

Le NGS dans la génétique des amyloses

Pr Jill CORRE

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Table 2. Frequency of cytogenetic iFISH aberrations depending on disease (PC-AL vs PC-non-AL) and stage of plasma cell dyscrasia (MGUS/SMM/MM)

	PC-AL					PC-non-AL					P (PC-AL vs PC-non-AL)
	n/N (%)					n/N (%)					
	Total (n = 344)	MGUS (n = 104)	SMM (n = 210)	MM (n = 30)	P	Total (n = 513)	MGUS (n = 32)	SMM (n = 130)	MM (n = 301)	P	
Translocations											
t(11;14)	331/544 (60.8)	117/164 (71.3)	200/345 (58.0)	14/35 (40.0)	.001	76/519 (14.6)	4/62 (6.5)	7/156 (4.5)	65/301 (21.6)	<.001	<.001
t(4;14)	19/544 (3.5)	1/164 (0.6)	16/345 (4.6)	2/35 (5.7)	.17	86/519 (16.6)	6/62 (9.7)	33/156 (21.2)	47/301 (15.6)	.81	<.001
t(14;16)	13/543 (2.4)	2/164 (1.2)	11/344 (3.2)	0/35 (0)	.58	30/511 (5.5)	9/60 (15.0)	15/153 (9.8)	6/298 (2.0)	<.001	<.001
IgH unknown	94/541 (17.4)	25/164 (15.2)	286/343 (16.6)	12/34 (35.3)	.25	166/489 (33.9)	37/61 (60.7)	75/153 (49.0)	54/275 (19.6)	<.001	<.001
Gains											
1q21	167/544 (30.7)	40/164 (24.4)	115/345 (33.3)	12/35 (34.3)	.25	195/519 (37.6)	23/62 (37.1)	69/156 (44.2)	103/301 (34.2)	.75	.034
5p15/5q35	83/533 (15.6)	17/160 (10.6)	54/341 (15.8)	12/32 (37.5)	.013	136/367 (37.1)	17/54 (31.5)	54/148 (36.5)	65/165 (39.4)	.75	<.001
9q34	172/543 (31.7)	42/163 (25.8)	112/345 (32.5)	18/35 (51.4)	.054	283/517 (54.7)	31/62 (50.0)	80/156 (51.3)	172/299 (57.5)	.75	<.001
11q22.3	211/544 (38.8)	71/164 (43.3)	128/345 (37.1)	12/35 (34.3)	.31	224/519 (43.2)	15/62 (24.2)	58/156 (37.2)	151/301 (50.2)	<.001	.59
11q23											
15q22	97/544 (17.9)	19/163 (11.7)	64/344 (18.6)	14/35 (40.0)	.004	245/517 (47.5)	21/62 (33.9)	65/156 (41.7)	159/299 (53.2)	.01	<.001
19q13	102/542 (18.8)	21/163 (12.9)	70/344 (20.3)	11/35 (31.4)	.054	258/519 (49.7)	23/62 (37.1)	73/156 (46.8)	162/301 (53.8)	.067	<.001
Deletions											
8p21	38/534 (7.1)	6/161 (3.7)	28/338 (8.3)	4/35 (11.4)	.22	108/519 (20.8)	3/62 (4.8)	29/156 (18.6)	76/301 (25.2)	.003	.034
13q14	209/544 (38.4)	56/164 (34.1)	133/345 (38.6)	20/35 (57.1)	.22	264/519 (50.9)	2/62 (41.9)	79/156 (50.6)	159/301 (52.8)	.75	.071
17p13	16/544 (2.9)	3/164 (1.8)	10/345 (2.9)	3/35 (8.6)	.25	47/519 (9.1)	1/62 (1.6)	10/156 (6.4)	36/301 (12.0)	.024	.19

Frequency of cytogenetic aberrations in the AL amyloidosis group (PC-AL) as compared with the MGUS/SMM/MM control group (PC-non-AL group). Frequencies are also detailed for the respective stages of plasma cell dyscrasia. *P* values indicate changes of the frequency of the respective aberrations depending on the stage of plasma cell dyscrasia. The *P* value in the last column addresses the frequency difference between the PC-AL and PC-non-AL groups (adjusted for the respective frequencies of MGUS/SMM/MM in both groups via logistic regression). All *P* values were adjusted for multiple testing across probes using Holm correction to control the family-wise error rate.

La t(11;14) sont associés à un pronostic défavorable chez les patients au diagnostic d'une amylose AL traités par des stratégies à base de Bortezomib

