Immuunfenotypische MRD Detectie van Plasmacellen: Optimalisatie en Klinisch nut

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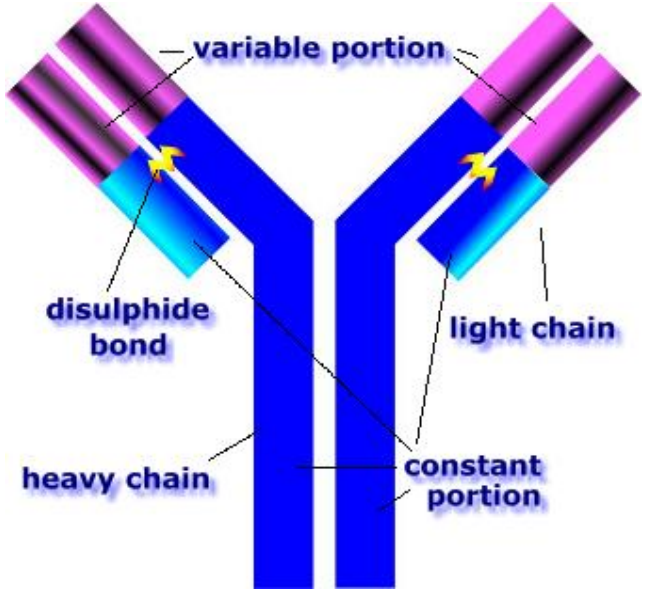
Plasmacel neoplasie?

- Monoclonale proliferatie van plasmacellen met secretie van één uniek Ig (zware en/of lichte keten)
- Ig zijn <u>meestal</u> detecteerbaar in serum en/of urine d.m.v. een electroforese waarbij een M-piek gevonden wordt
- Dikwijls is er een gedaalde concentratie van de polyclonale Ig



WHO classificatie

Monoclonale gammopathie van onbekende oorsprong
MGUS (Monoclonal Gammopathy of Undetermined Significance)

Plasmacelmyeloom of Multiple Myeloom (MM)

1. Niet-secreterend myeloom
2. Smoldering myeloom
3. Plasmacelleukemie

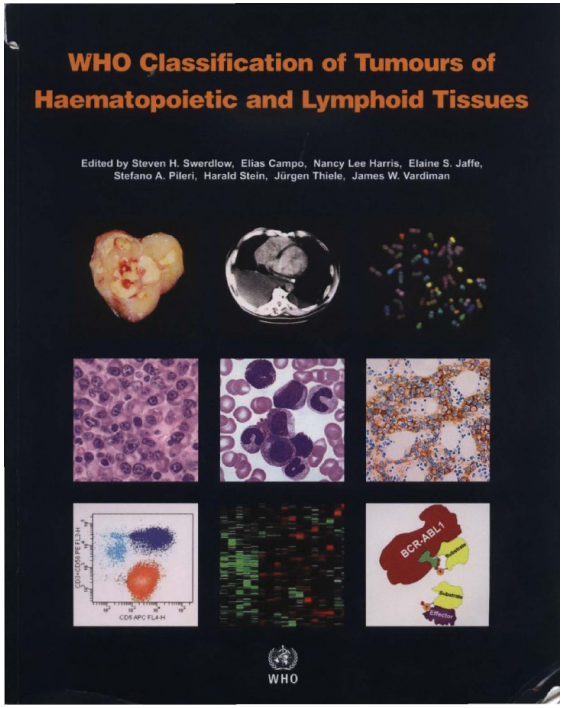
Plasmacytoom (één tumor)

1. Solitair plasmacytoom van het bot
2. Extra medullair plasmacytoom

Monoclonale immunoglobuline "depositie" ziekte
MIDD (Monoclonal Immunoglobulin Deposition Diseases)

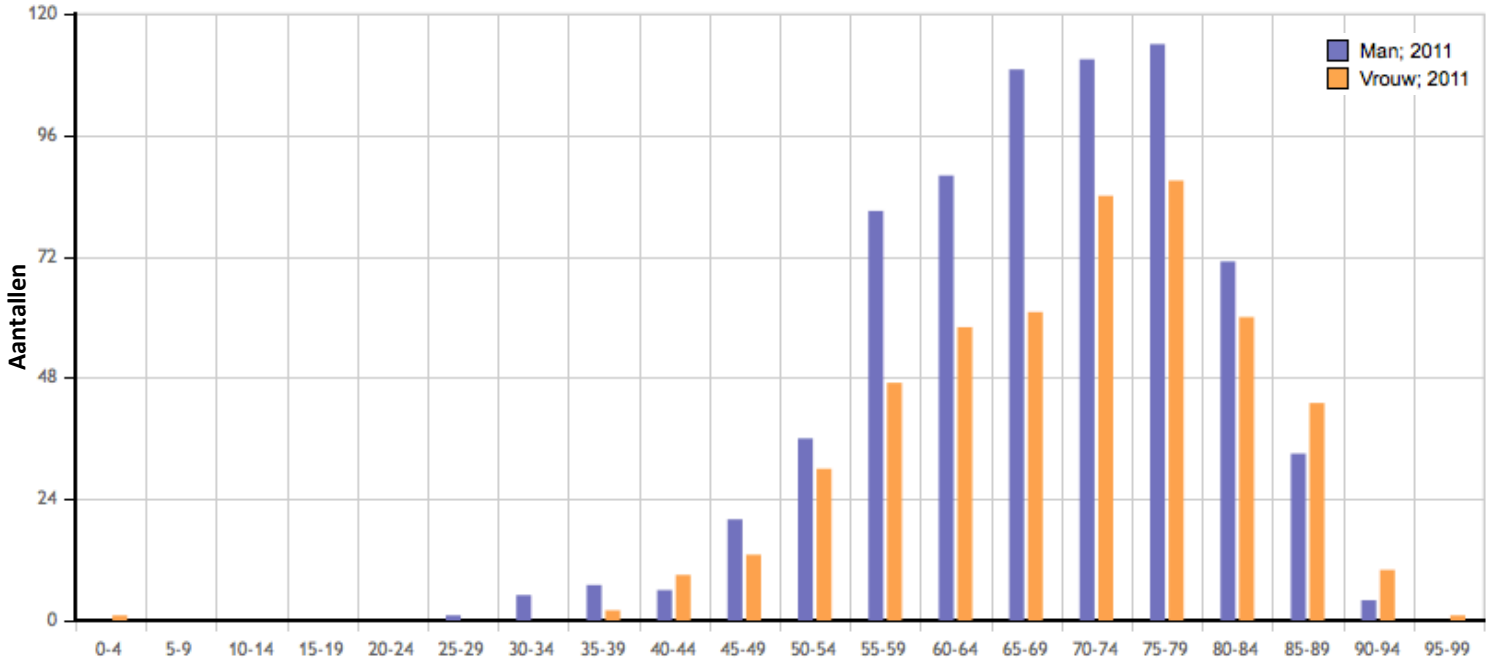
1. Primaire amyloïdose
2. Systemische lichte of zware keten depositie aandoeningen

Osteosclerotisch Myeloom (POEMS syndroom)



WHO, 2008

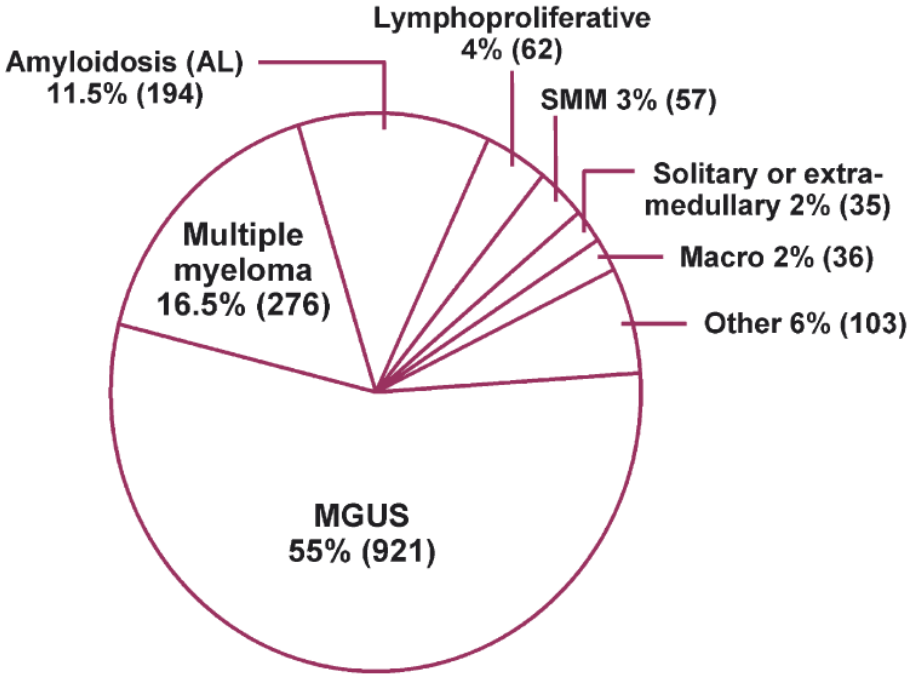
Epidemiologie



Plasmacel neoplasieën meest prevalent op hogere leeftijd…

Bron: Nederlandse Kankerregistratie 2011

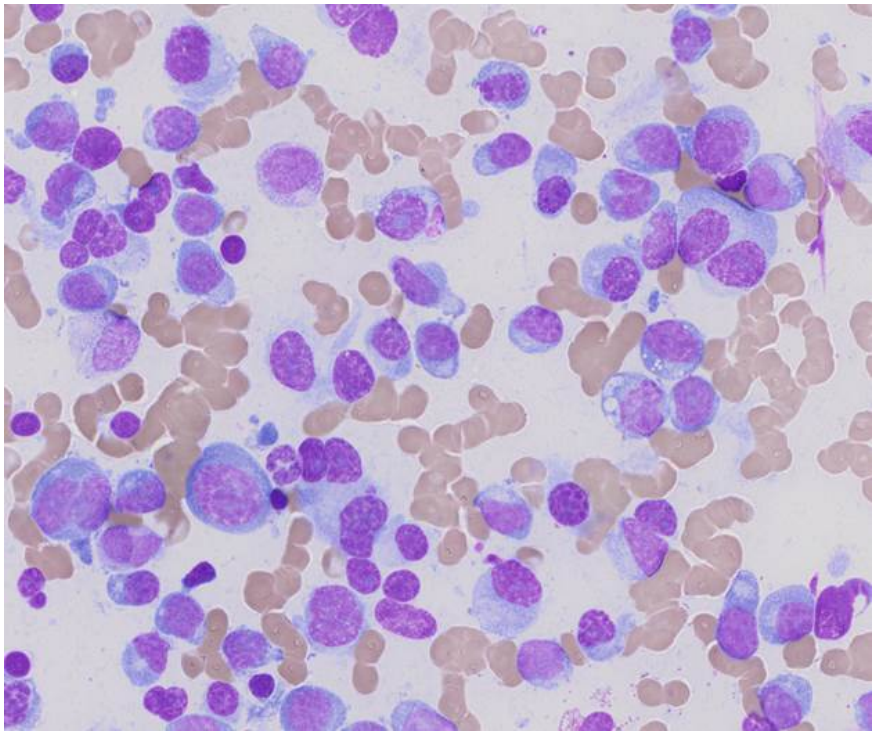
Epidemiologie



MGUS meest frequente plasmacelneoplasie…

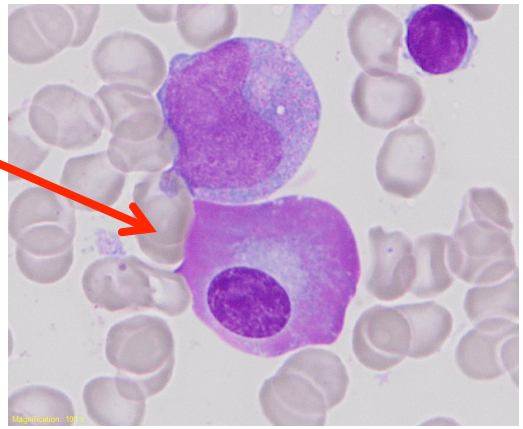
Kyle et al, 2007, Mayo Clinic

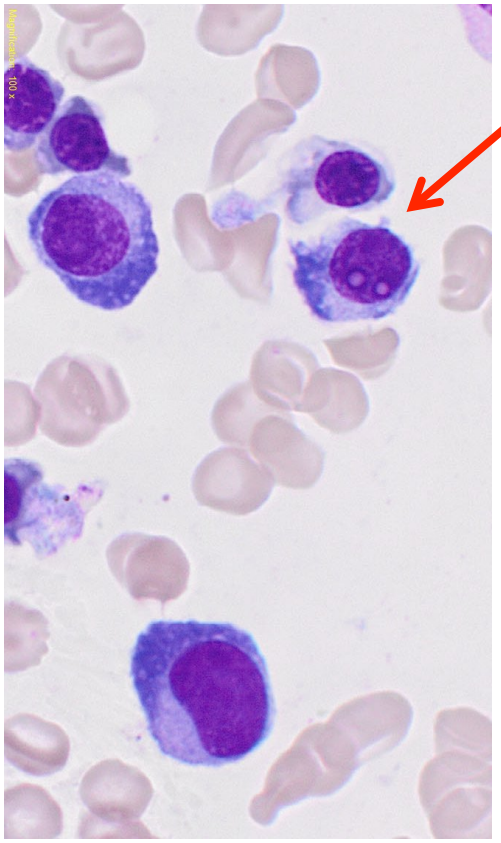
Multiple myeloom: maligne woekering van plasmacellen (in het beenmerg)

