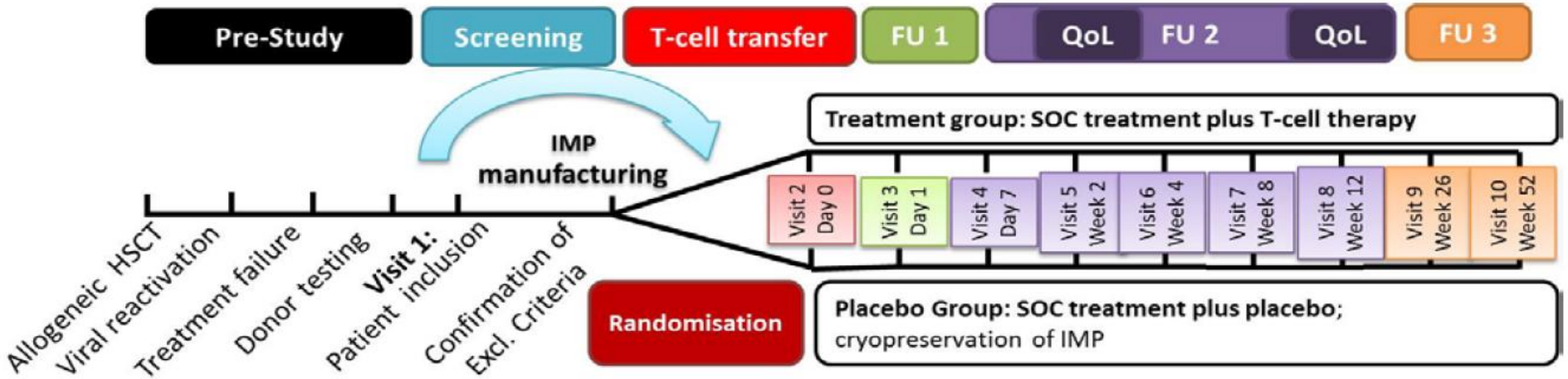
    European academic phase III clinical trial : Trace

  - **However, those CT suppose a randomization with a control group without infusion of VST: break on inclusions into trials**

Stanojevic et al.. Cytotherapy. 2022

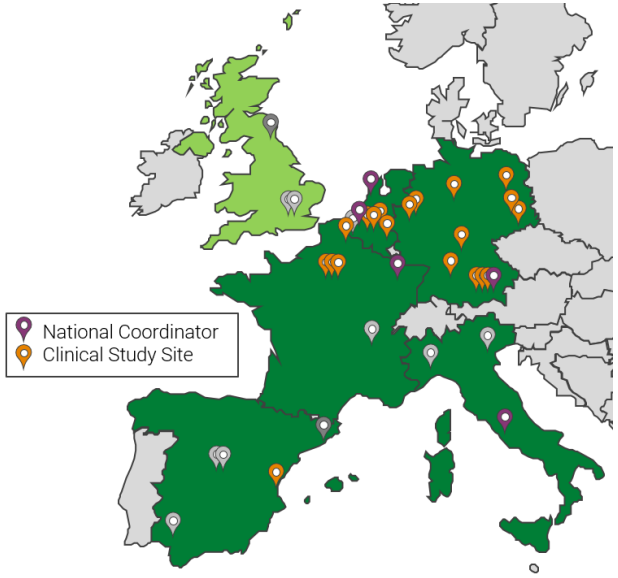
An academic European Phase III study

- A randomized, blinded placebo-controlled phase III study in a large cohort of patients: 130 patients
- TRACE Trial: *Adoptive Transfer of Multivirus Specific T cells*
- PI: Pr Tobias FEUCHTINGER from Munich
- Funding: H2020 2017 and Support from Miltenyi Biotec / Alternative fundings
- 7 European countries: Harmonized procedures for production and QC