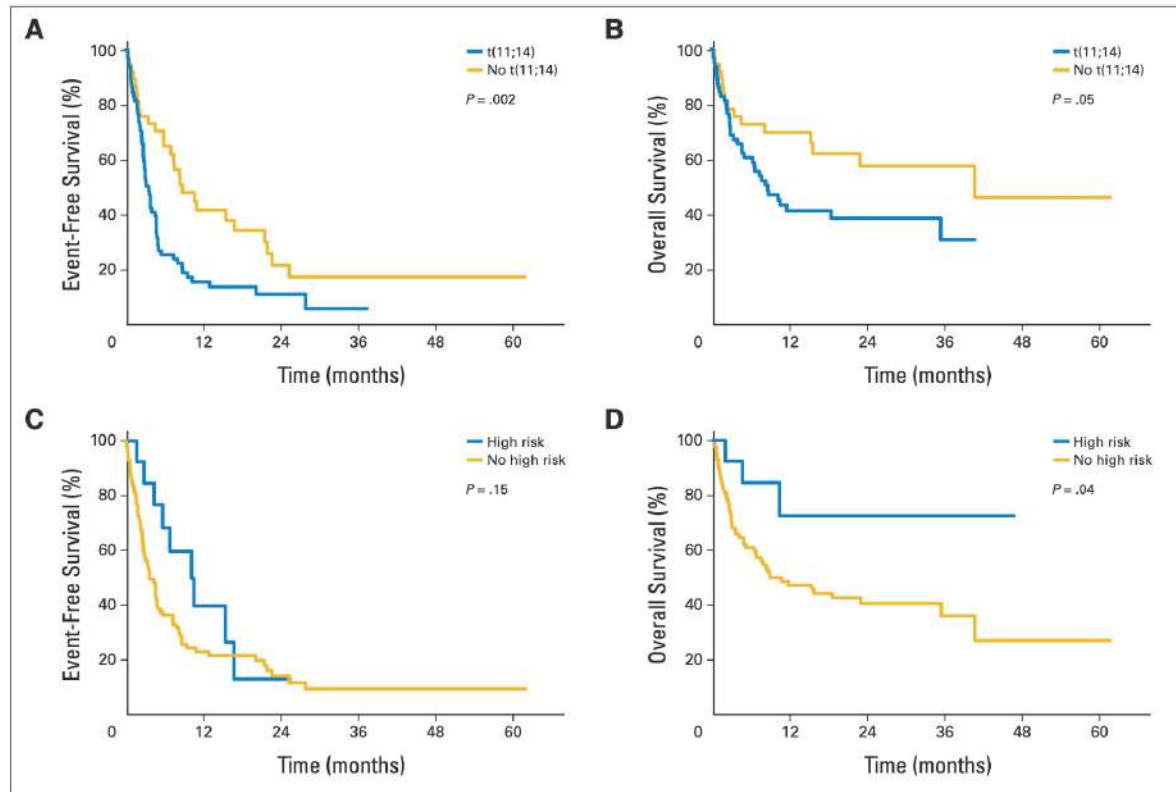
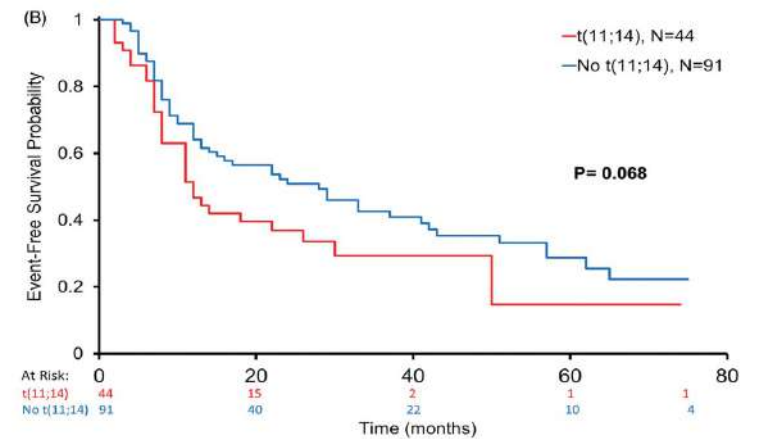
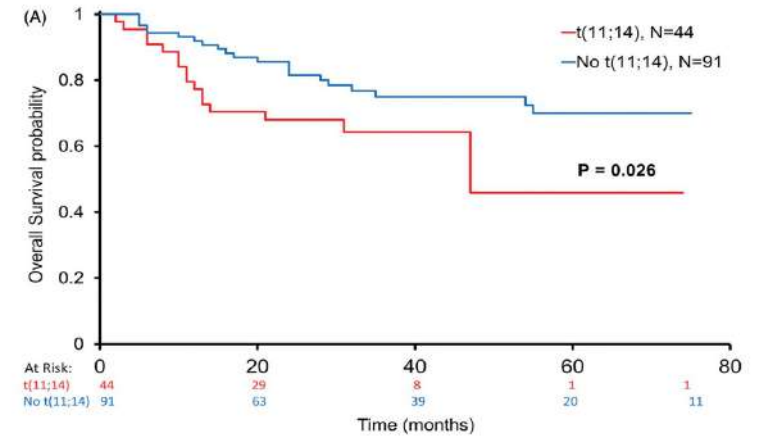
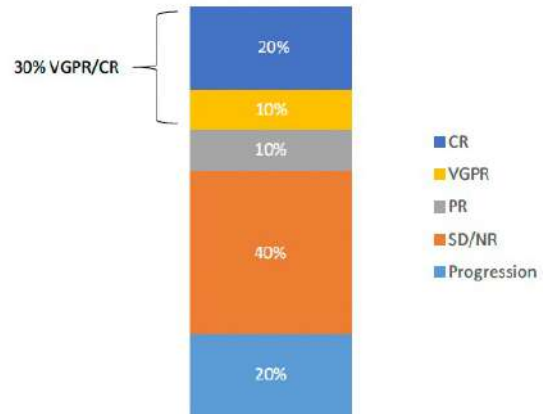


Fig 1. Hematologic event-free survival and overall survival in the bortezomib-dexamethasone cohort according to (A and B) t(11;14) and (C and D) t(4;14), t(14;16), and del(17p) subsumed as high-risk aberrations.

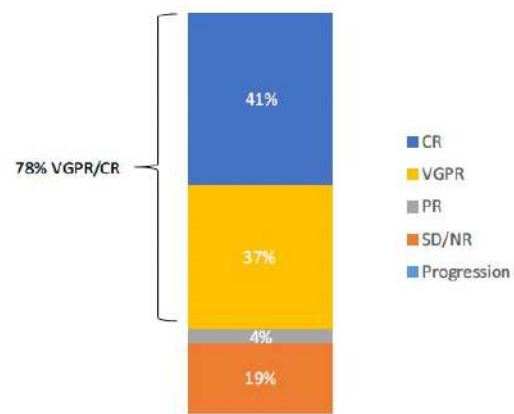


Le Venetoclax induit près de 80% VGPR/CR chez les patients réfractaires/en rechute d'une amylose AL avec t(11;14)

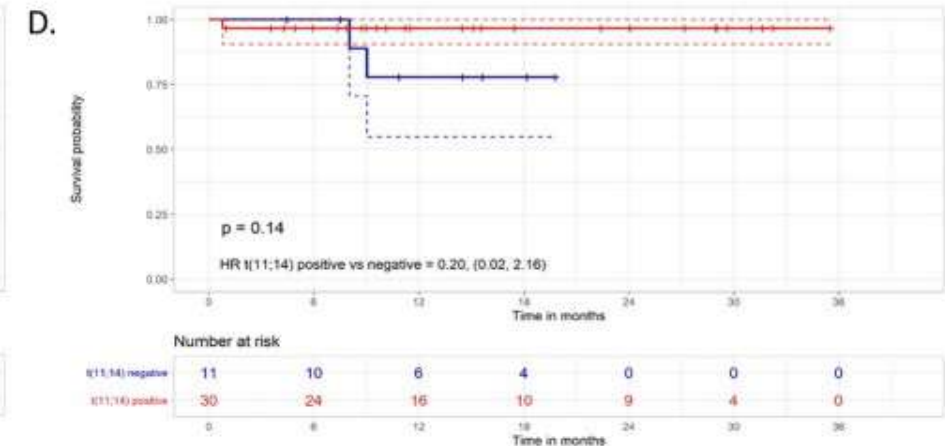
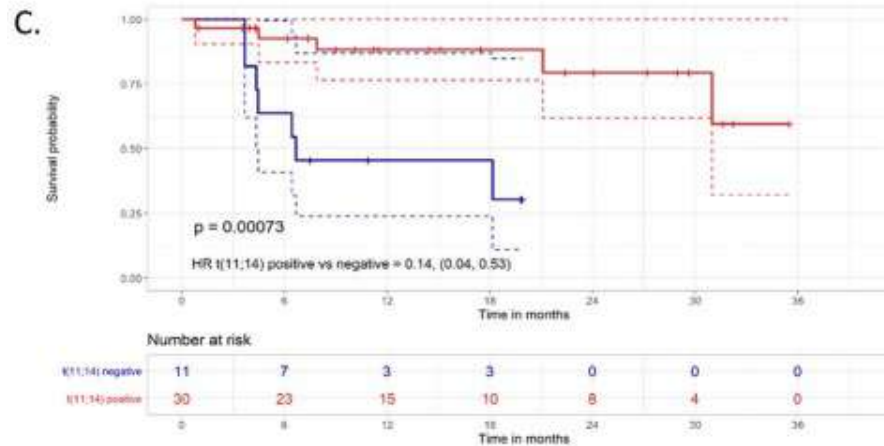
B
Best response in evaluable non-t(11;14) patients (n = 10)