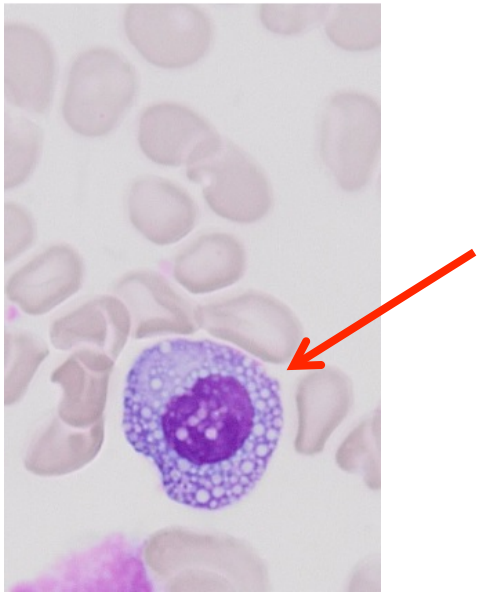


Typisch beeld van plasmacellen in het beenmerg bij de ziekte van Kahler of het multiple myeloom…

Multiple myeloom: maligne woekering van plasmacellen (in het beenmerg)





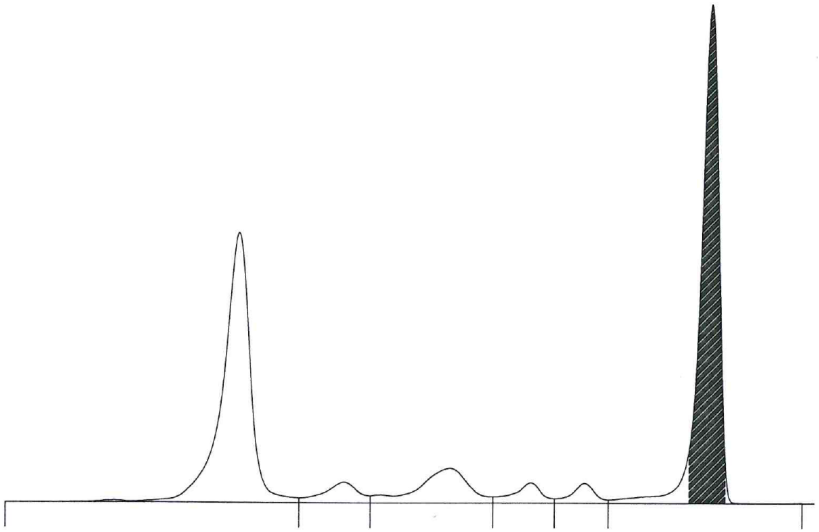


Risicofactoren voor plasmacel neoplasie

- **LEEFTIJD**
- Afrikaanse Amerikanen > Aziatische Amerikanen
- Familiale voorgeschiedenis
- Mannen > Vrouwen (in USA 11.200 *versus* 8700 / jaar)
- Obesitas
- MGUS (Monoclonale Gammopathie van Onbekende Oorsprong), 3% bij 50+; 5.5% bij 70+; >10% bij 80+

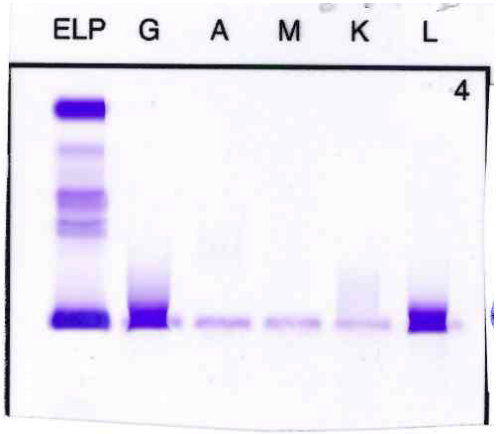
Kyle et al, 2007, Mayo Clinic

MGUS is een “pre-maligne” aandoening



Een toevallige vondst van een M-proteïne, <30 g/L

Te bevestigen met immunofixatie



MGUS is een “pre-maligne” aandoening

3 criteria moeten vervuld worden:

1. M-proteïne <30 g/L
   - 15 - 20% IgM (lymfoïd of lymfoplasmacytoïd fenotype)
   - >80% non-IgM (IgG > IgA > light chain Ig only > IgD > IgE) (plasmacytoïd fenotype)

2. <10% clonale plasmacellen in BM

3. Geen orgaanfalen tgv plasmacel neoplasie:
   1. **Geen** lytische botletsels
   2. **Geen** hypercalcemie
   3. **Geen** anemie
   4. **Geen** nierinsufficiëntie

   } Calcium, hemoglobine, creatinine

Kyle et al, 2010 Leukemia

MGUS is een “pre-maligne” aandoening

# of Risk Factors	# Patients (%)	20-year Progression	Relative Risk
0	449 (38)	5%	1
1	420 (37)	21%	5.4
2	226 (20)	37%	10.1
3	53 (5)	58%	20.8
Total	**1148 (100)**	**20%**	**N/A**

Risk Factors: M-protein >1.5 g/dL, non-IgG MGUS, FLC ratio <0.26 or >1.65

# of Risk Factors	# Patients (%)	5-year Progression	Relative Risk
0	127 (46)	2%	1
1	133 (48)	10%	5
2	16 (6)	46%	23
Total	**276 (100)**	**8.5%**	**N/A**

Risk Factors: ≥95% aberrante plasmacellen (↓CD38 expressie, CD56+, CD45- of CD19-) en DNA aneuploïdie.

Rajkumar et al, 2005, Blood
Pérez-Persona et al, 2007, Blood

“Smoldering myeloom”

2 criteria moeten vervuld worden:

1. M-proteïne: IgG of IgA ≥30 g/L en/of ≥10% clonale plasmacellen in BM

2. Geen orgaanfalen tgv plasmacel neoplasie:
   1. **Geen** lytische botletsels
   2. **Geen** hypercalcemie
   3. **Geen** anemie
   4. **Geen** nierinsufficiëntie

The 2010 IMWG guidelines state the following: “an MRI of the spine and pelvis is recommended because it can detect occult lesions and, if present, predict for a more rapid progression to symptomatic myeloma.”

Kyle et al, 2010 Leukemia

“Smoldering myeloom”

# of Risk Factors	# Patients (%)	5-year Progression	Relative Risk
1	76 (28)	25%	1
2	115 (42)	51%	2.0
3	82 (30)	76%	3.0
Total	**273 (100)**	**51%**	**N/A**

***Risk Factors:** marrow plasma cells ≥10%, M-protein ≥3 g/dL, FLC ratio <0.125 or >8
*Patients must have at least one of the first two risk factors to meet criteria for SMM

# of Risk Factors	# Patients (%)	5-year Progression	Relative Risk
0	28 (31)	4%	1
1	22 (25)	46%	11.5
2	39 (44)	72%	18
Total	**89 (100)**	**46%**	**N/A**

***Risk Factors:** ≥95% aberrante plasmacellen (↓CD38 expressie, CD56+, CD45- of CD19-) en immunoparese (↓ andere immunoglobulines).

Rajkumar et al, 2005, Blood
Pérez-Persona et al, 2007, Blood

Multiple myeloom

Diagnostische criteria (volgens IMWG)

1. M-proteïne: IgG of IgA ≥30 g/L, urine >1 g/24h (kappa of lambda) (behalve bij het niet-secreterend myeloom)

2. ≥10% monoclonale plasmacellen in BM

3. Min. één van de **"CRAB"** tekenen
   - **C** → Ca↑ >2.88 mmol/L
   - **R** → Nierinsufficiëntie: creatinine >2 mg/dL
   - **A** → Anemie: Hb <10 g/dL
   - **B** → Botletsels (lytisch of osteoporose)

Kyle & Rajkumar, Leukemia 2009

Multiple myeloom

Laboratoriumanalyses bij diagnose (2009 IMWG):

- MIPB (rouleaux, achtergrondkleuring)
- Calcium en creatinine (+ levertesten, elektrolyten, albumine)
- Serum-eiwitelectroforese, immunofixatie, free light chains
- Nefelometrische kwantificatie van de serum Ig
- Urine-analyse: 24-uurs urine collectie voor TE, EF & IF
- Albumine, β2-microglobuline en LDH: **PROGNOSTISCH**
- BM-aspiraat en/of botbiopt (↑ plasmacellen en morfologische atypie)

- Cytogenetica (karyotypering en FISH voor 17p, t(4;14) en t(14;16))

Dimopoulos, Blood 2010

Niet-secreterend myeloom en plasmocytoom

Diagnostische criteria bij het:

Niet-secreterend myeloom:

- Afwezigheid van een M-proteïne
- **≥30%** monoclonale plasmacellen in BM

Solitair plasmocytoom

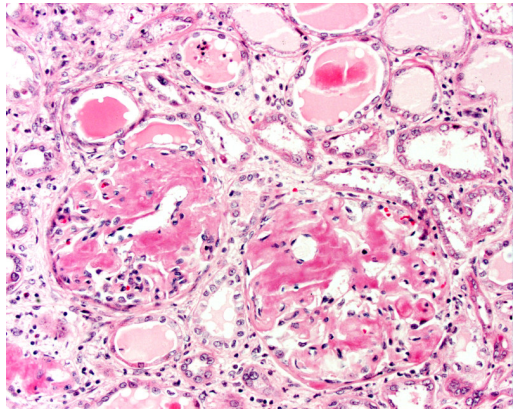
- M-proteïne (+ of -)
- Positief botbiopt
- Normaal BM-aspiraat zonder plasmacelverhoging
- Geen botafwijkingen
- Geen orgaanfalen

Monoclonal Immunoglobulin Deposition Diseases (MIDD)

Klinisch: neerslag Ig in viscerale en zachte weefsels

De meest frequent voorkomende vorm is **1° amyloïdose**, waarbij (meestal) lichte ketens neerslaan ovv AL amyloid (amyloid light chain)
(β-geplooide plaatstructuren)

- Kliniek: orgaanfalen
- MGUS (80%), plasmacel myeloom (20%)
- Diagnose: Congorood-kleuring (APO)
- Klinisch labo:
  - M-proteine mbv IF (90%)
  - IF & sFLC (99%) (70% λ)
  - β2-microglobuline, creatinine, calcium, …



Systemische lichte of zware keten depositie-aandoeningen, waarbij **GEEN** β-geplooide plaatstructuren worden gevormd

- Frequent aangetaste orgaan: nier
- Diagnose: APO

Immuunfenotypering van plasmacellen

Toepassingen?

1. (Differentiële) diagnose van plasma cel neoplasiën

   - Reactieve, polyclonale plasmacytose of monoclonale plasma cel leukemie?
   - MGUS of benigne plasma cellen *versus* maligne plasmacellen?
   - IgM multiple myeloma of Ziekte van Waldenström?
   - Reactieve plasmacytose of “relapsed” multiple myeloma?

2. Risico stratificatie: transformatie of relapse?

3. Targeted therapies?

CAT-vraagstelling

I. Which immunophenotypic markers can be used for the differentiation between normal, reactive and aberrant plasma cells?

II. What is the prognostic value of the different immunophenotypic markers used for characterization of plasma cells in plasma cell neoplasms?

III. What is the role of MRD monitoring of plasma cells in patients with plasma cell neoplasms? Is flow-based MRD a well suited technique for MRD assessment in plasma cell neoplasms?

