

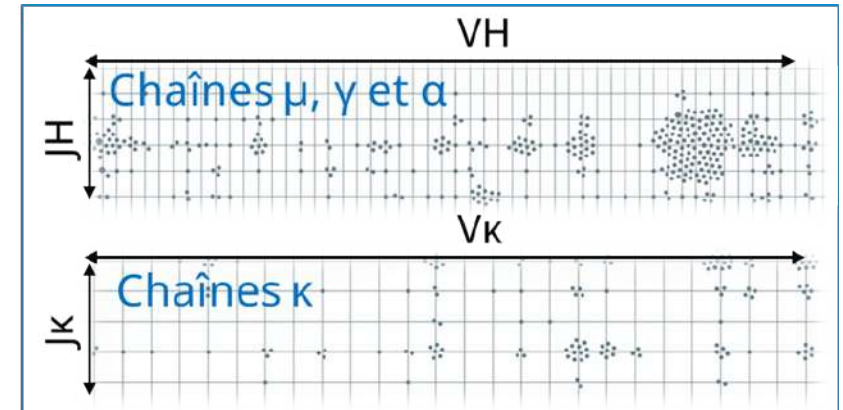
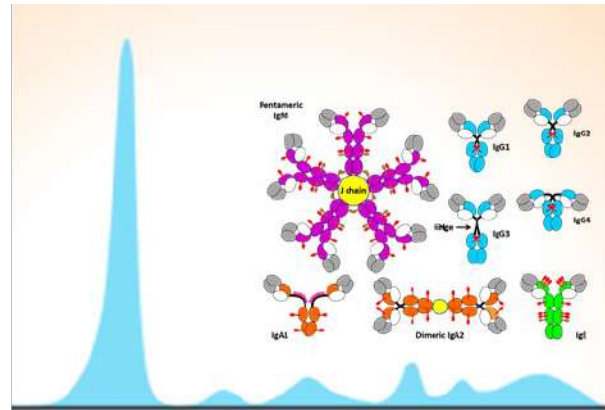
# Le NGS pour le séquençage des immunoglobulines : diagnostic et suivi des amyloses AL

V. PASCAL



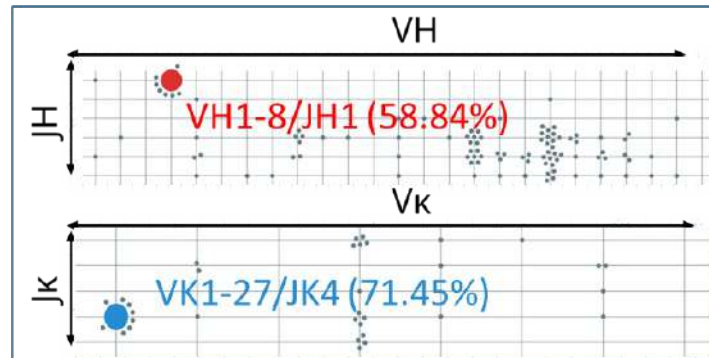
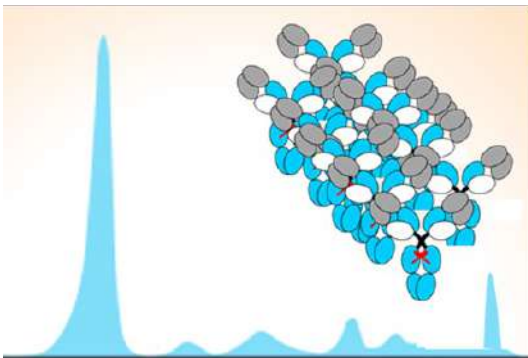
# Répertoires d'immunoglobulines par NGS

**Région  $\gamma$ -globulin / statut immunologique** (inflammation, infections, maladie auto-immune, composant monoclonal)