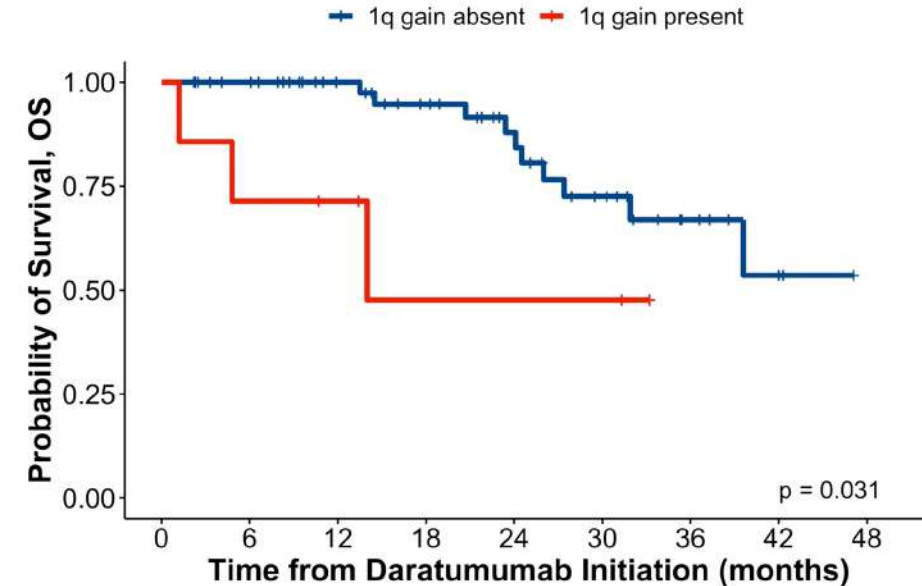
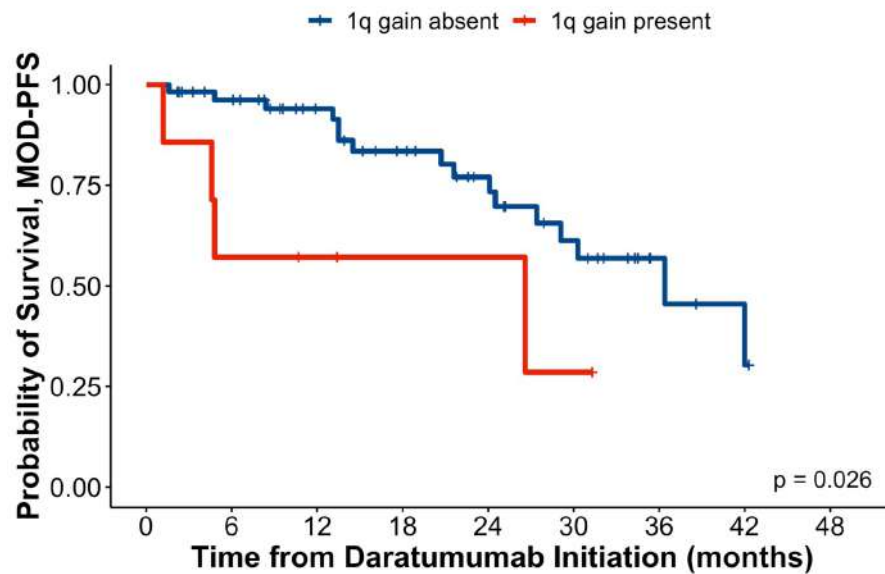
C
Best response in evaluable t(11;14) patients (n = 27)



Premkumar et al. Blood Cancer Journal (2021) 11:10



La présence d'un gain 1q est associée à un mauvais pronostic chez les patients traités par Daratumumab en monothérapie



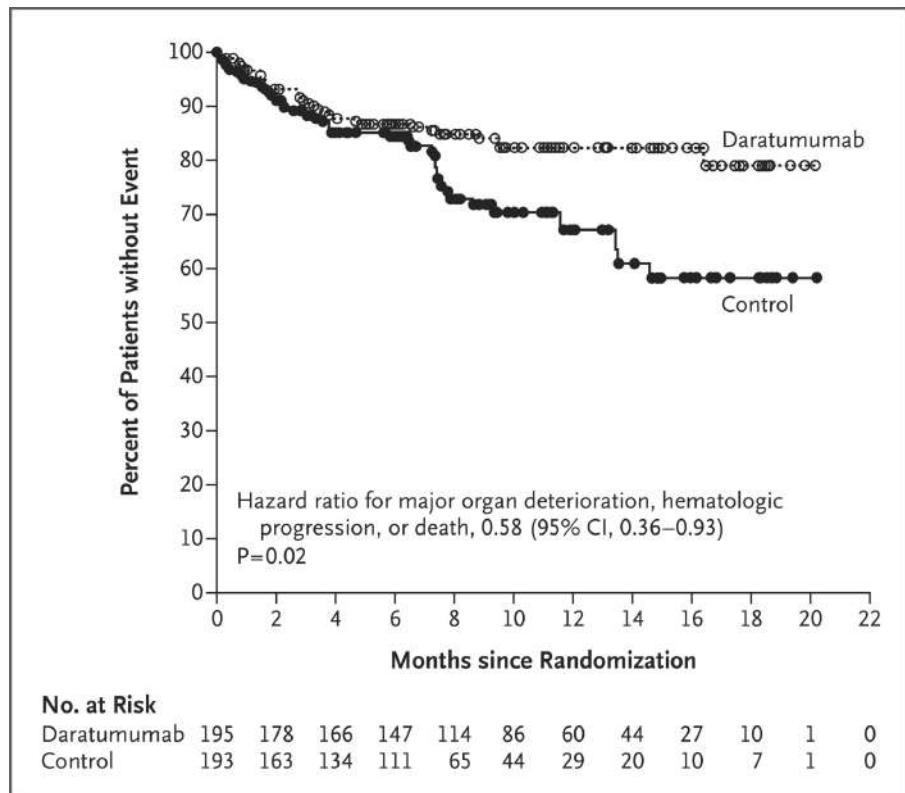


Table 1. Distribution of cytogenetic abnormalities.