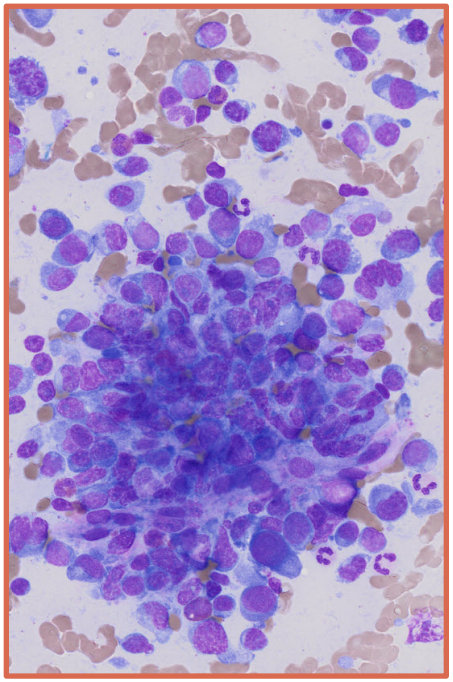
IV. Can flow cytometric detection of plasma cells tailor therapy in patients with plasma cell neoplasms?

I. Immuunfenotypering

Beperking(!): kwantificatie: morfologie *versus* flowcytometrie

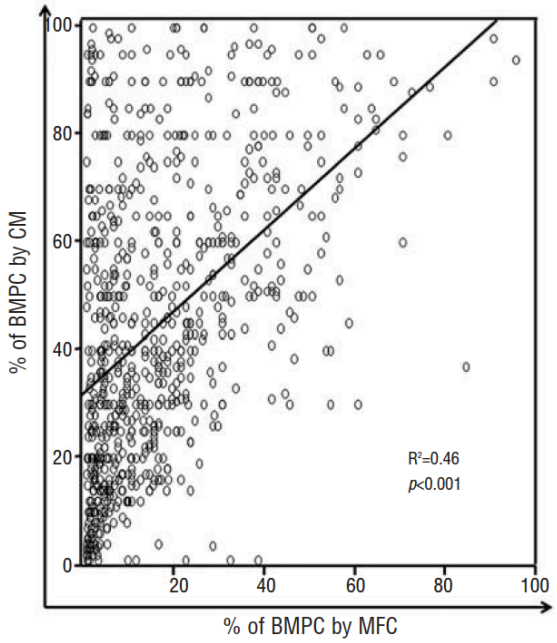
Reden?

- Heterogene verdeling plasmacellen in BM
- Vaak secundair aspiraat voor flowcytometrie
- Contaminatie door perifeer bloed (inherent aan de afnametechniek!)
- Adhesie lipiden
- Plasmocyten: fragiel!
  - Verlies tijdens opwerking (lysis RBC en naald)
  - Gedaalde CD138 expressie door lysis
  - Verlies tijdens centrifugatie



Smock et al, 2007, APLM

I. Immuunfenotypering



Morfologie *versus* flow cytometrie

- Onderschatting plasmacellen met MFC => uitdagend om plasmacellen te detecteren
- Altijd correleren met morfologie!
- Membraanmerkers: nuttige informatie!

Paiva et al, 2009, Haematologica

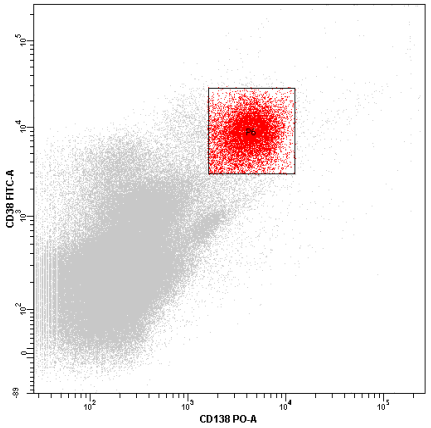
I. Immuunfenotypering merkers

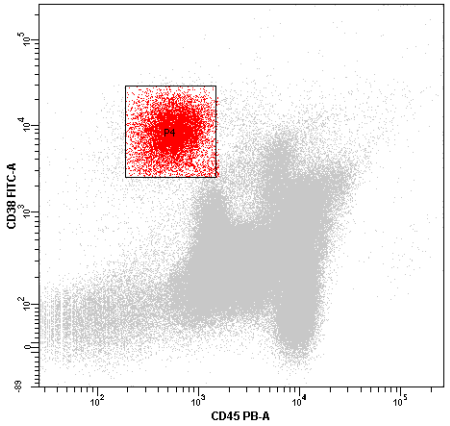
- **CD138 (syndecan-A)**
  - erg specifiek voor plasmacellen (B-B4 cloon!)
  - CD138-: plasmablasten ; CD138+: plasmacellen

- **CD38**
  - Minder specifiek
  - Ook op niet-hematopoïetische cellen (T & B-cellen)

- **CD45**
  - Leukocytenantigen
  - **2 populaties**
    ⇒ negatieve, aberrante populatie
    ⇒ zwak positieve populatie

- **Light scatter eigenschappen**
  - FSC meer dan SSC





Pellat-Deceunynck, 1994, Blood
Terstappen et al, 1990, Blood
Towbridge et al, 1994, Annu Rev Immunol

I. Immuunfenotypering merkers

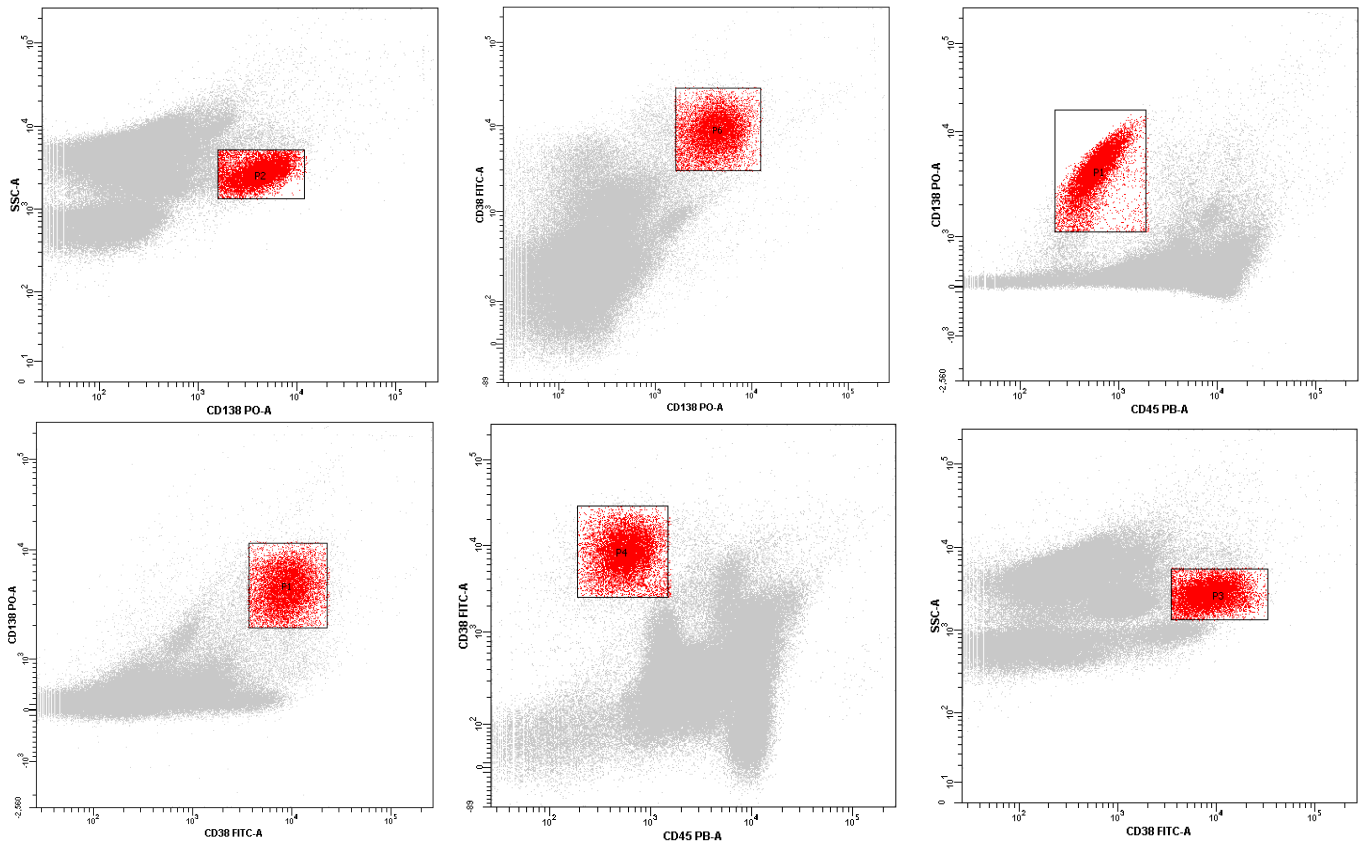
Table 2. Identification of the optimal marker combination for gating plasma cells: overall performance of different combinations of plasma cell gating markers evaluated at the EMN workshop held in Leeds in May 2007.

Gating markers	*CD38*	*CD38 and CD45*	*CD38 and CD138*	*CD38, and CD45 CD138*
Proportion of cases with detectable disease	42%	28%	42%	61%
Median percentage of plasma cells in cases with detectable disease	8.1% (1.6-35%)	0.8% (0.2-26%)	7.6% (0.5-39%)	2.7% (0.07-33%)
Precision*	67%	67%	67%	92%

**percentage of cases with concordant results between participants.*

European Myeloma Network, Rawstron et al, 2008, Haematologica

I. Immuunfenotypering merkers



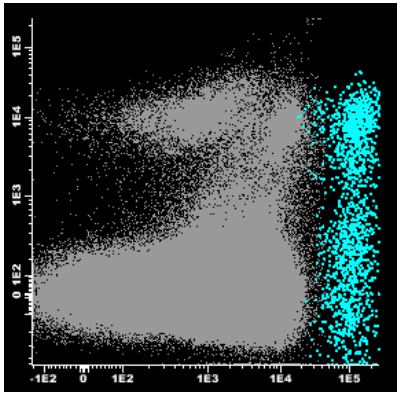
Verschillende "gating" strategieën plasmacellen…

I. Immuunfenotypering merkers

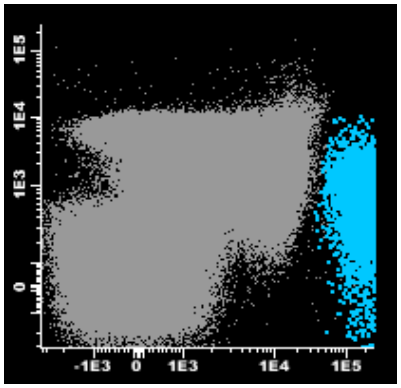
Antigen down-regulatie

Normaal beenmerg

CD19
Heterogeen
(≈ 80% cellen positief)



CD45
Homogeen
(≈ 80% cellen positief)

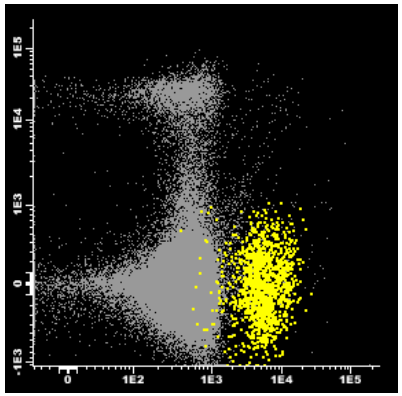


CD38

Multiple Myeloma

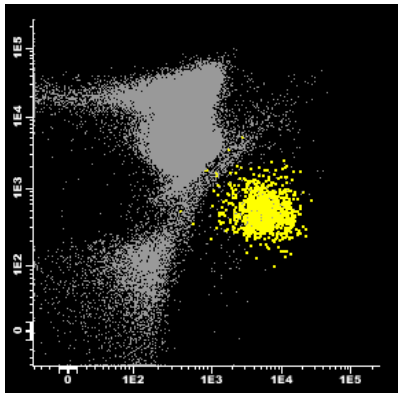
CD19
Homogeen
negatief

(in +/- 93% van de patiënten)



CD45
Homogeen
negatief

(in +/- 83% van de patiënten)