**Replg - Seq** (~ EPS "moléculaire")


**Profil Ig-MO**

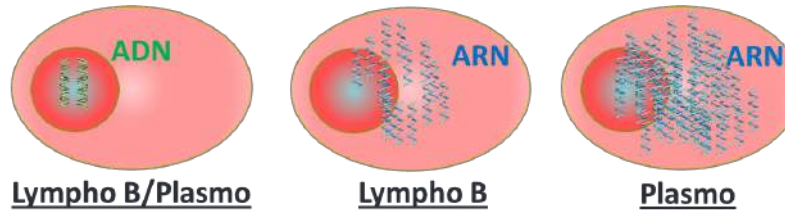


```
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ACCCTGTCCCTCAGTGCAGTGTCTCTGGTGGCTCCA
TCAGTAGTGGTACTACTACTGGAGCTGGATCCGGCA
GCCCGCCGGGAAGGGACTGGAGTGGATTGGCGTA
TCTATACAGTGGGAGTACCAACTACAACCCCTCCT
CAAGAGTCGAGTCCCATATCAGTTGACCCGTC CAAG
AACAA GTTCTCCCTGAAACTGAGGTCCGTGACCGCG
CAGACACGGCCGTGTATTACTGTGCAGAGGGGGTT
GTAGTGGTGGTAGCTGCCACCACTTCCATTATTACGG
TATGGACGTCTGGGGCCAAAGGACCACGGTCACAGT
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```

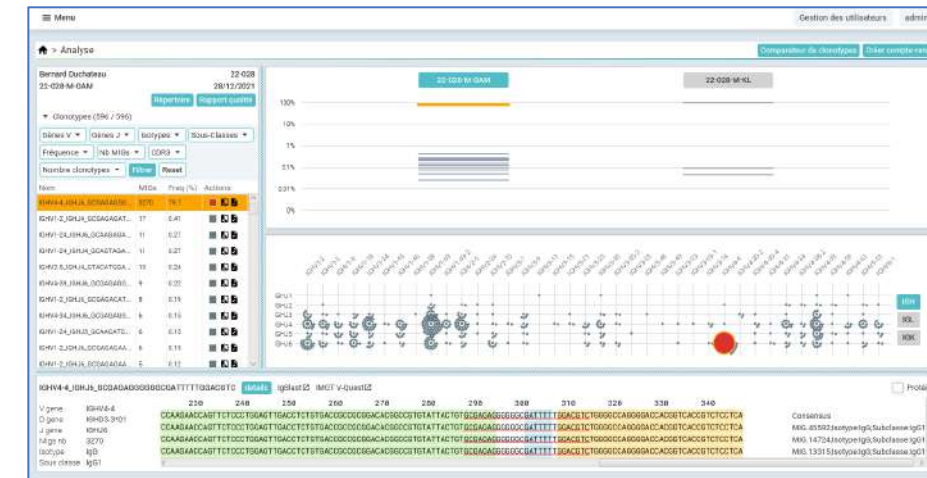
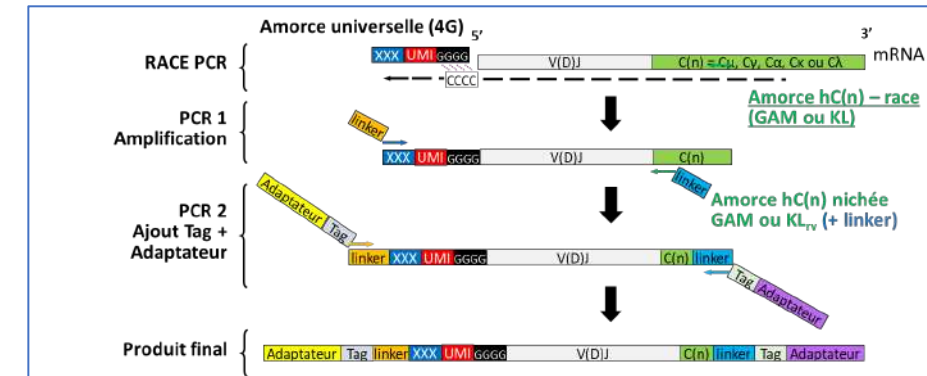
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AGCCACTCTGACCATCAGCGGGAACCCAGTC
TATGGATGAGGCTGACTATTACTGTACGC
GTGGGACACCAGCACTGTGGTATTCGGCGG
AGGGACCAAGCTGACCTGCTAGGTACGCC
CAAGGCTCCCTCGGTCACTCTGTCCCG
CCCTCTCTGAGGAGCTCCAAGCCAAC
```

# RACE RepSeq au labo d'immunologie (CHU Limoges)

- Développée / **CRIBL** en collaboration avec le **CNR amylose AL**
- Recherche **composante monoclonale** (MO, sang, tissus)
- Sur matrice **ARN** :  **sensibilité**



- En routine depuis juin 2021 (~ 30 prélèvements / mois)
- **Indications:**
  - **Outil diagnostique** -> MGRS/MGCS :
    - Clone de **faible abondance**
    - Cas incertains/compliqués (Sd POEMS, chaîne lourde tronquée...)
  - **Suivi et MRD** -> MGRS/MGCS : en cours d'évaluation...



# Outil diagnostic -> MGRS / « petits » clones

-> 13 patients:

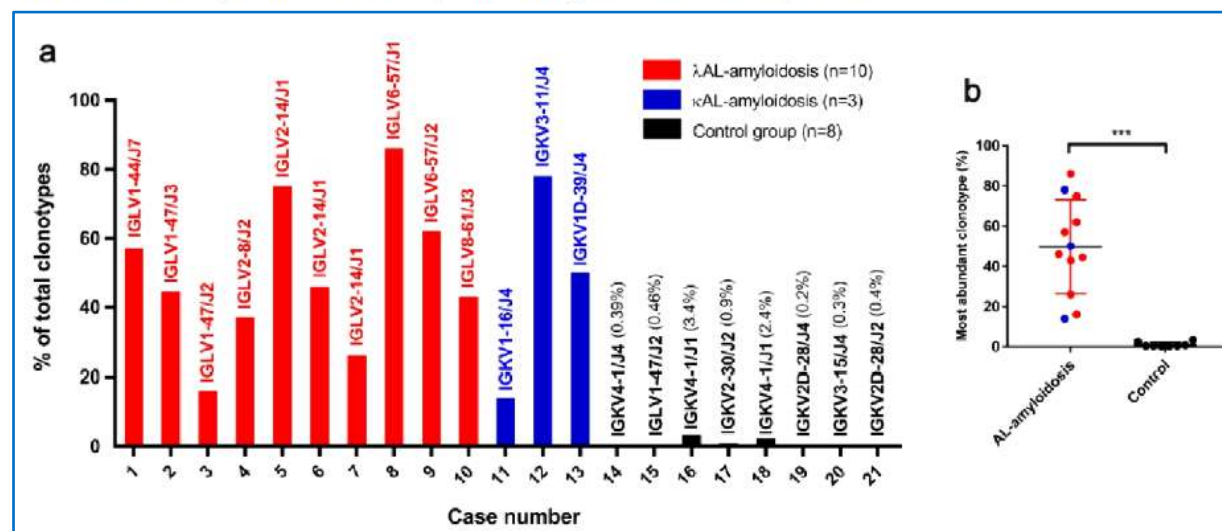
PBR -> amylose AL + dFLC <50 mg/L

www.kidney-international.org

technical notes

## RNA-based immunoglobulin repertoire sequencing is a new tool for the management of monoclonal gammopathy of renal (kidney) significance

Vincent Javaugue<sup>1,2,9,10</sup>, Virginie Pascal<sup>2,3,9,10</sup>, Sébastien Bender<sup>2,9</sup>, Sarah Nasraddine<sup>3</sup>, Mathilde Dargelos<sup>1,9</sup>, Mehdi Alizadeh<sup>4</sup>, Alexis Saintamand<sup>5</sup>, Matthieu Filloux<sup>3</sup>, Paco Derouault<sup>6</sup>, Sabrina Bouyer<sup>7</sup>, Estelle Desport<sup>1,9</sup>, Arnaud Jaccard<sup>2,8,9</sup>, Frank Bridoux<sup>1,2,9</sup>, Michel Cogné<sup>2,5,9</sup> and Christophe Sirac<sup>2,9</sup>



	Case 1	Case 2
Sex	Male	Male
Age (years)	71	63
Serum immunofixation	Negative	<del>IgGκ</del>
Urine immunofixation	Negative	λ
Kappa (mg/L)*	8	16
Lambda (mg/L)*	24	42
dFLC	16	26
Lambda/Kappa ratio*	3	2.62
Peripheral blood flow cytometry	No clone	ND
Bone marrow findings		
Plasma cell (%)	1	3
IHC	No clone	ND
Flow cytometry	No clone	No λ clone κ-PC clone (1% of cells)
RACE RepSeq analysis		
Number of λ sequences	142205	176577
Clone	IGLV1-44	IGLV1-47
Clonal sequences (%)	80	65

# RACE-RepSeq : Base de données d'IgMo

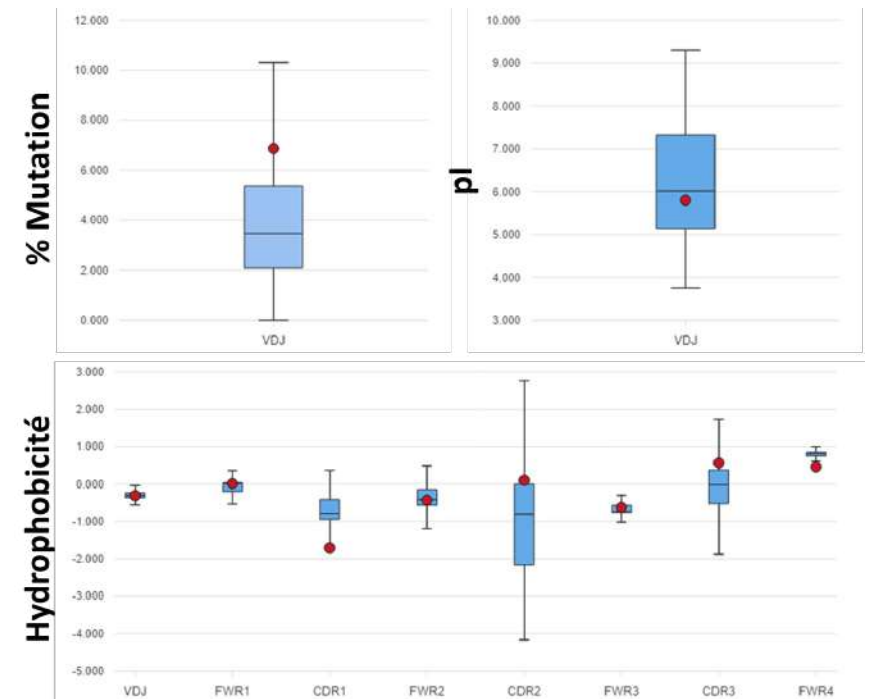
- 602 IgMo – chaînes légères séquencées (338 chaînes Lourdes)

- 258 AL -> 145 IgMo confirmé (Anapath)
- 87 POEMS -> 36 (Clinique)
- 24 PGNMID -> 19 (Anapath)
- 155 MM-MGUS-MW
- 27 Cryo
- 22 LCPT
- 18 LCDD

-> Confirmation Clinique / Anapath en cours...