	D-VCd (n = 195)	VCd (n = 193)	Total (N = 388)
FISH/karyotype test performed, n (%)	155 (79.5)	166 (86.0)	321 (82.7)
Cytogenetic abnormality, n/N (%)			
del17p13	9/134 (6.7)	9/148 (6.1)	18/282 (6.4)
t(11;14)	54/126 (42.9)	56/140 (40.0)	110/266 (41.4)
del13q14	18/111 (16.2)	28/127 (22.0)	46/238 (19.3)
amp1q21	32/126 (25.4)	28/138 (20.3)	60/264 (22.7)

Kumar S. et al, Amyloid. 2023 Sep;30(3):268-278.

Kaplan–Meier Estimates of Survival Free from Major Organ Deterioration or Hematologic Progression.

D-VCd : traitement standard chez les patients atteints d'amylose AL nouvellement diagnostiquée, indépendamment des anomalies cytogénétiques

Subgroup	D-VCd		VCd		HR (95% CI)
	EVT/N (%)	Median, months	EVT/N (%)	Median, months	
del17p13	2/9 (22.2)	NE	6/9 (66.7)	7.0	0.23 (0.05–1.17)
t(11;14)	10/54 (18.5)	NE	27/56 (48.2)	8.6	0.32 (0.16–0.67)
del13q14	3/18 (16.7)	NE	14/28 (50.0)	9.4	0.23 (0.07–0.80)
amp1q21	9/32 (28.1)	NE	13/28 (46.4)	13.4	0.53 (0.23–1.25)
ITT	46/195 (23.6)	NE	92/193 (47.7)	8.8	0.39 (0.27–0.56)

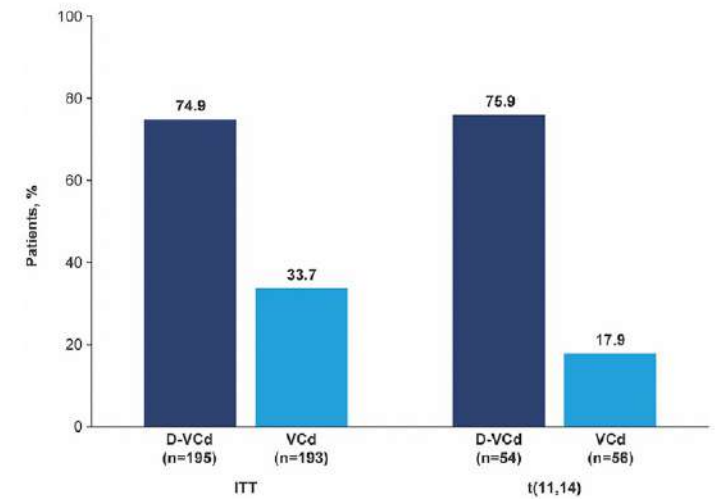


Figure 6. Proportion of patients in the ITT and t(11;14) populations with a deep response.^a ^aDeep response defined as patients achieving either dFLC <10 mg/L or iFLC ≤20 mg/L. Twelve-month landmark data cut (CCO November 2020). D-VCd: daratumumab, bortezomib, cyclophosphamide, and dexamethasone; ITT: intent to treat; VCd: bortezomib, cyclophosphamide, and dexamethasone.

Avant toute analyse génétique, les plasmocytes doivent être triés

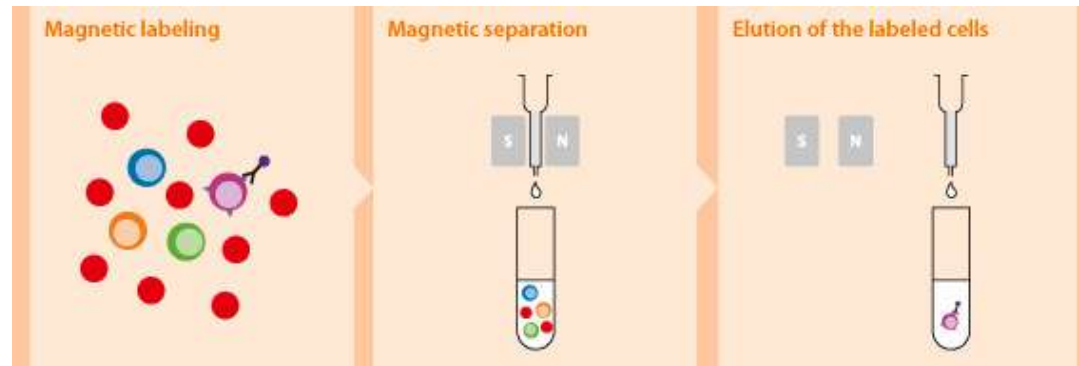
Moelle
osseuse



> 5 millions de GB



AutoMACS Miltenyi



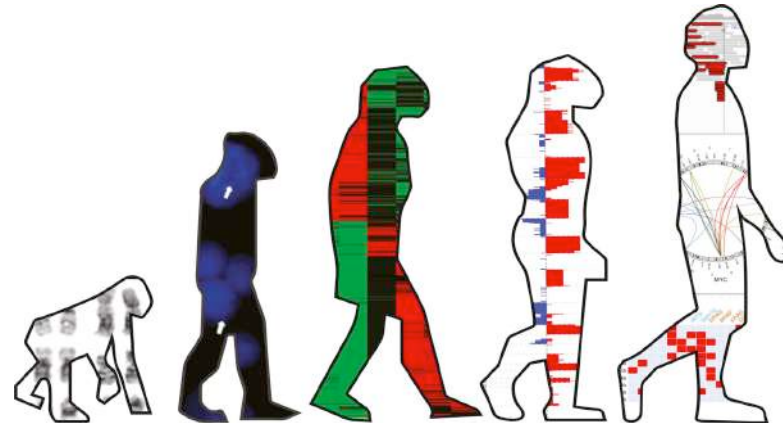
contrôle de la pureté
plasmocytaire

20 000 cellules



≥ 70%





FISH (gold standard)