CD38

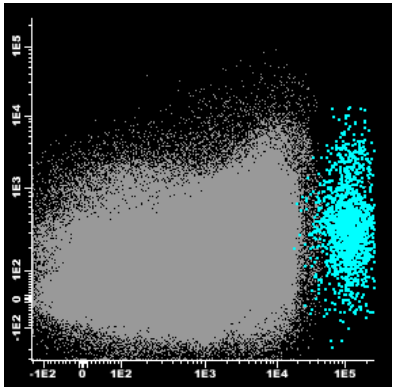
Mateo et al, 2008, J Clin Oncol

I. Immuunfenotypering merkers

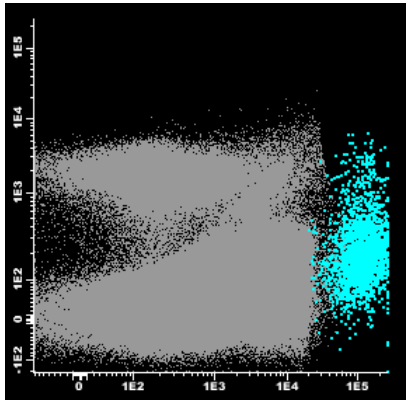
Antigen up-regulatie

Normaal beenmerg

CD56
Heterogeen
(≤ 10% cellen
zwak positief)



CD28
Homogeen
(≈ 15% cellen
negatief tot zwak
positief)

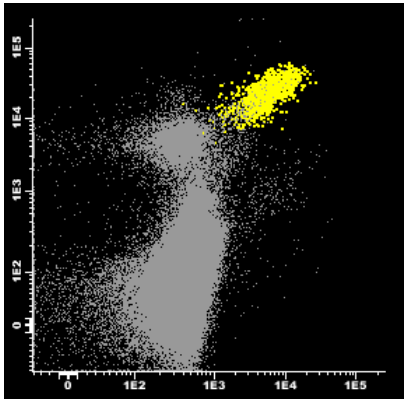


CD38

Multiple Myeloma

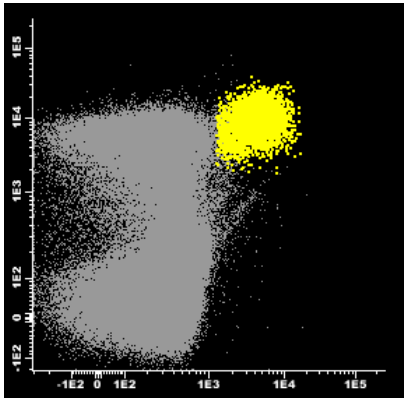
CD56
Homogeen
sterk positief

(in +/- 65% van
de patiënten)



CD28
Homogeen
sterk positief

(in +/- 25% van
de patiënten)



CD38

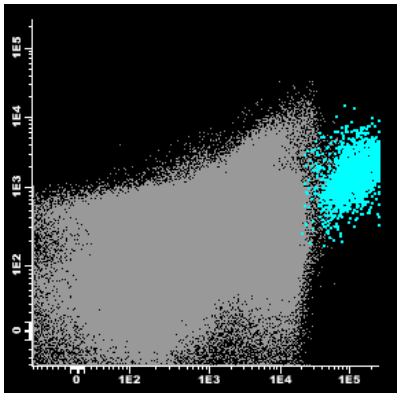
Mateo et al, 2008, J Clin Oncol

I. Immuunfenotypering merkers

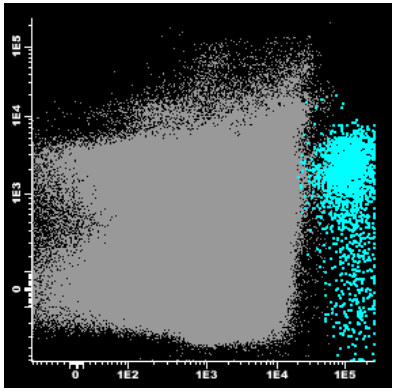
Antigen down-regulatie

Normaal beenmerg

CD27
Homogeen
Positief
(100% cellen
positief)



CD81
Homogeen
(≈ 90% cellen
positief)

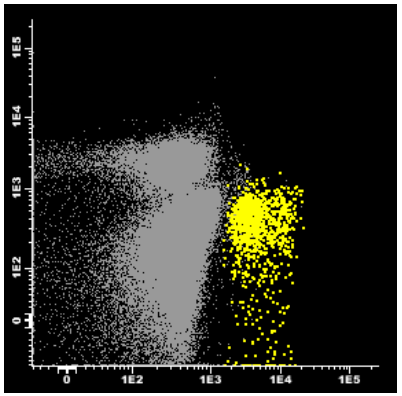


CD38

Multiple Myeloma

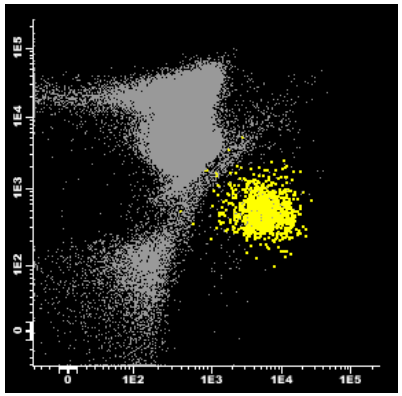
CD27
Homogeen
negatief

(in +/- 68% van
de patiënten)



CD81
Homogeen
negatief

(in +/- 61% van
de patiënten)



CD38

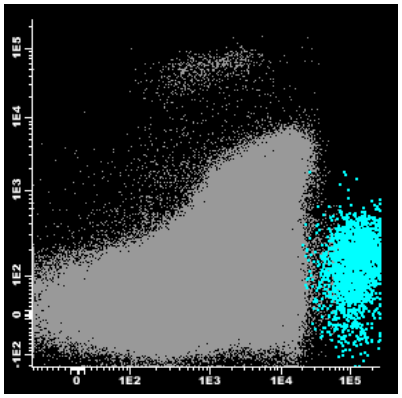
Mateo et al, 2008, J Clin Oncol

I. Immuunfenotypering merkers

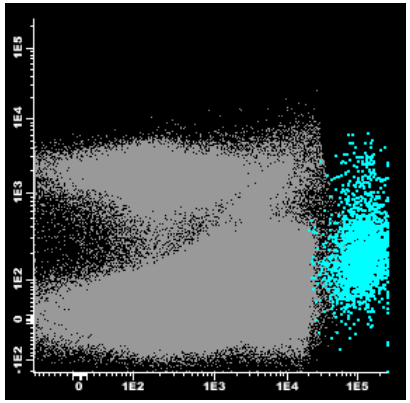
Asynchrone expressie

Normaal beenmerg

CD117
Homogeen
negatief
(0% cellen zwak
positief)



CD20
Homogeen
negatief
(≤ 10% cellen
zwak positief)

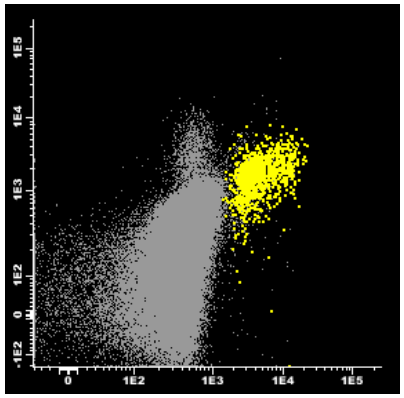


CD38

Multiple Myeloma

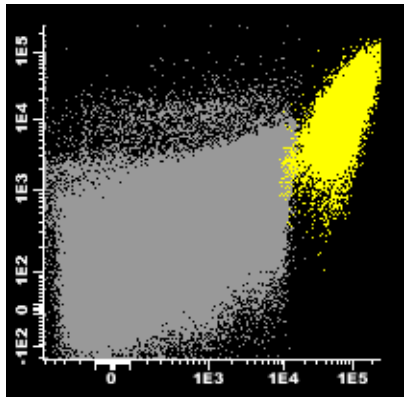
CD117
Homogeen
Sterk positief

(in +/- 30% van
de patiënten)



CD20
Homogeen
sterk positief of
zwak positief

(in +/- 20% van
de patiënten)



CD38

Mateo et al, 2008, J Clin Oncol

I. Immuunfenotypering merkers

Andere merkers?

- Myeloïd geassocieerde merkers:
  - **CD33:** expressie geassocieerd met zwakke OS en hogere mortaliteit

  => misinterpretatie!

- Andere merkers:
  - **CD200-** (70% MGUS) geassocieerd met betere PFS
  - **CD221+** slechtere prognose
  - **CD52**, **CD10**, **CD22, CD229**

- Lichte keten **κ** & **λ** restrictie?

Raja et al, 2010, Br J Hematol

I. Immuunfenotypering merkers

Wat met clonaliteit?

- Neoplastische plasmacellen identificeren a.d.h.v.:

1. **Cytoplasmatisch immuunglobuline (κ & λ)**
   - **Minder gevoelig** wanneer laag aantal cellen (<30%)
   - enkel bij **diagnose**: achtergrond van polyclonale, normale polytypische plasmacellen
   - **"**bi-clonale" pathologie

2. **Aberrante antigen patronen**
   - \+ IMWG: gevoeliger en specifieker in **follow-up**
   - \+ 6-kleuren flow cytometrie: alle plasmacellen met aberrant fenotype monoclonaal
   - \- 10% van de plasmacel neoplasiën: immuunfenotype niet voldoende
     => "aberrant" immuunfenotype ook in gezonde patiënten

- Introductie **meer-kleuren flow cytometrie** als oplossing?

Robillard et al, 2013, Blood Cancer Journal
Morice et al, 2007, Leukemia
de Tute et al, 2007, Leukemia
Rawstron et al, 2008, Haematologica

CAT-vraagstelling

I. Which immunophenotypic markers can be used for the differentiation between normal, reactive and aberrant plasma cells?

II. What is the prognostic value of the different immunophenotypic markers used for characterization of plasma cells in plasma cell neoplasms?

III. What is the role of MRD monitoring of plasma cells in patients with plasma cell neoplasms? Is flow-based MRD a well suited technique for MRD assessment in plasma cell neoplasms?

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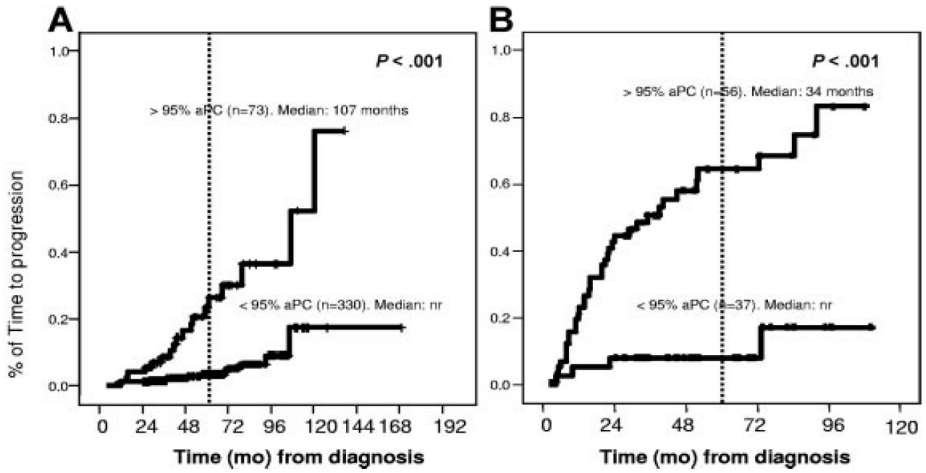
II. Prognose

- MGUS: normale, polyclonale plasmacellen en aberrante plasmacellen
  - > 80% patiënten met MGUS, > 5% normale plasmacellen
  - < 15% patiënten met multiple myeloma: > 5% normale plasmacellen