- > Base de reference = 21111 HC + 54740 LC

-> IgMo AAL183 (IGLV6-57)



# Décryptage moléculaire et prédiction?

## IgMo-LC dans l'amylose AL :

- Prédominance Vλ6-57, Vλ2-14, Vλ1-44, Vκ1-33....
- Prédominance certains Vλ ↔ atteinte d'organe particulière
- Outils prédictifs accessibles / web:

IgMo POEMS001 (Vλ1-44 / H(40)->N)

blood Regular Article  
Immunoglobulin variable domain high-throughput sequencing reveals specific novel mutational patterns in POEMS syndrome

nature COMMUNICATIONS

83% prediction **LICTOR**

ARTICLE  
Machine learning analyses of antibody somatic mutations predict immunoglobulin light chain toxicity

Maura Garofalo<sup>1</sup>, Luca Piccoli<sup>1</sup>, Margherita Romeo<sup>2</sup>, Maria Monica Barzago<sup>2</sup>, Sara Ravasio<sup>1,3</sup>, Mathilde Foglierini<sup>1,4</sup>, Milos Matkovic<sup>1,5</sup>, Jacopo Sgrignani<sup>1</sup>, Raoul De Gasparo<sup>1</sup>, Marco Prunotto<sup>1,5</sup>, Luca Varani<sup>1</sup>, Luisa Diomedea<sup>2</sup>, Olivier Michielin<sup>6,7</sup>, Antonio Lanzavecchia<sup>1,8</sup> & Andrea Cavalli<sup>1,4,9</sup>

scientific reports

**VLamyPred**

OPEN Exploring the sequence features determining amyloidosis in human antibody light chains

Puneet Rawat<sup>1</sup>, R. Prabakaran<sup>2</sup>, Sandeep Kumar<sup>2</sup> & M. Michael Gromiha<sup>1,2,3</sup>

79% prediction

Leukemia

**NetNGlyc** 42% AL vs 12% MM

ARTICLE  
An N-glycosylation hotspot in immunoglobulin κ light chains is associated with AL amyloidosis

Alice Neviani<sup>1,2</sup>, Maria Grett<sup>1,2</sup>, Silvia Mangiacavalli<sup>1</sup>, Bruno Paiva<sup>1,3</sup>, Paolo Milani<sup>1,4,5</sup>, Pasquale Cascino<sup>1,4</sup>, Maggie Piccetti<sup>1,2</sup>, Valentina Spreafico<sup>1,2</sup>, Claudio Salvatore Carina<sup>1,2</sup>, Pietro Benvenuto<sup>1,2</sup>, Bial Goicoechea<sup>1</sup>, Francesca Fazio<sup>1</sup>, Marco Bassetti<sup>1,2</sup>, Andrea Rai<sup>1,2</sup>, Martina Nanni<sup>1,2</sup>, Giulia Mazzini<sup>1,2</sup>, Serena Cammino<sup>1,2</sup>, Melania Antonietta Scialò<sup>1,2</sup>, Simona Casarini<sup>1,2</sup>, Paola Roggiani<sup>1,2</sup>, Francesca Lavatelli<sup>1,2</sup>, Maria Teresa Petrucci<sup>1</sup>, Pier Paolo Ottaviani<sup>1,2</sup>, Stefano Ricigiani<sup>1,2</sup>, Luca Arcari<sup>1,2</sup>, Giampaolo Merlini<sup>1,2</sup>, Giovanni Palladini<sup>1,2</sup> and Mario Nuvolone<sup>1,2,6</sup>

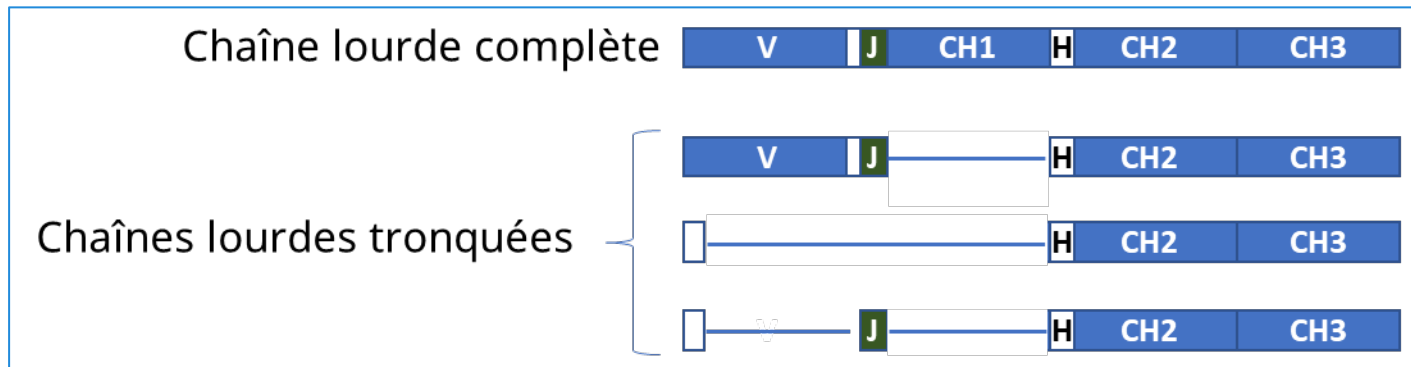
Limoges-Poitiers database (100 seq AL)