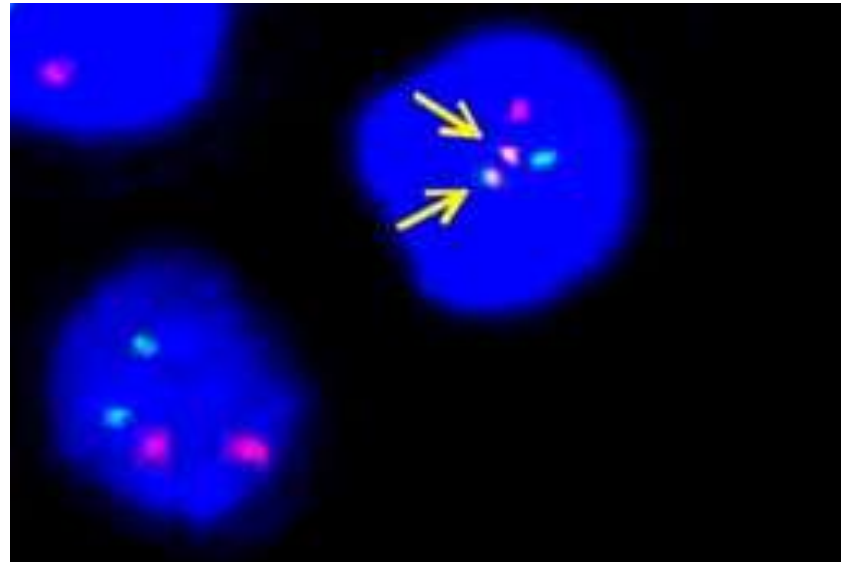
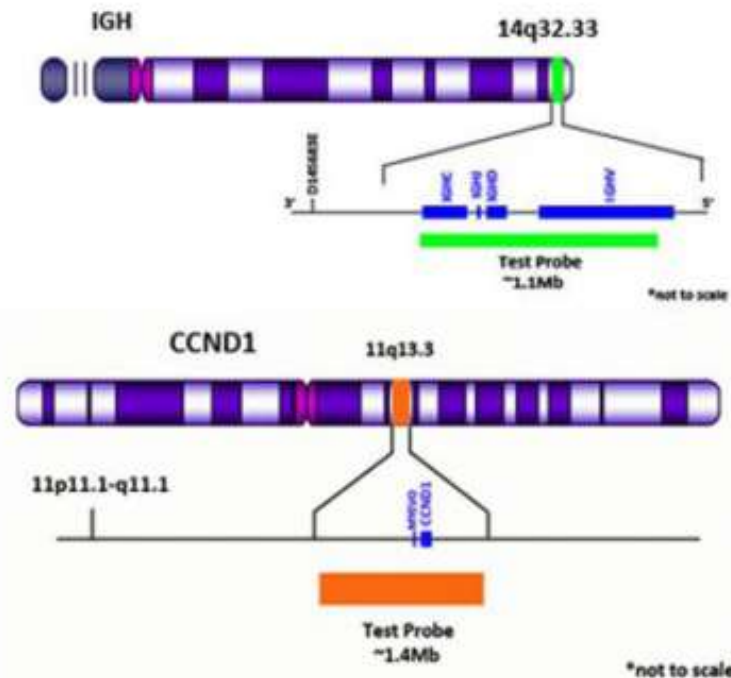
20 000 plasmocytes / sonde
BHN340 + B500 = 92€ + 135€ = 227€

Next Generation Sequencing

≥ 45 000 plasmocytes
RIHN 8170 = 2206€

Détection des anomalies chromosomiques par FISH (Fluorescence in situ hybridization)

- Plus adaptée au plasmocyte tumoral que caryotype conventionnel
- Haute résolution, rapide (2 jours), mais n'informe QUE sur les sondes choisies



Nombre de plasmocytes avec
signal de fusion /100 noyaux

Seul de positivité 30%

Limite : quand négative et que le
myélogramme n'est pas infiltré →
résultat non informatif (analyse
sur majorité de plasmocytes
polyclonaux ??)

Etude phénotypique sur 24 patients Amylose AL au diagnostic :

- Plasmocytes clonaux détectés dans 100% des cas
 - Médiane 0.81%, range 0.01%-30%
- Distribution médiane plasmocytes clonaux / plasmocytes normaux = 85%/15%

Détection des anomalies génétiques par NGS

Next Generation Sequencing ou séquençage « haut débit »

=

séquençage de centaines de milliers de fragments simultanément
et permettant une approche en « multiplex »

Détection des anomalies génétiques par NGS

1 Library Preparation



= préparer l'ADN (fragmentation, marquage, contrôle pureté)

2 Cluster Generation



= amplifier la librairie sur une « flow cell »
(lame où sont fixées les amorces)

3 Sequencing

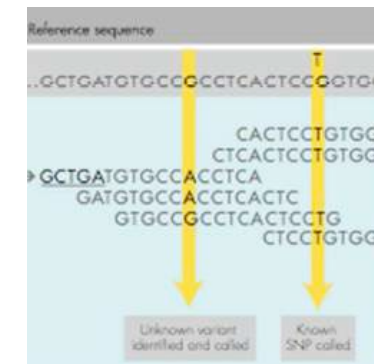
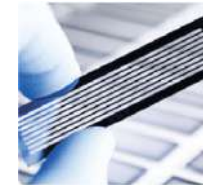
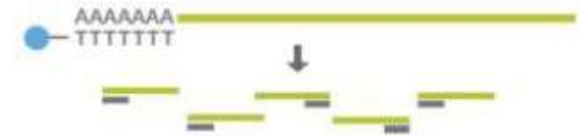


= déterminer l'ordre des nucléotides de ces
amplifiats (séquençage synthétique =
incorporation de 4 nucléotides fluo)

4 Data Analysis



= scanner les images et déterminer les intensités
de fluo, puis appliquer le pipeline d'analyse
bioinformatique