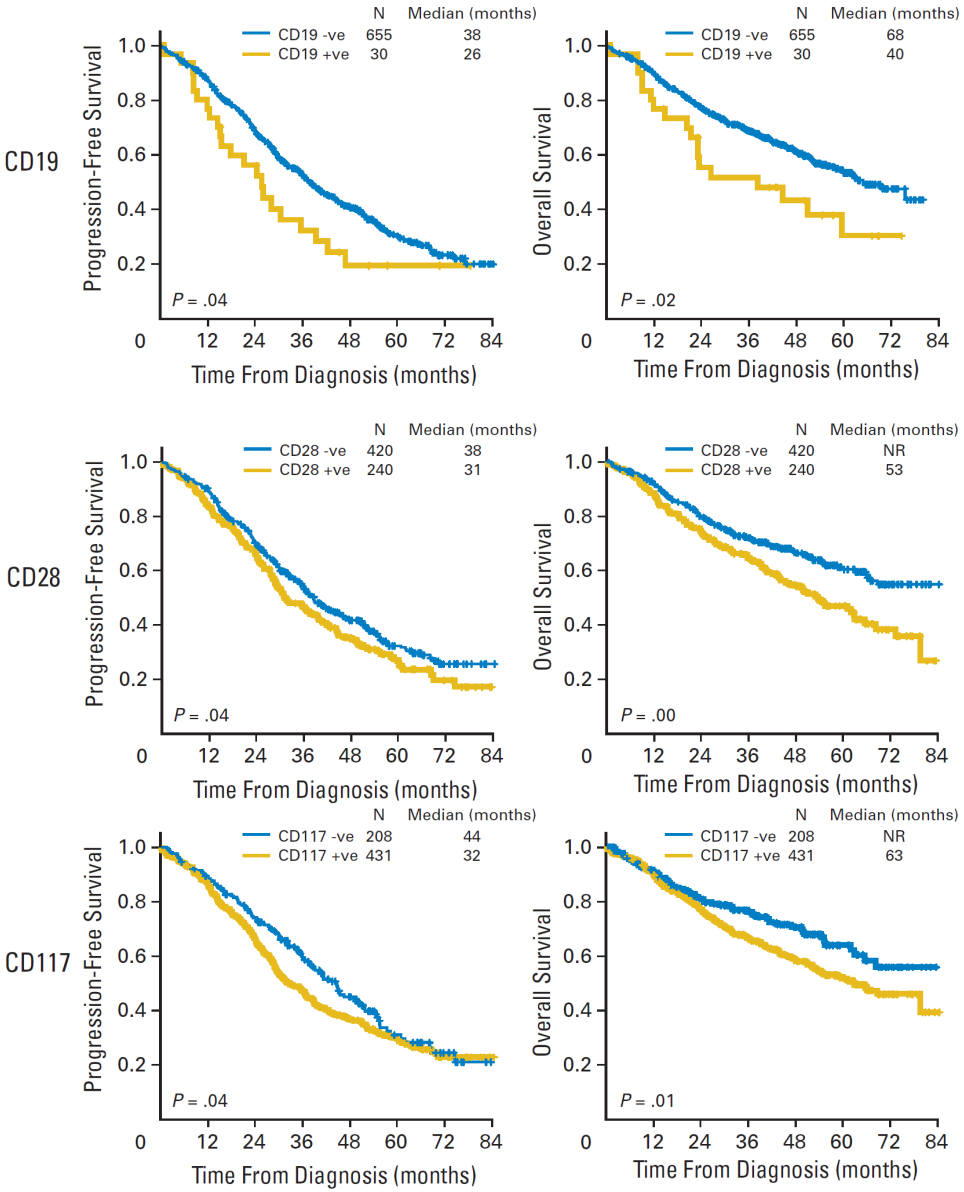
  ⇒ **> 5% residuele polyclonale plasmacellen : onderscheid MGUS en MM**

- > 5% normale plasmacellen bij diagnose:
  - MGUS: 25% *versus* 5% TTP
  - Smoldering myeloma: 64% *versus* 8% TTP



Pérez-Persona, 2007, Blood

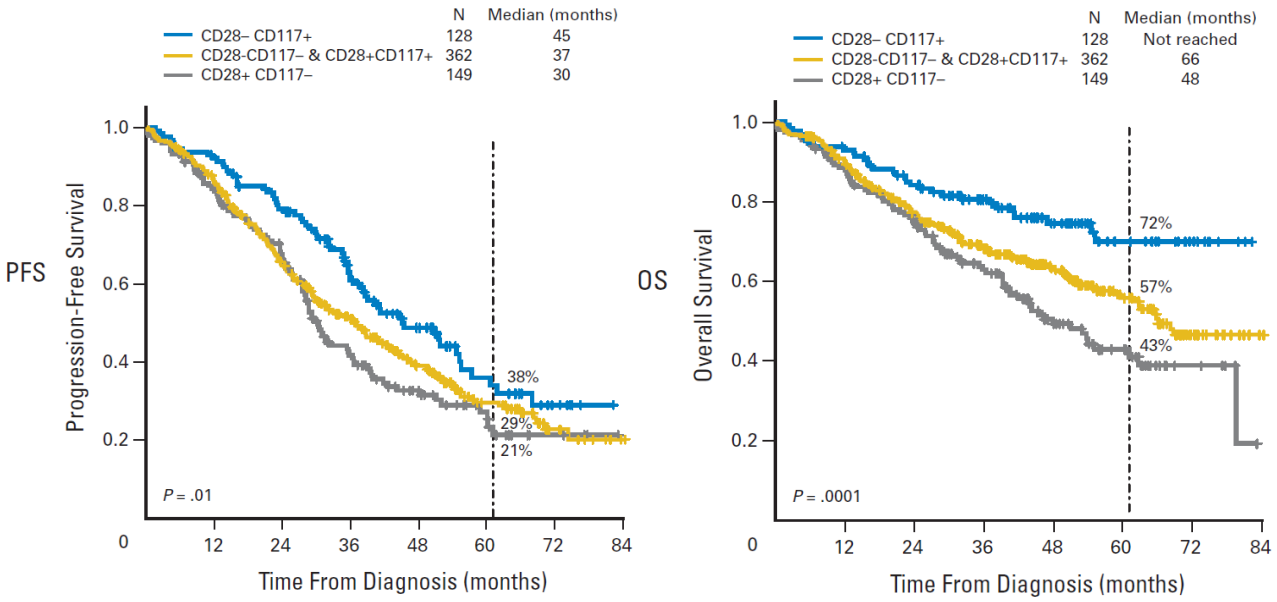
II. Prognose



- 3 merkers prognostische waarde
  => CD19, CD28, CD117
- CD56 & CD33
  => niet significant
- CD20 en CD45
  = geen prognostische waarde

Mateo et al, 2008, J Clin Oncol

II. Prognose

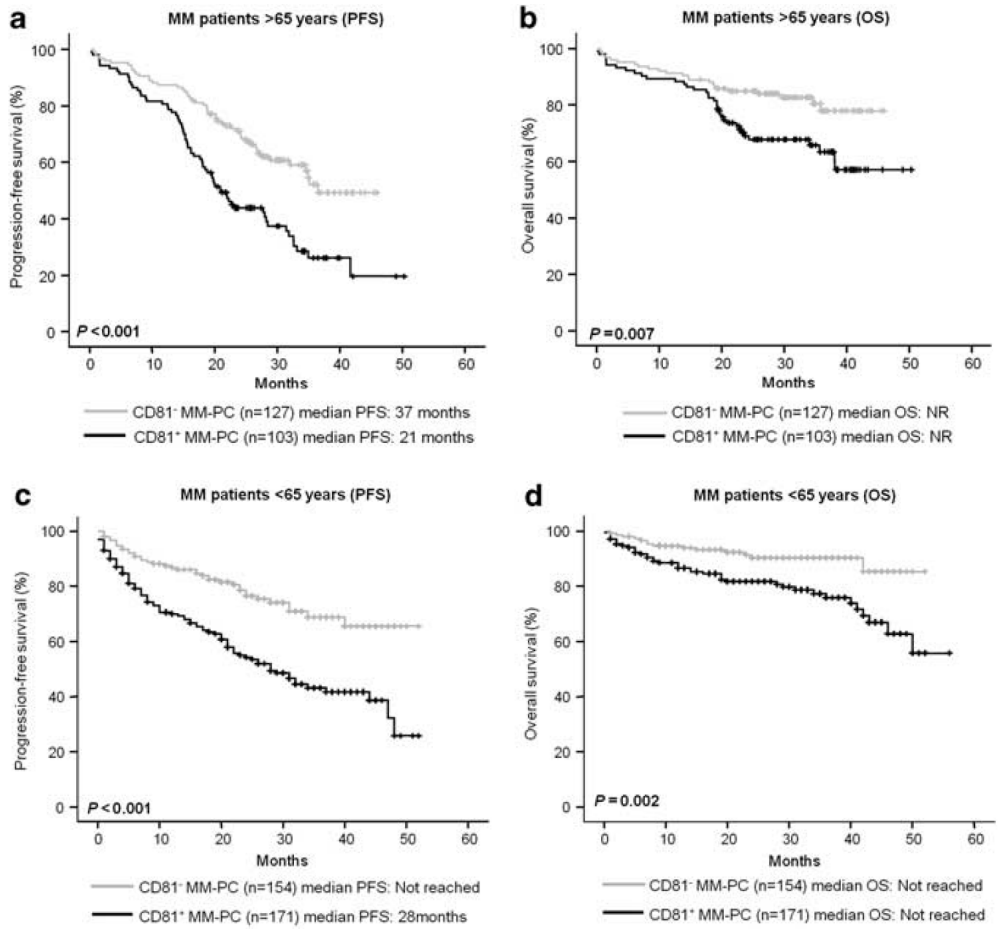


Prognostische impact van de verschillende antigen combinaties:

- Goede prognose **(21%)**: CD28-/CD117+
- Intermediaire prognose **(56%)**: CD28-/CD117- & CD28+/CD117+
- Slechte prognose **(23%)**: CD28+/CD117-

Mateo et al, 2008, J Clin Oncol

II. Prognose



CD81 expressie bij 45% multiple myeloma patiënten

- ≈ kortere PFS
- ≈ kortere Time To Progression
- ≈ kortere OS

Paiva et al, 2012, Leukemia
Moreau et al, 2006, Br J Haematol

Merkers?

Plasma cellen

CD138

CD38

CD45

Aantonen maligniteit

CD19

CD56

~~**CD20**~~

CD28

CD117

CD81

~~**CD27**~~

Minimum

“Entry-level” panel

CAT-vraagstelling

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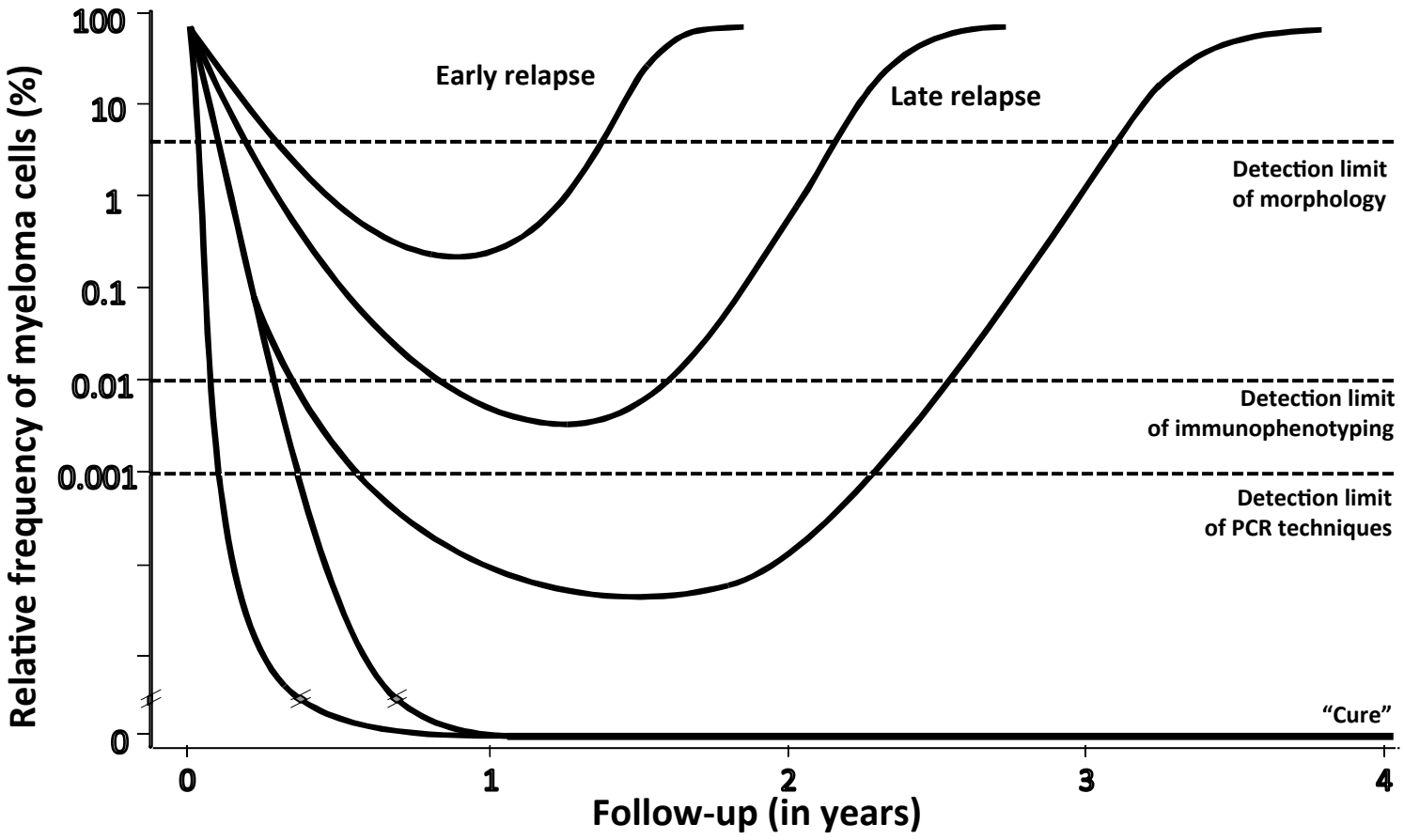
III. Minimal Residual Disease