VLamyPred = 66%  
(Lictor ~ 33%)  
NetNGly = 19% / kappa

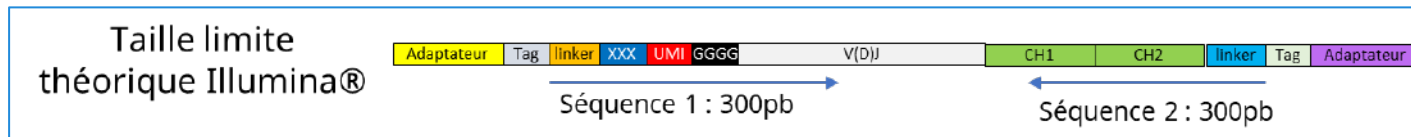
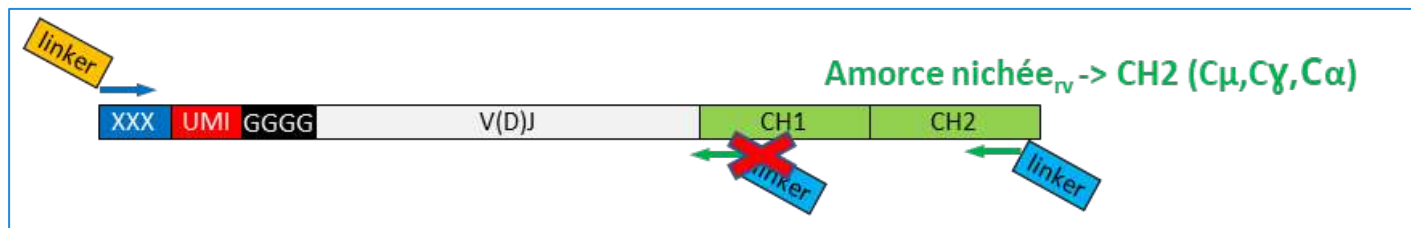
Accession	Gene	Region	Position	Sequence	VLamyPred	NetNGly	Lictor
1	IGHV1-18	CDR1	15	Q	Non-amyloid	0	0
2	IGHV1-18	CDR1	16	V	Non-amyloid	0	0
3	IGHV1-18	CDR1	17	I	Non-amyloid	0	0
4	IGHV1-18	CDR1	18	L	Non-amyloid	0	0
5	IGHV1-18	CDR1	19	S	Non-amyloid	0	0
6	IGHV1-18	CDR1	20	T	Non-amyloid	0	0
7	IGHV1-18	CDR1	21	Y	Non-amyloid	0	0
8	IGHV1-18	CDR1	22	F	Non-amyloid	0	0
9	IGHV1-18	CDR1	23	C	Non-amyloid	0	0
10	IGHV1-18	CDR1	24	A	Non-amyloid	0	0
11	IGHV1-18	CDR1	25	G	Non-amyloid	0	0
12	IGHV1-18	CDR1	26	P	Non-amyloid	0	0
13	IGHV1-18	CDR1	27	L	Non-amyloid	0	0
14	IGHV1-18	CDR1	28	K	Non-amyloid	0	0
15	IGHV1-18	CDR1	29	R	Non-amyloid	0	0
16	IGHV1-18	CDR1	30	H	Non-amyloid	0	0
17	IGHV1-18	CDR1	31	Q	Non-amyloid	0	0
18	IGHV1-18	CDR1	32	E	Non-amyloid	0	0
19	IGHV1-18	CDR1	33	D	Non-amyloid	0	0
20	IGHV1-18	CDR1	34	N	Non-amyloid	0	0
21	IGHV1-18	CDR1	35	K	Non-amyloid	0	0
22	IGHV1-18	CDR1	36	R	Non-amyloid	0	0
23	IGHV1-18	CDR1	37	G	Non-amyloid	0	0
24	IGHV1-18	CDR1	38	S	Non-amyloid	0	0
25	IGHV1-18	CDR1	39	T	Non-amyloid	0	0
26	IGHV1-18	CDR1	40	Y	Non-amyloid	0	0
27	IGHV1-18	CDR1	41	F	Non-amyloid	0	0
28	IGHV1-18	CDR1	42	C	Non-amyloid	0	0
29	IGHV1-18	CDR1	43	A	Non-amyloid	0	0
30	IGHV1-18	CDR1	44	G	Non-amyloid	0	0
31	IGHV1-18	CDR1	45	P	Non-amyloid	0	0
32	IGHV1-18	CDR1	46	L	Non-amyloid	0	0
33	IGHV1-18	CDR1	47	K	Non-amyloid	0	0
34	IGHV1-18	CDR1	48	R	Non-amyloid	0	0
35	IGHV1-18	CDR1	49	H	Non-amyloid	0	0
36	IGHV1-18	CDR1	50	Q	Non-amyloid	0	0
37	IGHV1-18	CDR1	51	E	Non-amyloid	0	0
38	IGHV1-18	CDR1	52	D	Non-amyloid	0	0
39	IGHV1-18	CDR1	53	N	Non-amyloid	0	0
40	IGHV1-18	CDR1	54	K	Non-amyloid	0	0
41	IGHV1-18	CDR1	55	R	Non-amyloid	0	0
42	IGHV1-18	CDR1	56	G	Non-amyloid	0	0
43	IGHV1-18	CDR1	57	S	Non-amyloid	0	0
44	IGHV1-18	CDR1	58	T	Non-amyloid	0	0
45	IGHV1-18	CDR1	59	Y	Non-amyloid	0	0
46	IGHV1-18	CDR1	60	F	Non-amyloid	0	0
47	IGHV1-18	CDR1	61	C	Non-amyloid	0	0
48	IGHV1-18	CDR1	62	A	Non-amyloid	0	0
49	IGHV1-18	CDR1	63	G	Non-amyloid	0	0
50	IGHV1-18	CDR1	64	P	Non-amyloid	0	0
51	IGHV1-18	CDR1	65	L	Non-amyloid	0	0
52	IGHV1-18	CDR1	66	K	Non-amyloid	0	0
53	IGHV1-18	CDR1	67	R	Non-amyloid	0	0
54	IGHV1-18	CDR1	68	H	Non-amyloid	0	0
55	IGHV1-18	CDR1	69	Q	Non-amyloid	0	0
56	IGHV1-18	CDR1	70	E	Non-amyloid	0	0
57	IGHV1-18	CDR1	71	D	Non-amyloid	0	0
58	IGHV1-18	CDR1	72	N	Non-amyloid	0	0
59	IGHV1-18	CDR1	73	K	Non-amyloid	0	0
60	IGHV1-18	CDR1	74	R	Non-amyloid	0	0
61	IGHV1-18	CDR1	75	G	Non-amyloid	0	0
62	IGHV1-18	CDR1	76	S	Non-amyloid	0	0
63	IGHV1-18	CDR1	77	T	Non-amyloid	0	0
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65	IGHV1-18	CDR1	79	F	Non-amyloid	0	0
66	IGHV1-18	CDR1	80	C	Non-amyloid	0	0
67	IGHV1-18	CDR1	81	A	Non-amyloid	0	0
68	IGHV1-18	CDR1	82	G	Non-amyloid	0	0
69	IGHV1-18	CDR1	83	P	Non-amyloid	0	0
70	IGHV1-18	CDR1	84	L	Non-amyloid	0	0
71	IGHV1-18	CDR1	85	K	Non-amyloid	0	0
72	IGHV1-18	CDR1	86	R	Non-amyloid	0	0
73	IGHV1-18	CDR1	87	H	Non-amyloid	0	0
74	IGHV1-18	CDR1	88	Q	Non-amyloid	0	0
75	IGHV1-18	CDR1	89	E	Non-amyloid	0	0
76	IGHV1-18	CDR1	90	D	Non-amyloid	0	0
77	IGHV1-18	CDR1	91	N	Non-amyloid	0	0
78	IGHV1-18	CDR1	92	K	Non-amyloid	0	0
79	IGHV1-18	CDR1	93	R	Non-amyloid	0	0
80	IGHV1-18	CDR1	94	G	Non-amyloid	0	0
81	IGHV1-18	CDR1	95	S	Non-amyloid	0	0
82	IGHV1-18	CDR1	96	T	Non-amyloid	0	0
83	IGHV1-18	CDR1	97	Y	Non-amyloid	0	0
84	IGHV1-18	CDR1	98	F	Non-amyloid	0	0
85	IGHV1-18	CDR1	99	C	Non-amyloid	0	0
86	IGHV1-18	CDR1	100	A	Non-amyloid	0	0