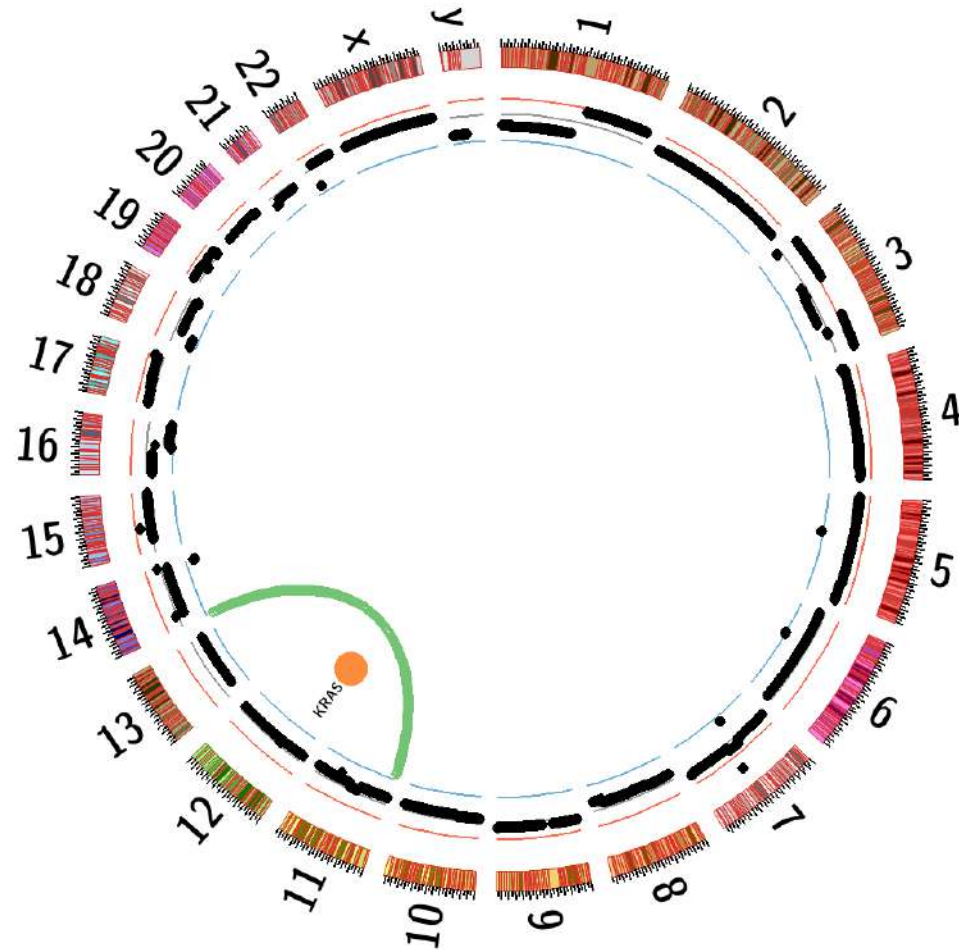


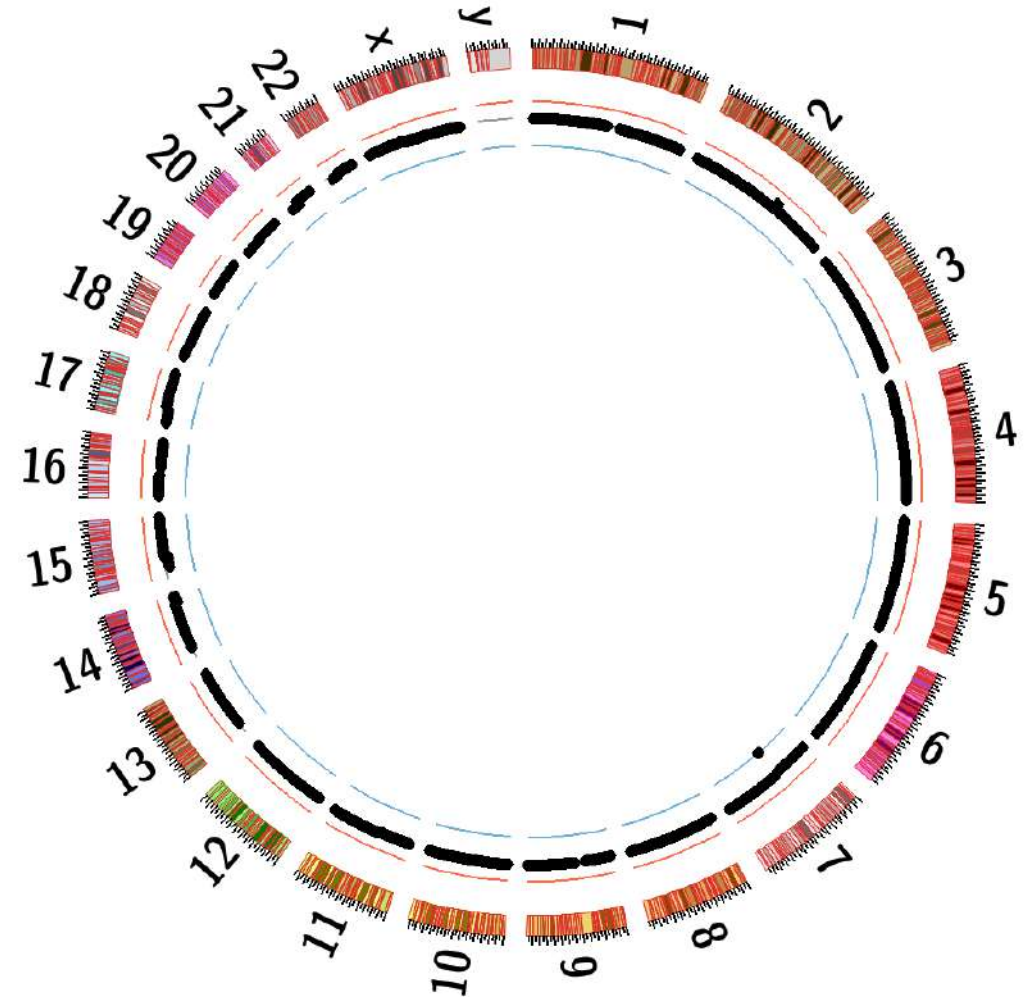
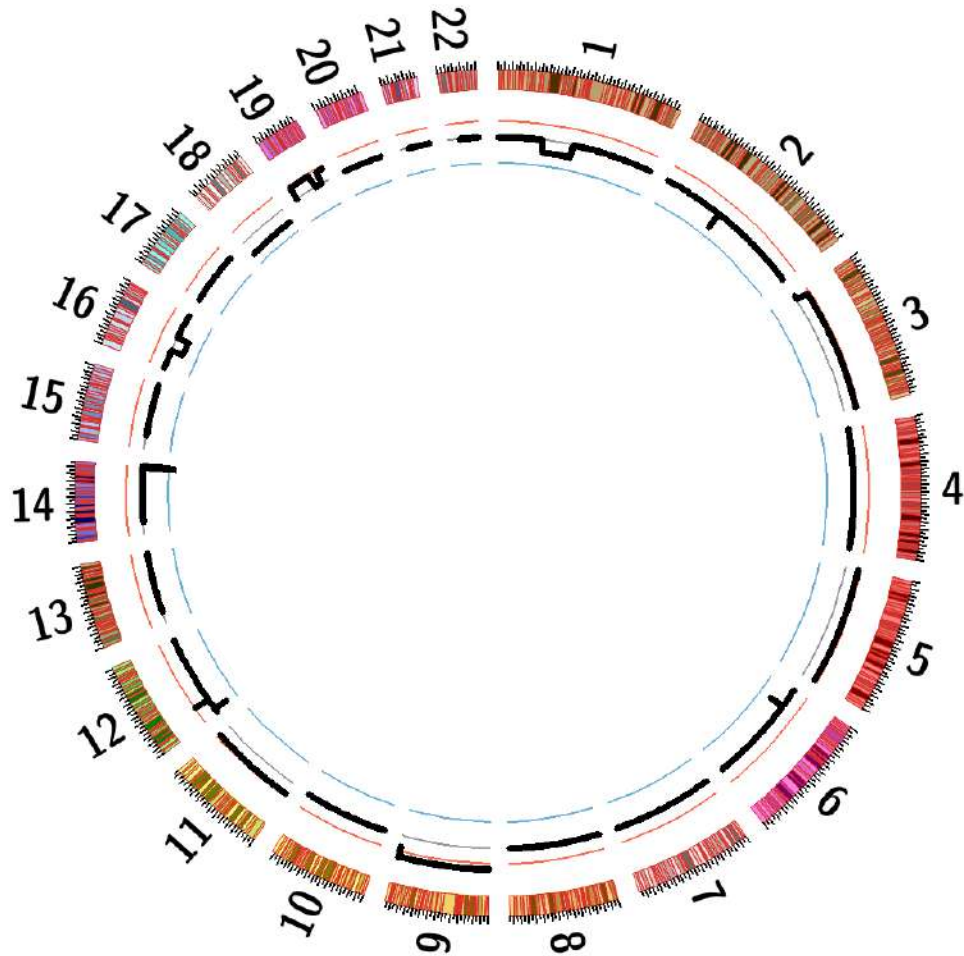
Détection des anomalies génétiques par NGS