Factoren die de effectiviteit van behandeling beïnvloeden

- **Karakteristieken tumorcellen**

v.b. - genetische afwijkingen
- *In vitro* GM gevoeligheid
- Immuunfenotype/percentage plasmacellen

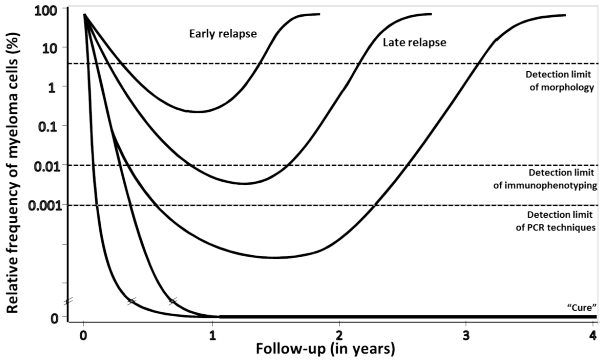
- ***In vivo* geneesmiddelen verdeling**

v.b. - GI absorptie
- Verdeling in het lichaam (vb. CNS)
- Metabolisme
- Excretie lever
- Excretie nier

- **Behandelingscomplicaties**

v.b. - Neveneffecten (infecties, allergische reacties)
- RX

Evaluatie van behandelings-efficiëntie a.d.h.v. MRD



Doel van MRD onderzoek

- Identificatie/detectie van (kleine) subsets residuele plasmacellen
- “Kinetics”: vermindering tumor load voor en na inductie behandeling bevat cruciale informatie over “response to treatment”
- Prognose:
  - Voorspellen uitkomst in patiënten met myeloma
  - Voorspellen relapse
  - MRD-stratificatie: identificatie van “low-risk” (therapie-reductie) en “high-risk” (therapy-intensification) patiënten
  - Prognostische informatie voor patiënten die stam cel transplantatie ondergaan.
- Identificatie van die patiënten die baat hebben bij een welbepaalde specifieke therapie?

Kumar et al, 2014, Leukemia

MRD technieken

Allele Specific Oligonucleotide-Real Time Quantitative Polymerase Chain Reaction **(ASO-RQ-PCR)** & Multiparameter Flow Cytometrie **(MFC)**

⇒ Moleculaire analyses

ASO RQ PCR van de junctionele regio van IgH genen

- \+ Sensitiviteit: 10^{-4}-10^{-5}
- \- **Toepasbaarheid: ≈ 40%**
- \- Expertise: ++
- \- Kosten: ++
- \- TAT: weken

Lage toepasbaarheid?

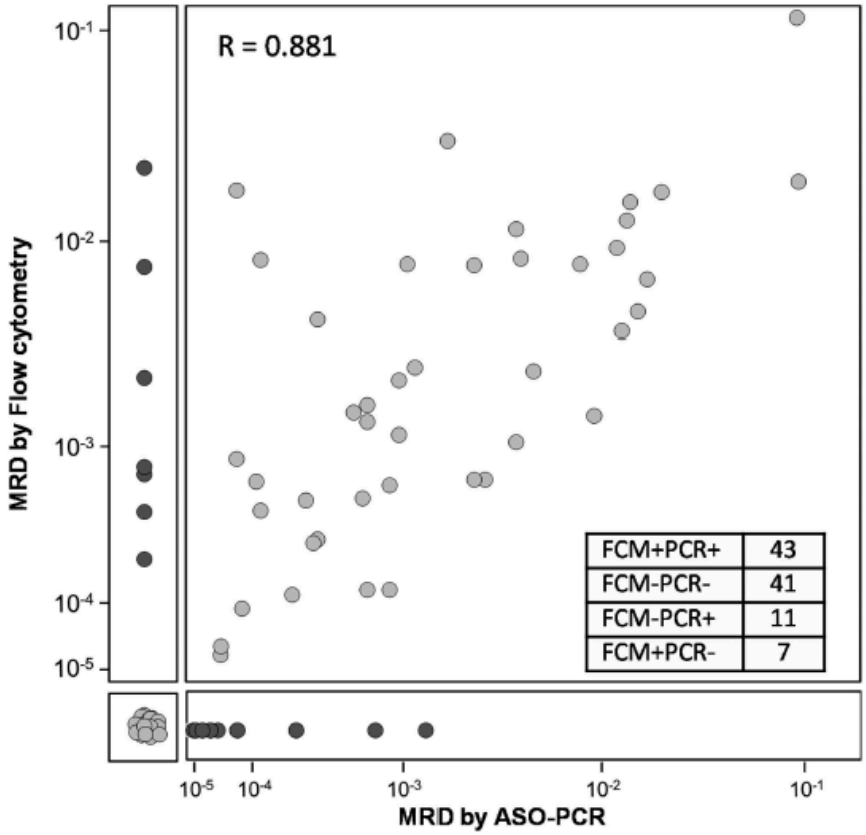
1. **clonaliteit (20%)**
2. **sequencing (10%)**
3. **ASO performance (30%)**

⇒ Multiparameter Flow Cytometrie

- \+ Sensitiviteit: 10^{-4}
- \+ Toepasbaarheid: >90%
- \- Expertise: ++
- \+ Kosten: +
- \+ TAT: dag(en)

Puig et al, 2014, Leukemia

ASO-RQ-PCR versus MFC



Puig et al, 2014, Leukemia

Minimal Residual Disease

- Gevoeligheid minstens 10^{-3} (bij voorkeur 10^{-4} tot 10^{-6})
- Specifiek
- Reproduceerbaarheid en standaardisatie
- Kwantitatieve techniek
- Stabiele, plasmacel-specifieke merkers (MAIP)

Marker	Immunophenotypic shift				
	Pts with changes/ Total pts (%)	(+)* to (−)	(−) to (+)	Change in level of expression	Multiple changes
CD19	5/45 (11.1)	0	4	1	0
CD20	2/45 (4.4)	1	1	0	0
CD45	9/45 (20.0)	1	5	2	1†
CD56	5/45 (11.1)	3	0	2	0

*(+) includes partial (+) and subset (+); †From (−) to partial (+) to (−).

Spears et al, 2011, Int J Lab Hematol

IMWG response criteria in multiple myeloma

Complete response (CR)	Stringent complete response (sCR)	Very good partial response (VGPR)	Partial response (PR)
Negative immunofixation of serum and urine ***and***	CR as defined ***plus***	Serum and urine M-component detectable by immunofixation but not on electrophoresis, ***or***	≥50% reduction of serum M-protein and reduction in 24-hour urinary M-protein by ≥90% or to <200mg/24h
Diappearance of any soft tissue plasmacytomas ***and***	Normal FLC ratio	≥90% reduction in serum-M-component *plus* urine component < 100mg/24h	If the serum and urine M-protein are not measurable, a decrease ≥50% in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria
<5% plasma cells in bone marrow	**Absence of clonal plasma cells by immunohisto-chemistry or 2- to 4-color flow cytometry**		If serum and urine M-protein are not measurable, and serum free light assay is also not measurable, ≥50% redcution in bone marow plasma cells is required in place of M-protein, provided baseline percentage was ≥30%
			In addition to the above criteria, if present at baseline, ≥50% reduction in the size of soft tissue plasmacytomas is also required

Rajkumar et al, 2011, Blood

Impact respons

- Kwaliteit respons ≈ langere overleving
  - ⇒ CR ≈ betere overleving
    - M-component ≠ residuele tumor
    - $t^{1/2}$ verschillende immunoglobulines
  - ⇒ meer gevoelige technieken voor MRD onderzoek!

	IgA	IgG	IgM	IgD	IgE	Light chains
$t^{1/2}$(dagen)	5	23	5	8	2	2-6 uur

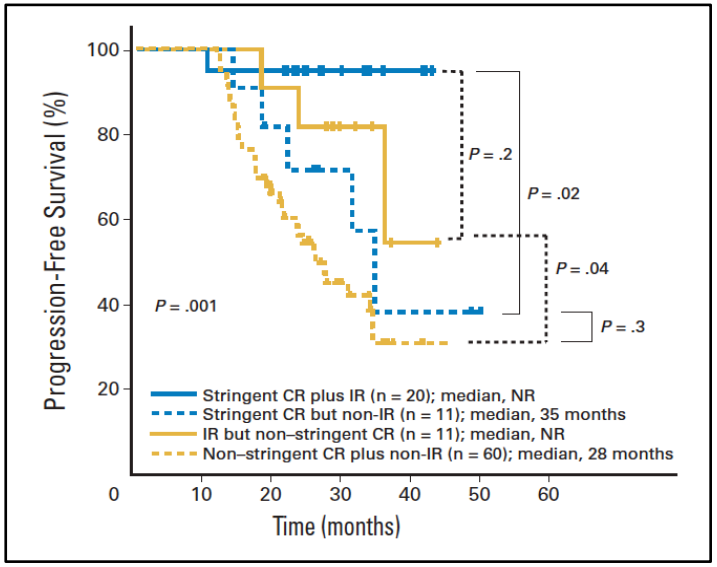
- 260 patiënten (>65 jaar), nieuwe diagnose MM
  - Partiële response (41%)
  - **CR** (43%) *versus* **sCR** (30%) *versus* **immunophenotypic response (IR)** (30%)

Paiva et al, 2011, J Clin Oncol
Paiva et al, 2008, Blood

Impact respons

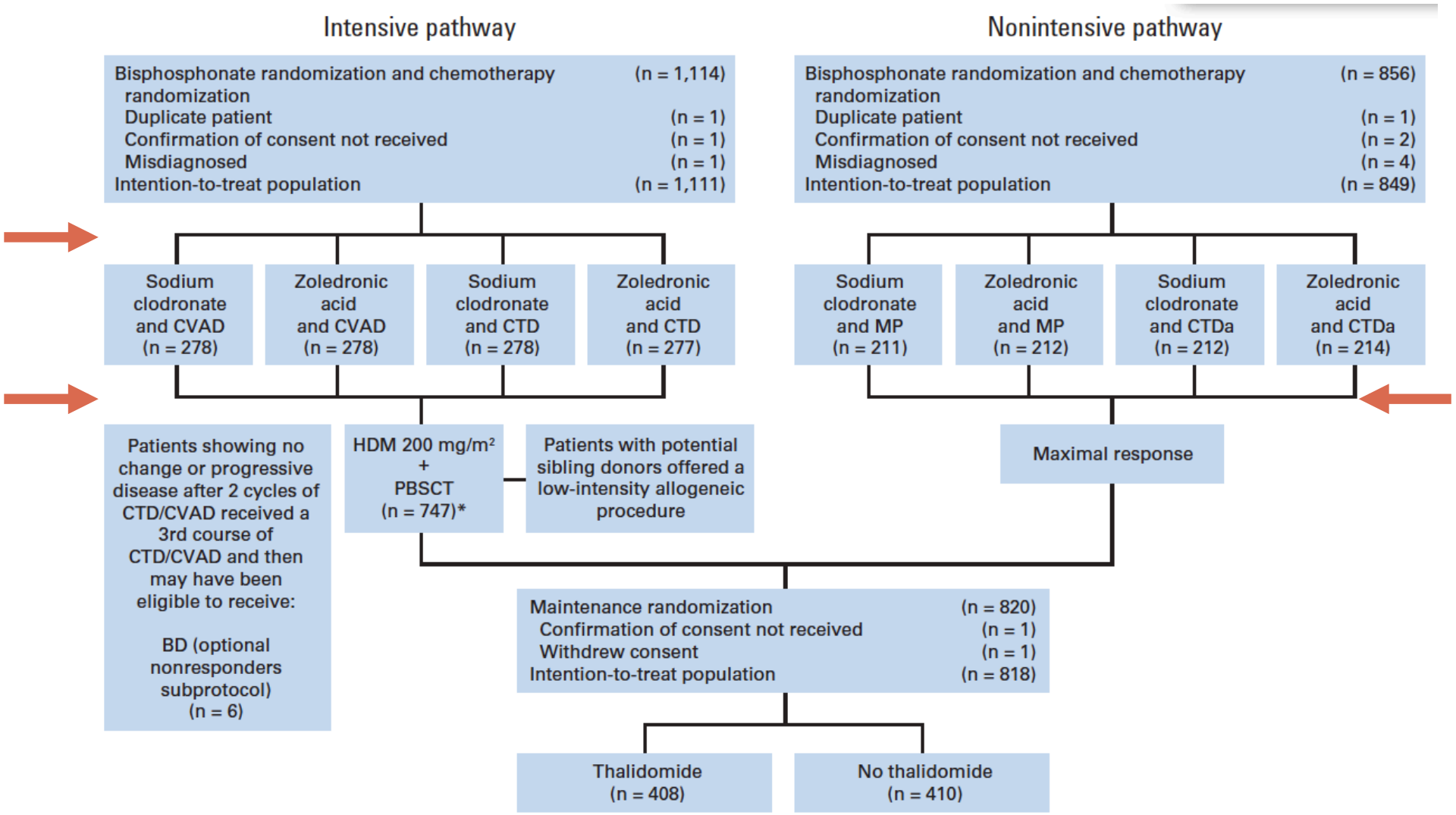
- Outcome by response:
  - ⇒ IR, sCR of CR => betere outcome versus PR:
    - PFS (90%, 69%, 60% *versus* 35%; p<0.001)
    - TTP (96%, 71%, 68% *versus* 37%; p<0.001)
    - OS (94%, 94%, 93% *versus* 70%; p= 0.08)
  - ⇒ multivariaat analyse: IR als onafhankelijke prognostische factor PFS en TTP