An academic European Phase III study



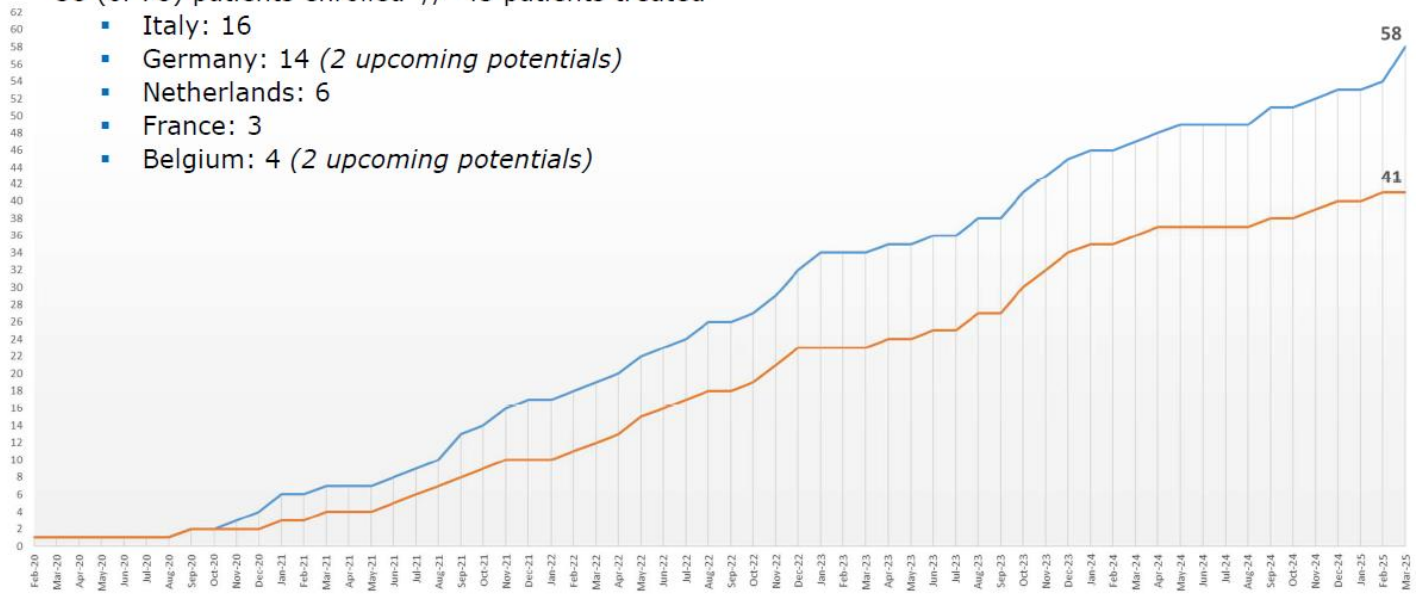
French Coordinating center:
Nancy University Hospital

- French PI: **Pr Maud d'Aveni**
- Production center: **UTCT**

FR04	Pr JH Dalle	Paris (Robert Debré)
FR05	Pr S Nguyen Quoc	Paris (Pitie Salpetrière)
FR07	Pr B Bruno	Lille
FR08	Dr M Ouachee-Chardin/ Dr C Renard	Lyon
FR09	Pr B Neven	Paris (Necker)

58 (of 78) patients enrolled // 43 patients treated

- Italy: 16
- Germany: 14 *(2 upcoming potentials)*
- Netherlands: 6
- France: 3
- Belgium: 4 *(2 upcoming potentials)*



3 patients included in France:

Lille: 03-22
La Pitié: 17-11-22
La Pitié: 12-23

Need to improve recruitment !

Aknowledgements

Cell Therapy Unit: UTCT



PhD Students



Y Wang



Q Chongseng

SIR



JA Guimaraes

Pharmacy &



Ségolène Pitz

Medical Students



Esther Hazane Leroyer

- **ATMP group**
  - Pr JH DALLE, Hôpital Robert Debré
  - Dr L CLEMENT, CHRU de Bordeaux
  - Dr K BILGER, HUS de Strasbourg
  - Dr C POCHON, CHRU de Nancy
  - Pr G MARTIN-BLONDEL, CHU de Toulouse
  - Pr H KAMINSKI, CHRU de Bordeaux
  - Pr L COUZI, CHRU de Bordeaux
  - Dr A LEGENDRE, EFS
  - Pr H ROUARD, EFS
  - Pr J LARGHERO, Hôpital Saint Louis
  - Dr N PETITPAIN, CHRU de Nancy
  - Dr V PRONTSKUS, CHRU de Nancy
  - Pr D BENSOUSSAN, CHRU de Nancy
  - Mme N RAUS, SFGM-TC
  - Mme V FOSSAT, SFGM-TC
- **Cell therapy unit, UTCT**
  - All the technical staff
  - Pr V Decot
  - Dr L Reppel
  - Dr C Laroye
  - Dr M Gauthier
- **Pr M D'Aveni, PI Trace**
- **Dr A Campidelli**
- **Miltenyi Biotec**
- **All the patients, their family and the donors**