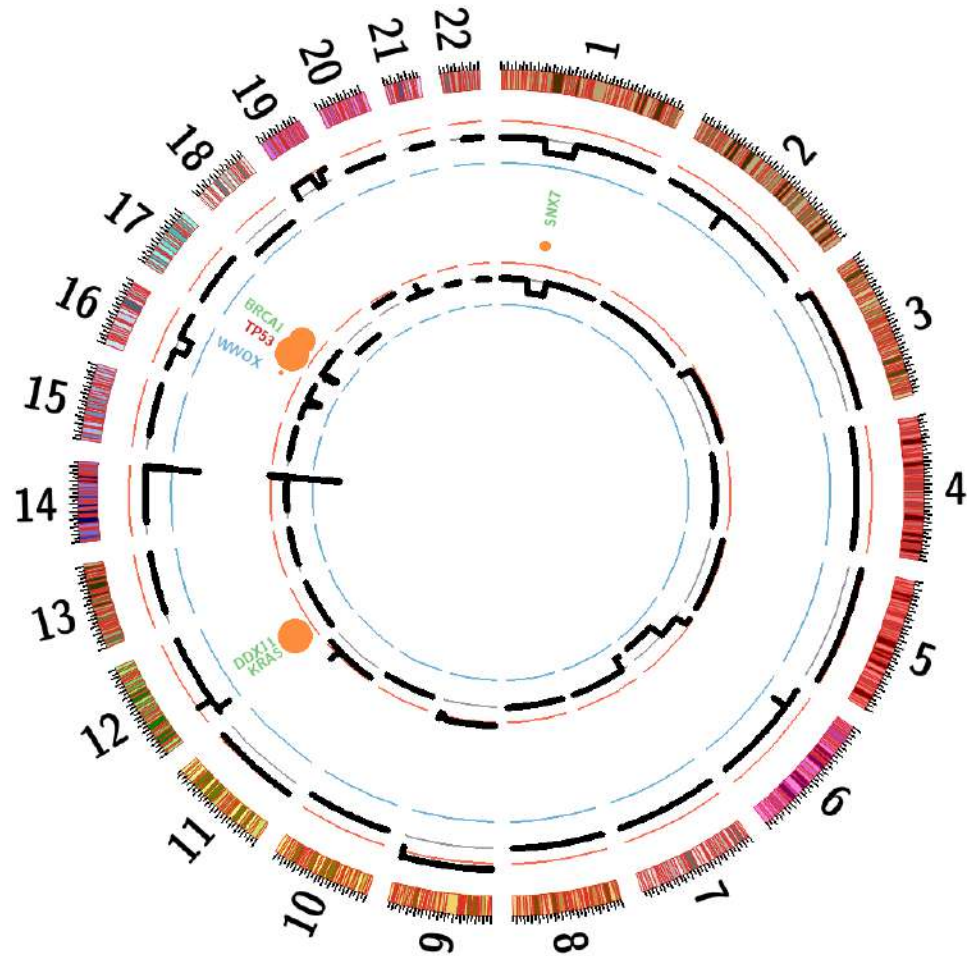
Séquençage ciblé* grâce à un panel qui inclue :

- 2358 SNPs : **ANOMALIES DE NOMBRE** (gains, délétions, trisomies, monosomies...)
- séquence IGH : **TRANSLOCATIONS « 14q32 »** = t(11;14), t(4;14), t(14;16), t(14;20), t(6;14), t(8;14)
- 246 gènes le plus souvent mutés dans le MM : **PROFIL MUTATIONNEL** (TP53, NRAS, KRAS, BRAF, FAM46C, DIS3, ATM, ATR, MYC, TRAF3, BIRC2, BIRC3, CYLD, IRF4, CRBN...)

* Whole Genome Sequencing : 3000 MB
Whole Exome Sequencing : 70MB
Targeted Sequencing : 3MB







Anomalies primitives : translocations 14q32 (sauf la t(8;14))
Anomalies pouvant être acquises : del17p, gain 1q, del1p32



Base de données de l'Unité de Génomique du Myélome à Toulouse (centralisation des analyses de différents centres IFM)

= 1135 amyloses AL au diagnostic (2001-2024)



→ 674 demandes de NGS

→ 64 échecs (9,5%) : nombre de globules blancs ou nombre plasmocytes triés ou pureté du tri insuffisants

→ 60 caryotypes normaux : non informatif ?

t(11;14) positive dans 51% des cas (282/550)

- Del17p : 2% (dont 40% double hit)
- Gain 1q : 22%
- Del1p32 : 1,7% (mono allélique++)
- t(4;14) : 3,9% (plus souvent HR, 41% vs 29%)
- t(14;16) : 2%
- Mutation TP53 : 1,7%
- Score LP >1 : 1,7%
- Score IMS: 6,5%

Données personnelles, non publiées

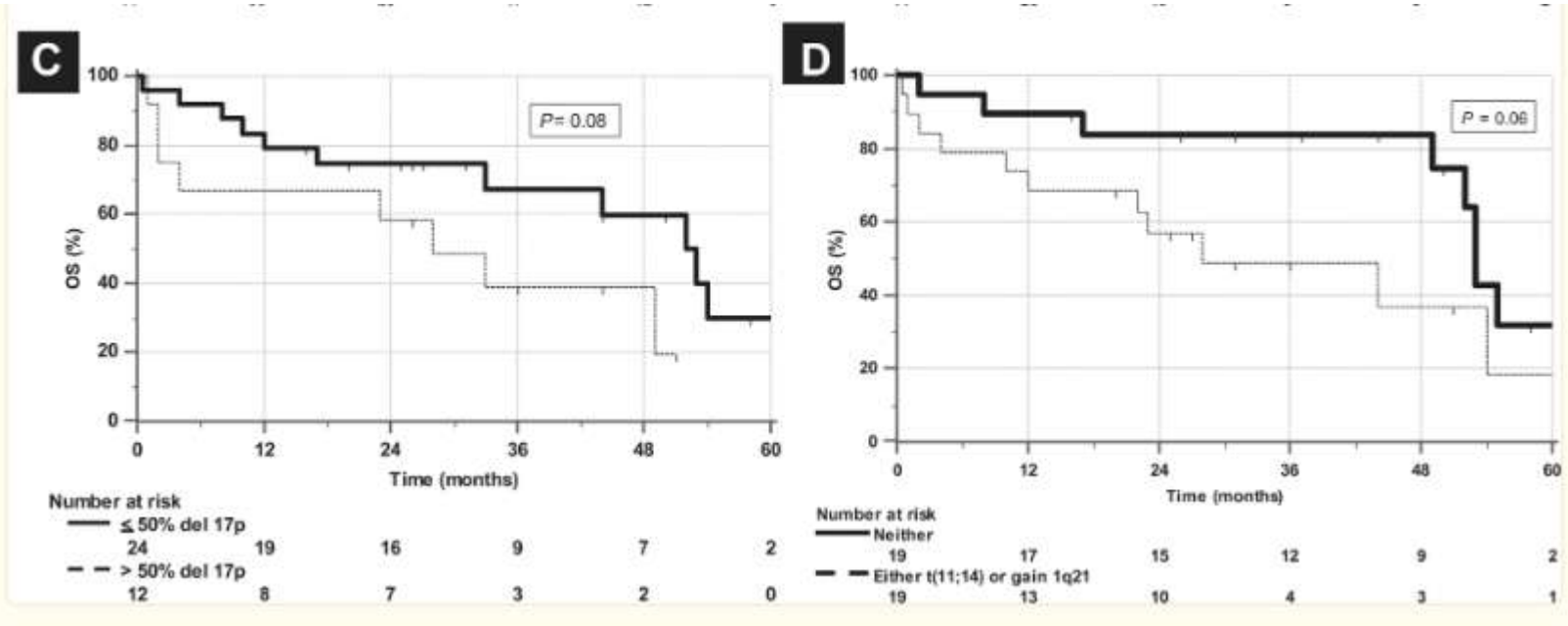
Etude Whole Exome Sequencing sur 24 patients Amylose AL au diagnostic :