- Prognostische impact **IR** => sCR: inclusie IR?



Paiva et al, 2011, J Clin Oncol
Paiva et al, 2008, Blood

MRC Myeloma IX Trial



Rawstron et al, 2013, J Cllin Oncol

MRC Myeloma IX Trial

- Minimal Residual Disease

Intensief behandelde patiënten

- CTD (**C**yclophosphamide, **T**halidomide, **D**examethasone) & CVAD (**C**yclophosphamide, **V**incristine, **D**oxorubicin, **D**examethasone): **25%** *versus* **13%**
- Na ASCT: hogere MRD negativiteit: **71%** *versus* **54%**

⇒ ASCT meest effectieve therapie in MM patiënten

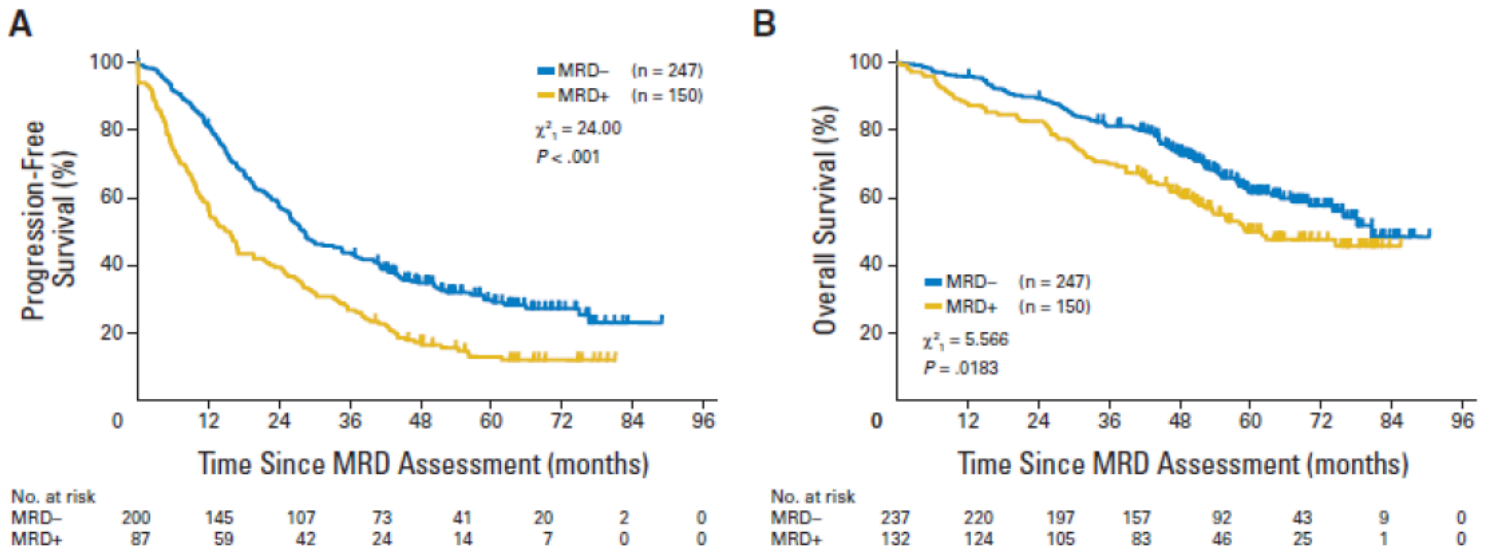
Niet-intensief behandelde patiënten

- Melphalan/prednisone versus CTDa: **26%** *versus* **3%**

- Minimal Residual Disease: intensief *versus* niet-intensief

Rawstron et al, 2013, J Cllin Oncol

MRC Myeloma IX Trial



"In intensive-pathway patients, absence of MRD at day 100 after ASCT was highly predictive of a favorable outcome (PFS: P = .001; OS: P=.0183)"

Rawstron et al, 2013, J Cllin Oncol

MRC Myeloma IX Trial

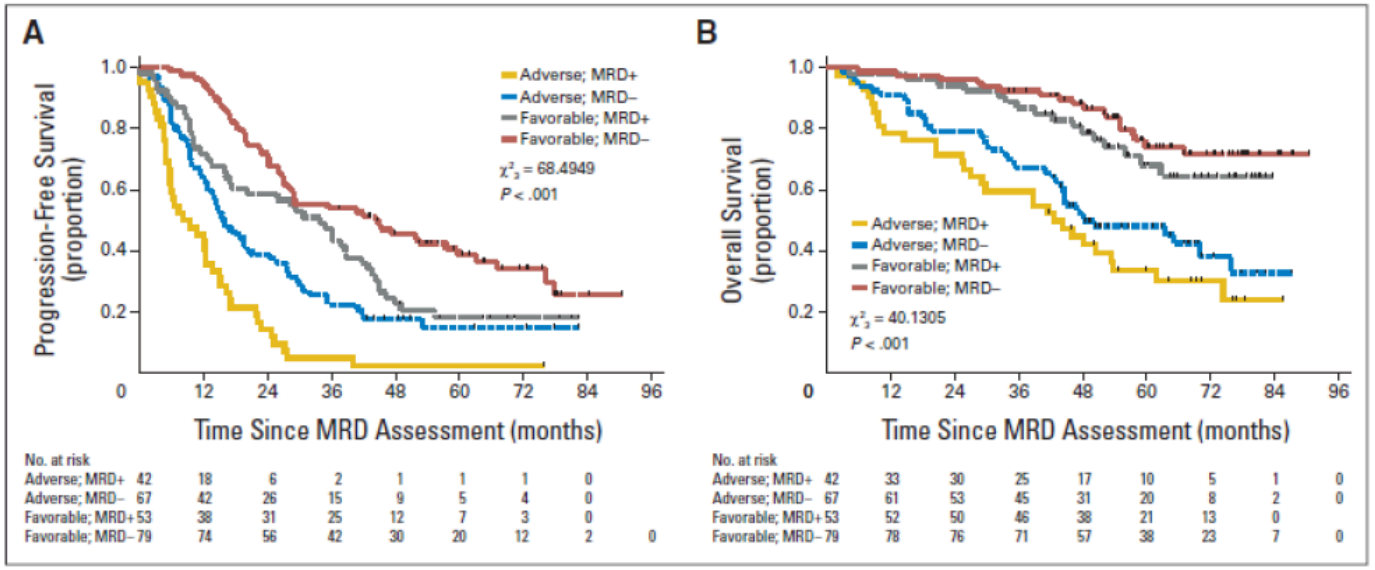


Fig 3. Outcome according to minimal residual disease (MRD) status after autologous stem-cell transplantation and cytogenetic risk profile. (A) Progression-free survival; (B) overall survival.

"This outcome advantage was demonstrable in patients with favorable (t(11;14)(q13;q32)) and adverse (t(4;14)(p16;q32) or t(14;16)(q32;q23)) cytogenetics (PFS: P=0.014 and P = 0.001, respectively)"

Rawstron et al, 2013, J Clin Oncol

MRC Myeloma IX Trial

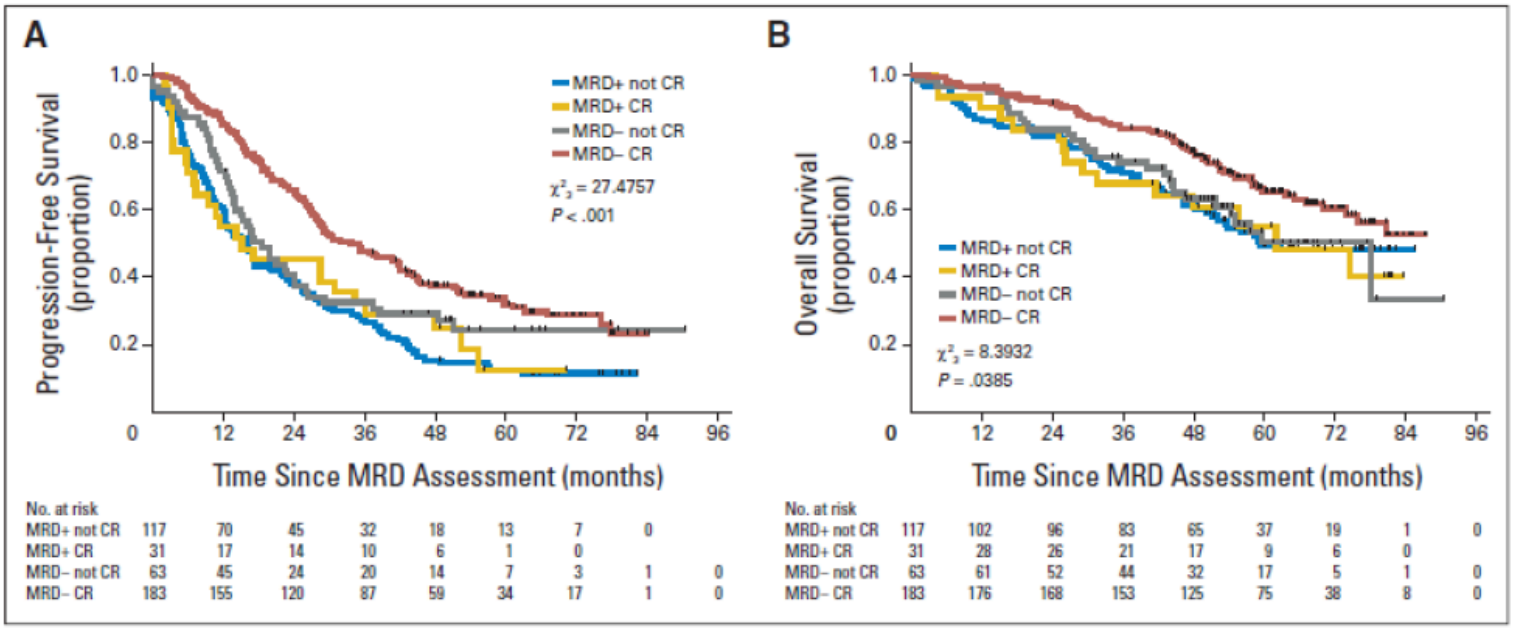


Fig 4. Outcome according to minimal residual disease (MRD) and immunofixation (IF) status after autologous stem-cell transplantation. Best outcome was demonstrated in those patients with IF-negative complete response (CR) and no demonstrable MRD. (A) Progression-free survival ($P < .001$); (B) overall survival ($P = .0385$).