Mutations somatiques, Hydrophobicité, Modifications post-traductionnelles, Score d'aggrégation  
-> « Machine learning » pour prédire la toxicité individuelle des IgMo-LC

# Outil diagnostique -> chaîne lourde tronquée (amylose AH)

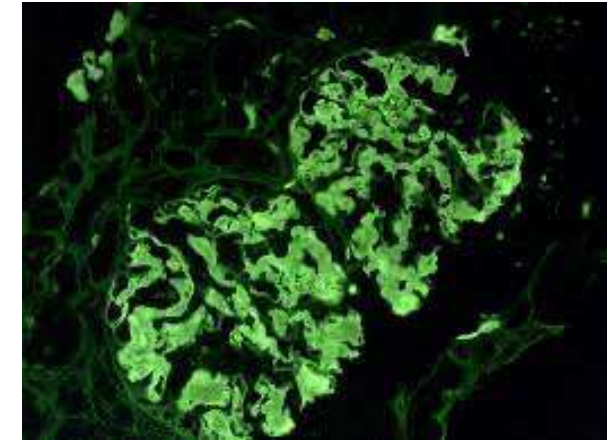


Séquençage des chaînes d'Ig complètes (domaine variable + constant)

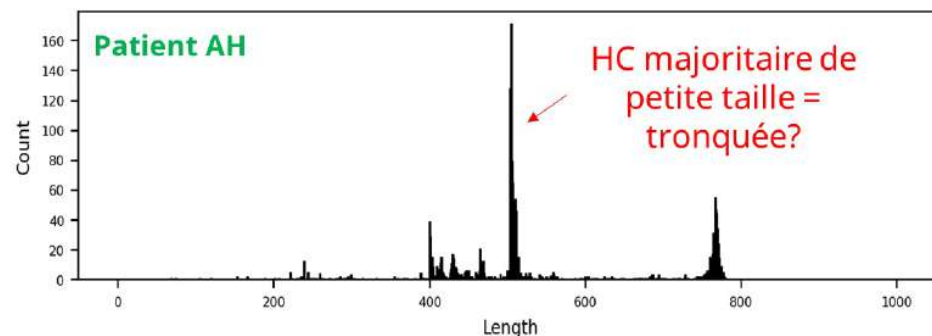
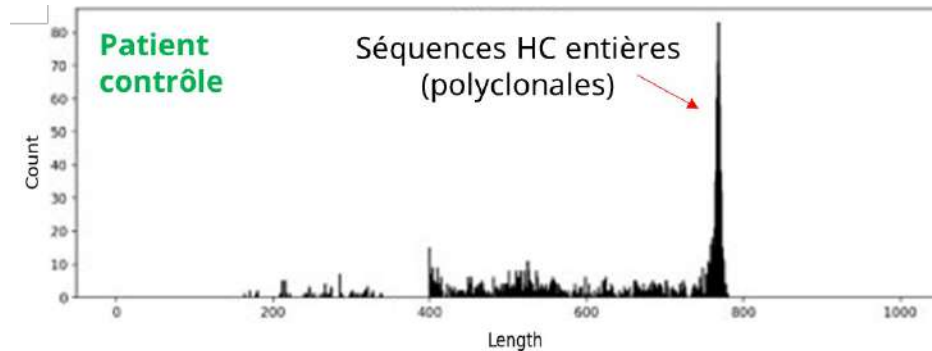


# Outil diagnostique -> chaîne lourde tronquée

Patient	EPS	IF	PBR
<b>Amylose AH <math>\gamma_1</math></b>	Pic = 4,5 g/L	IgA-kappa	Dépôts glomérulaires amyloïdes IF = <b>Chaînes lourdes <math>\gamma_1</math></b> ( $\emptyset$ $\kappa$ ou $\lambda$ )