- Charge mutationnelle : MGUS < Amylose AL < Myélome
- Incidence des mutations drivers : pas de difference significative
- 30% de t(11;14)
- Incidences des CNV: pas de difference significative sauf moins de del1p

- t(11;14) présent chez la moitié des patients
- Beaucoup moins de haut risque que dans le myélome
- FISH rapide et pas chère mais difficile à interpréter quand négative
- NGS : vision globale permettant de savoir sur quoi on a travaillé

Merci de votre attention !

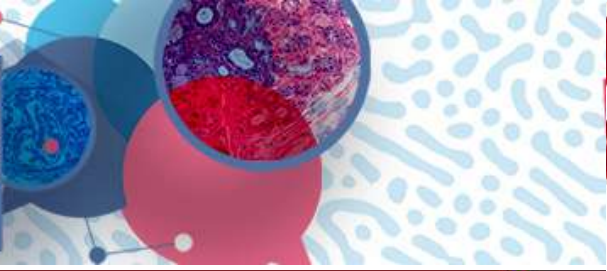
Merci à Raphael Szalat pour son aide



Clin Lymphoma Myeloma Leuk. 2018 November ; 18(11): e493–e499. doi:10.1016/j.clml.2018.07.292.

Outcome of Patients With Newly Diagnosed Systemic Light-Chain Amyloidosis Associated With Deletion of 17p

Sandy W. Wong¹, Ute Hegenbart², Giovanni Palladini³, Gunjan L. Shah⁴, Heather J. Landau⁴, Melissa Warner⁵, Denis Toskic⁵, Arnaud Jaccard⁶, Timon Hansen⁷, Joan Bladé⁸, M. Teresa Cibeira⁸, Efstathios Kastritis⁹, Angela Dispenzieri¹⁰, Ashutosh Wechalekar¹¹, Cindy Varga⁵, Stefan O. Schönland², Raymond L. Comenzo⁵



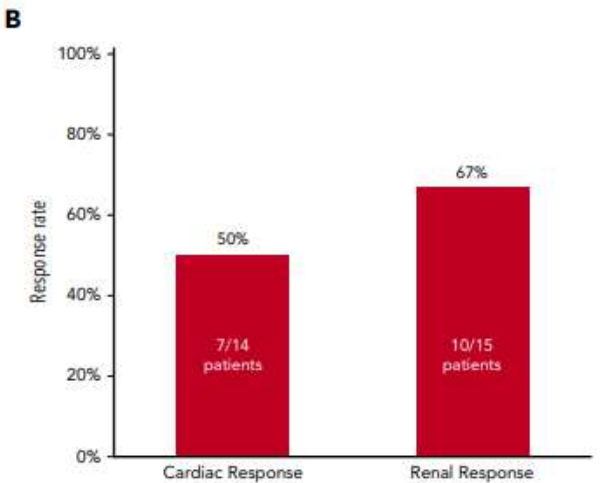
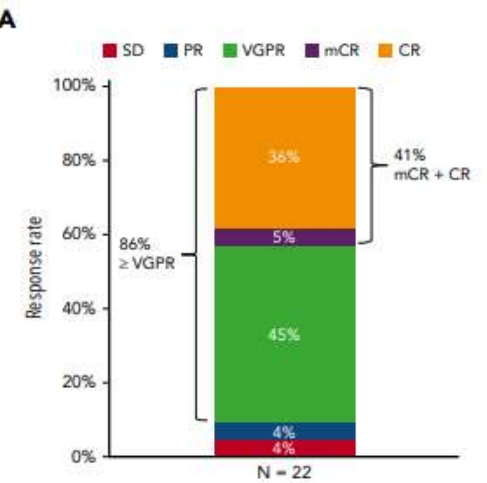
CLINICAL TRIALS AND OBSERVATIONS

Safety, tolerability, and response rates of daratumumab in relapsed AL amyloidosis: results of a phase 2 study

Vaishali Sanchorawala, Shayna Sarosiek, Amanda Schulman, Meredith Mistark, Mary Ellen Migre, Ramon Cruz, J. Mark Sloan, Dina Brauneis, and Anthony C. Shelton

Amyloidosis Center, School of Medicine, Boston University, Boston, MA; and Section of Hematology and Oncology, Boston Medical Center, Boston, MA

Daratumumab was administered by IV infusion once weekly for weeks 1 to 8, every 2 weeks for weeks 9 to 24, and every 4 weeks thereafter until progression or unacceptable toxicity, **for up to 24 months.**



CLINICAL TRIALS AND OBSERVATIONS

A prospective phase 2 trial of daratumumab in patients with previously treated systemic light-chain amyloidosis

Murielle Roussel,¹ Giampaolo Merlini,^{2,3} Sylvie Chevret,⁴ Bertrand Arnulf,⁵ Anne Marie Stoppa,⁶ Aurore Perrot,⁷ Giovanni Palladini,^{2,3} Lionel Karlin,⁸ Bruno Royer,⁵ Antoine Huart,⁹ Margaret Macro,¹⁰ Pierre Morel,¹¹ Laurent Frenzel,¹² Cyrille Touzeau,¹³ Eileen Boyle,¹⁴ Véronique Dorvaux,¹⁵ Fabien Le Bras,¹⁶ David Lavergne,^{17,18} Frank Bridoux,^{17,19} and Amaud Jaccard^{17,18}

Patients received 6 28-day cycles of IV daratumumab, every week for cycles 1 and 2 and every 2 weeks **for cycles 3 through 6.**