"This outcome advantage was demonstrable in patients achieving immunofixation-negative complete response (CR; PFS: P=0.068)"

Rawstron et al, 2013, J Cllin Oncol

Minimal Residual Disease

Authors	No of patients	Method	Sensitivity	Treatment regimen	CR	MRD negativity rate
San Miguel et al	87	4-color MFC	10^{-4}	ASCT	45%	36%
Paiva et al	295	4-color MFC	10^{-4}	ASCT	50%	42%
Liu et al	47	4-color MFC	NR	ASCT	66%	8%
					CR/VGPR	
Mateo et al	685	4-color MFC	NR	ASCT	36%	NR
Kumar et al	132	MFC	NR	Chemotherapy	22% to 47%	46%

Authors	No of patients	Method	Sensitivity	Treatment regimen	CR	MRD negativity rate
Bird et al	5	PCR (not ASO)	NR	Allo-BMT	100%	100%
Corradini et al	18	ASO nested PCR	NR	auto-SCT or allo-SCT	50%	0%
Björkstrand et al	15	ASO-PCR	NR	Auto-SCT x2	53%	80%
Swedin et al	36	ASO semi –nested PCR	$10^{-4} – 10^{-5}$	Auto-SCT or allo-SCT	42%	21%
Corradini et al	51	ASO-PCR	$10^{-5} – 10^{-6}$	Auto-SCT or allo-SCT	71%	7%; 50%
Martinelli et al	26	ASO-PCR	10^{-5}	Allo-SCT	38%	50%
Martinelli et al	229	ASO-PCR	10^{-5}	Auto-SCT or allo-SCT	Allo = 38% Auto = 22.5%	27%
Cavo et al	13	ASO-PCR	$10^{-5} – 10^{-6}$	Allo-SCT	92%	69%
Ladetto et al	29	Real time PC (not ASO) ASO nested PCR	10^{-4} $10^{-3} – 10^{-4}$	Auto-SCT	NR	NR
Davies et al	96	PCR (not ASO)	10^{-4}	Auto-SCT	53%	NR
Novella et al	36	PCR (not ASO) ASO nested PCR	$10^{-4} – 10^{-6}$ $10^{-4} – 10^{-5}$	Auto-SCT	24%	NR
Corradini et al	70	ASO-PCR	10^{-6}	Allo-SCT	100%	33%
Fenk et al	11	ASO real time PCR	$10^{-4} – 10^{-6}$	Auto-SCT or allo-SCT	45%	27%
Bakkus et al	87	ASO-PCR	10^{-4}	Auto-SCT	28%	35%
Raab et al	11	ASO real time PCR	$10^{-4} – 10^{-5}$	Allo-SCT	27%	65%
Ladetto et al	39	ASO nested Real-time PCR (not ASO)	10^{-6} 5×10^{-6}	Auto-SCT	49%	27%; 15% and NR
Korthals et al	70	Real-time ASO-PCR	$10^{-4} – 10^{-5}$	Auto-SCT	25% nCR before auto-PBSCT 29% nCR after auto-PBSCT	17% and 21%

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IV. Therapie?

- Thalidomide, lenalidomide, bortezomib, pomalidomide, …
- Autologe stamceltransplantatie
- Nieuwe strategieën: monoclonale antilichamen

Target	Agent	Clinical study phase	Single agent (S)/combination (C)
Activin A	Sotatercept	I/II	S
BAFF	Tabalumab (mAb)	I/II	S, C (lenalidomide)
CD38	Daratumumab	I	S
	SAR650984	I	S
	MOR202	I	S
CD40	Dacetuzumab (SGN-40)	Ib	S, C (lenalidomide)
	Lucatumumab (HCD122)	I	S
CD56	huN901-DM1 (C-mAb)	I	S
CD74	Milatuzumab	I/II	S
CD138	BT062 (mAb-DM4)	I	S
CS1	Elotuzumab	II/III	S, C (lenalidomide, bortezomib)
CXCR3	Plerixafor	II	C (bortezomib)
DKK-1	BHQ-880 (mAb)	I/II	S
FGF, PDGF	Dovitinib	I	S
HM1.24	anti-HM1.24 (mAb)		
IGF-1/R	CP-751,871 (mAb)	I	S
	EM164 (mAb)	I	S
IL-6/R	Siltuximab (mAb)	II	S, C (bortezomib)
KIR	IPH101 (mAb)	I/II	S
MUC1	AR20.5 (mAb)	I/II	S
RANKL	Denosumab (mAb)	I/II	S
TRAIL	Apo2L/TRAIL (Apo2 ligand)	I	S
	Mapatumumab	I/II	S
VEGF/R	Bevacizumab (mAb)	II	S
	SU5416	II	S
	Vandetanib (ZD6474)	II	S

Munshi et al, 2013, Clin Canc Research

“Take home messages”

- Verschillende merkers voorgesteld voor detecteren plasmacellen
  - Goede en specifieke manier detecteren!
  - CD138, CD38, CD45 + light scatter characteristics
  - CD19, CD56, CD28, CD117, CD20, CD27, CD81, CD33,CD31,CD39,CD40,CD44, cyclin D1, CD34

- Prognostische waarde voor CD19, CD56, CD28, CD117, CD81 en CD45 duidelijk aangetoond

- Immuunfenotypering kan gebruikt worden voor MRD detectie
  - Beter de respons, langere PFS en OS!
  - **Kan MRD onderzoek leiden tot (bij)sturen van therapie?**
  - **Heeft het (bij)sturen van de therapie een betere overleving tot gevolg?**

- Kan MFC een hulp bieden bij het kiezen van specifieke therapie?
  => mogelijks, fase 3 studies?

“To do’s”

- Bij **wie** nuttig (<65 en/of >65?) en **wanneer** (diagnose en/of follow-up?)
- **Evaluatie** meer-kleurenpanels
- **Implementatie** van MFC van plasmacellen in MRD-onderzoek in UZ Leuven

QUESTIONS

FOUND IN GOOGLE AUTOCOMPLETE

WHY DO WHALES JUMP
WHY ARE WITCHES GREEN
WHY ARE THERE MIRRORS ABOVE BEDS
WHY DO I SAY UH
WHY IS SEA SALT BETTER
WHY ARE THERE TREES IN THE MIDDLE OF FIELDS
WHY IS THERE NOT A POKEMON MMO
WHY IS THERE LAUGHING IN TV SHOWS
WHY ARE THERE DOORS ON THE FREEWAY
WHY ARE THERE SO MANY SVCHOST.EXE RUNNING
WHY AREN'T THERE ANY COUNTRIES IN ANTARCTICA
WHY ARE THERE SCARY SOUNDS IN MINECRAFT
WHY IS THERE KICKING IN MY STOMACH
WHY ARE THERE TWO SLASHES AFTER HTTP
WHY ARE THERE CELEBRITIES
WHY DO SNAKES EXIST
WHY DO OYSTERS HAVE PEARLS
WHY ARE DUCKS CALLED DUCKS
WHY DO THEY CALL IT THE CLAP
WHY ARE KYLE AND CARTMAN FRIENDS
WHY IS THERE AN ARROW ON AANG'S HEAD
WHY ARE TEXT MESSAGES BLUE
WHY ARE THERE MUSTACHES ON CLOTHES
WHY ARE THERE MUSTACHES ON CARS
WHY ARE THERE MUSTACHES EVERYWHERE
WHY ARE THERE SO MANY BIRDS IN OHIO
WHY IS THERE SO MUCH RAIN IN OHIO
WHY IS OHIO WEATHER SO WEIRD
WHY ARE THERE MALE AND FEMALE BIKES
WHY ARE THERE BRIDESMAIDS
WHY DO DYING PEOPLE REACH UP
WHY AREN'T THERE VARICOSE ARTERIES
WHY ARE OLD KLINGONS DIFFERENT

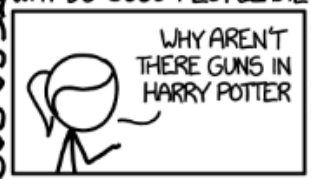
WHY IS PROGRAMMING SO HARD
WHY IS THERE A 0 OHM RESISTOR
WHY DO AMERICANS HATE SOCCER
WHY DO RHYMES SOUND GOOD
WHY DO TREES DIE
WHY IS THERE NO SOUND ON CNN
WHY AREN'T POKEMON REAL
WHY AREN'T BULLETS SHARP
WHY DO DREAMS SEEM SO REAL

WHY ARE THERE TINY SPIDERS IN MY HOUSE
WHY DO SPIDERS COME INSIDE
WHY ARE THERE HUGE SPIDERS IN MY HOUSE
WHY ARE THERE LOTS OF SPIDERS IN MY HOUSE
WHY ARE THERE SPIDERS IN MY ROOM
WHY ARE THERE SO MANY SPIDERS IN MY ROOM
WHY DO SPIDER BITES ITCH
WHY IS DYING SO SCARY
WHY IS THERE NO GPS IN LAPTOPS
WHY DO KNEES CLICK
WHY AREN'T THERE E GRADES
WHY IS ISOLATION BAD
WHY DO BOYS LIKE ME
WHY DON'T BOYS LIKE ME
WHY IS THERE ALWAYS A JAWA UPDATE
WHY ARE THERE RED DOTS ON MY THIGHS
WHY IS LYING GOOD

WHY DO TESTICLES MOVE
WHY ARE THERE PSYCHICS
WHY ARE HATS SO EXPENSIVE
WHY IS THERE CAFFEINE IN MY SHAMPOO
WHY DO YOUR BOOBS HURT
WHY ARE THERE SLAVES IN THE BIBLE
WHY DO TWINS HAVE DIFFERENT FINGERPRINTS
WHY ARE AMERICANS AFRAID OF DRAGONS
WHY IS HTTPS CROSSED OUT IN RED
WHY IS THERE A LINE THROUGH HTTPS
WHY IS THERE A RED LINE THROUGH HTTPS ON FACEBOOK
WHY IS HTTPS IMPORTANT

WHY ARE THERE WEEKS
WHY DO I FEEL DIZZY
MN
WHY IS YKK ON ALL ZIPPERS
WHY ARE THERE SWARMS OF GNATS
WHY IS THERE PHLEGM
WHY ARE THERE SO MANY CROWS IN ROCHESTER,
WHY AREN'T ECONOMISTS RICH
WHY DO AMERICANS CALL IT SOCCER
WHY ARE MY EARS RINGING
WHY ARE THERE SO MANY AVENGERS
WHY ARE THE AVENGERS FIGHTING THE X MEN
WHY IS WOLVERINE NOT IN THE AVENGERS
WHY IS PSYCHIC WEAK TO BUG
WHY DO CHILDREN GET CANCER
WHY IS POSEIDON ANGRY WITH ODYSSEUS
WHY IS THERE ICE IN SPACE
WHY IS THERE LAVA
WHY ARE THERE ANTS IN MY LAPTOP
WHY IS EARTH TILTED
WHY IS SPACE BLACK
WHY IS OUTER SPACE SO COLD
WHY ARE THERE PYRAMIDS ON THE MOON
WHY IS NASA SHUTTING DOWN

WHY IS THERE AN OWL IN MY BACKYARD
WHY IS THERE AN OWL OUTSIDE MY WINDOW
WHY IS THERE AN OWL ON THE DOLLAR BILL
WHY DO OWLS ATTACK PEOPLE
WHY ARE AK 47s SO EXPENSIVE
WHY ARE THERE HELICOPTERS CIRCLING MY HOUSE
WHY ARE THERE GODS
WHY ARE THERE TWO SPOCKS
WHY IS MT VESUVIUS THERE
WHY DO THEY SAY T MINUS
WHY ARE THERE OBELISKS
WHY ARE WRESTLERS ALWAYS WET
WHY ARE OCEANS BECOMING MORE ACIDIC
WHY IS ARWEN DYING
WHY AREN'T MY QUAIL LAYING EGGS
WHY AREN'T MY QUAIL EGGS HATCHING
WHY AREN'T THERE ANY FOREIGN MILITARY BASES IN AMERICA
WHY ARE MY BOOBS ITCHY
WHY ARE CIGARETTES LEGAL
WHY ARE THERE DUCKS IN MY POOL
WHY IS JESUS WHITE
WHY IS THERE LIQUID IN MY EAR
WHY DO Q TIPS FEEL GOOD
WHY DO GOOD PEOPLE DIE



WHY ARE ULTRASOUNDS IMPORTANT
WHY ARE ULTRASOUND MACHINES EXPENSIVE
WHY IS STEALING WRONG

WHY ARE DOGS AFRAID OF FIREWORKS
WHY IS THERE NO KING IN ENGLAND
WHY IS LIFE SO BORING
WHY IS GPS FREE
WHY IS THERE HELL IF GOD FORGIVES
WHY ARE THERE FEMALE MR MIMES
WHY AREN'T THERE DINOSAUR GHOSTS
WHY AREN'T THERE