Répartition  
des  
séquences  
d'Ig en  
fonction de  
leur taille



**Chaîne tronquée  $\gamma_1$  = 49% du  
répertoire GAM (MO)**

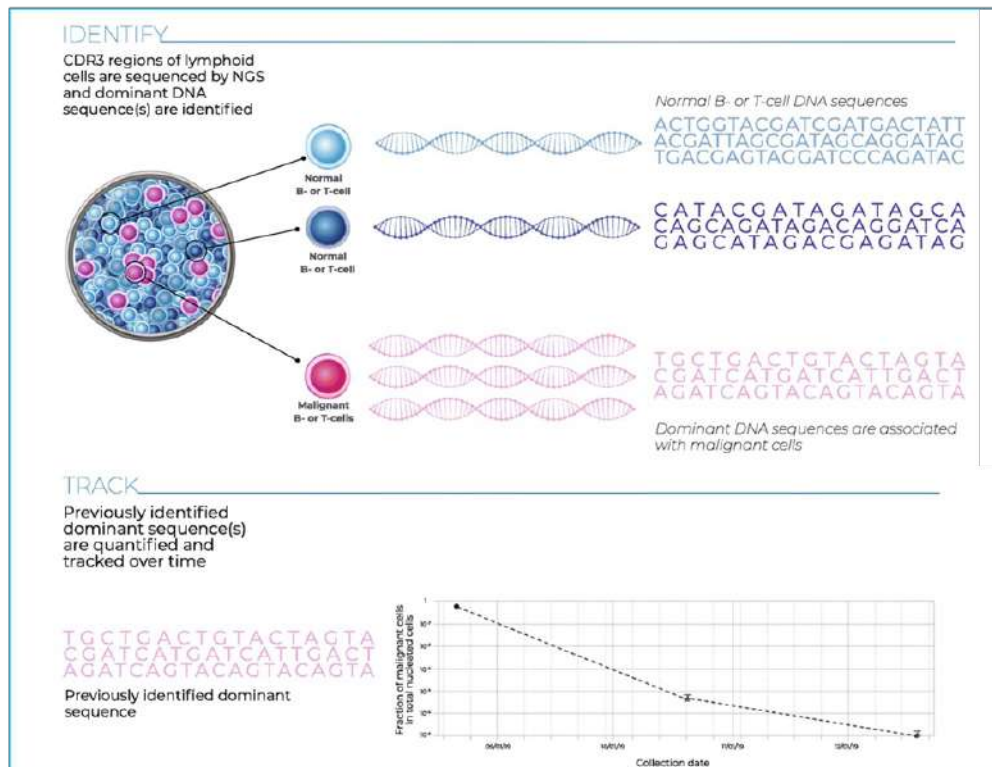


# Outil suivi + MRD (évaluation en cours -> IsAMYP)

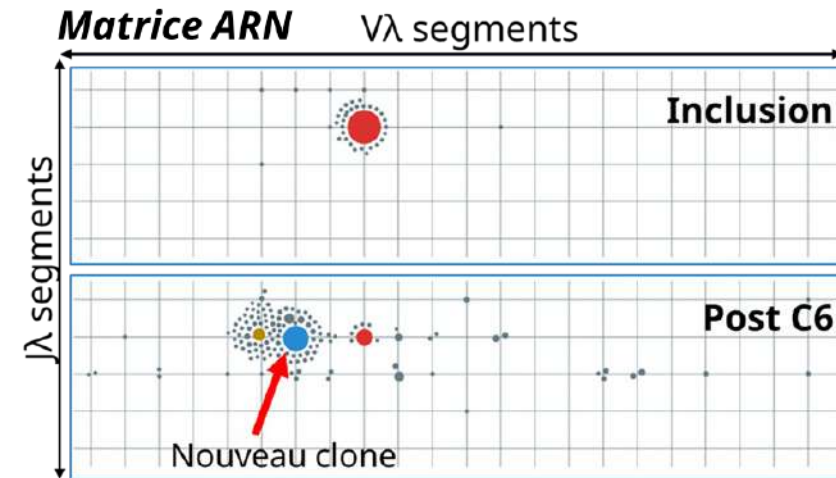
- **Technique de référence (MM...):**



## Matrice ADN



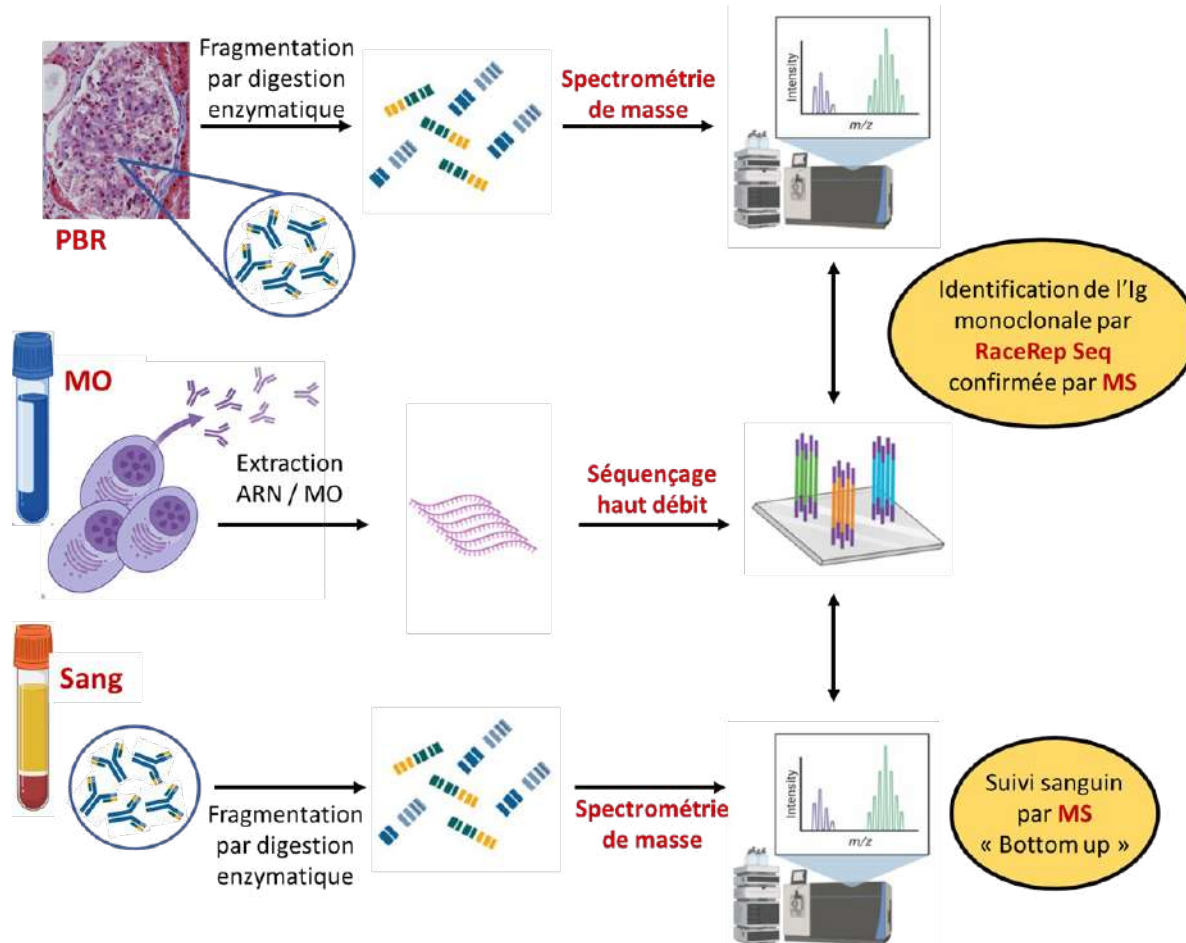
- **RACE RepSeq (AMYDARA):**



## Patient 167-34

- Réponse hématologique (CLL) = PR
- MRD (clonoSeq) < 7 (/10<sup>6</sup> cellules saines)
- Discordance CLL / MRD (ADN) -> **émergence d'un nouveau clone lambda** (Race RepSeq)

# Couplage spectrométrie de masse et RACE-RepSeq



## MS « Bottom up » :

- Isolement des Ig + digestion tryptique
- **Peptides candidats clonotypiques déduits** de la séquence ADN/ARN du clone cellulaire médullaire
- **Ss ~ MRD médullaire**
- **-> En cours:**
  - ❖ LMD-MS (Poitiers) <-> RACE-RepSeq (Limoges)
  - ❖ RACE-RepSeq (Limoges) <-> Entreprise SEBIA



Merci



Alexia Rinsant, Sihem Kaaki, Cécile Ory, Estelle Desport, JM Goujon, Pr Guy Touchard



Sébastien Bender



Christophe Sirac



Vincent Javaugue



Séléna Teillaud

Labo immunogénétique CHU Limoges



Frank Bridoux



Arnaud Jaccard



Murielle Roussel



Paco Derouault

