

Praktijkgericht onderwerp 3

Antistof therapie: gevolgen voor flow cytometrische diagnostiek.

Andries Bloem

Hoofd Diagnostiek
Laboratorium voor Translationele Immunologie

November 2017



Universitair Medisch Centrum
Utrecht
The Netherlands

Januari 2017

68 door FDA goedgekeurde therapeutische monoklonale antistoffen

Type antistof en targets

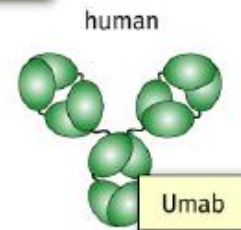
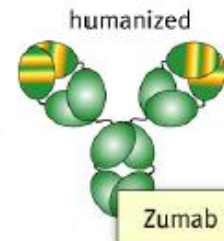
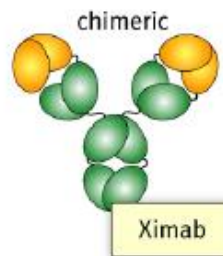
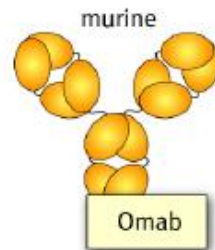


Box 5 Monoclonal antibodies approved by the US Food and Drug Administration

Product	Type	Target of action	Condition	Approved
Muronomab-CD3 (Orthoclone OKT3)	Mouse	CD3 antigen on T cells	Transplant allograft rejection	1986
Abciximac (ReoPro)	Chimeric	Glycoproteins IIb and IIIa on activated lymphocytes	Cardiovascular disease	1994
Daclizumab (Zenapax)	Humanized	CD25 (IL-2R α , Tac) on activated lymphocytes	Transplant allograft rejection	1997
Rituximab (Rituxan)	Chimeric	CD20 on B lymphocytes	Non-Hodgkin lymphoma	1997
Basiliximab (Simulect)	Chimeric	CD25 (IL-2R α) on activated lymphocytes	Transplant allograft rejection	1998
Palivizumab (Synagis)	Humanized	F protein on respiratory syncytial virus	Respiratory syncytial virus	1998
Infliximab (Remicade)	Chimeric	TNF- α	Rheumatoid arthritis, Crohn disease	1998
Trastuzumab (Herceptin)	Humanized	HER2 oncoprotein	Metastatic breast cancer	1998
Gemtuzumab ozogamicin (Mylotarg)	Humanized, toxin-linked	CD33 on leukemic blasts	Acute myelogenous leukemia	2000
Alezumab (Campath 1H)	Humanized	CD52 on B, T and NK cells and monocytes	Chronic lymphocytic leukemia	2001
Ibritumomab tiuxetan (Zevalin)	Chimeric, radionuclide-linked	CD20 on B lymphocytes	Non-Hodgkin lymphoma	2002

Daratumumab: volledig humaan IgG1, CD38, multipel myeloom, 2015/2016

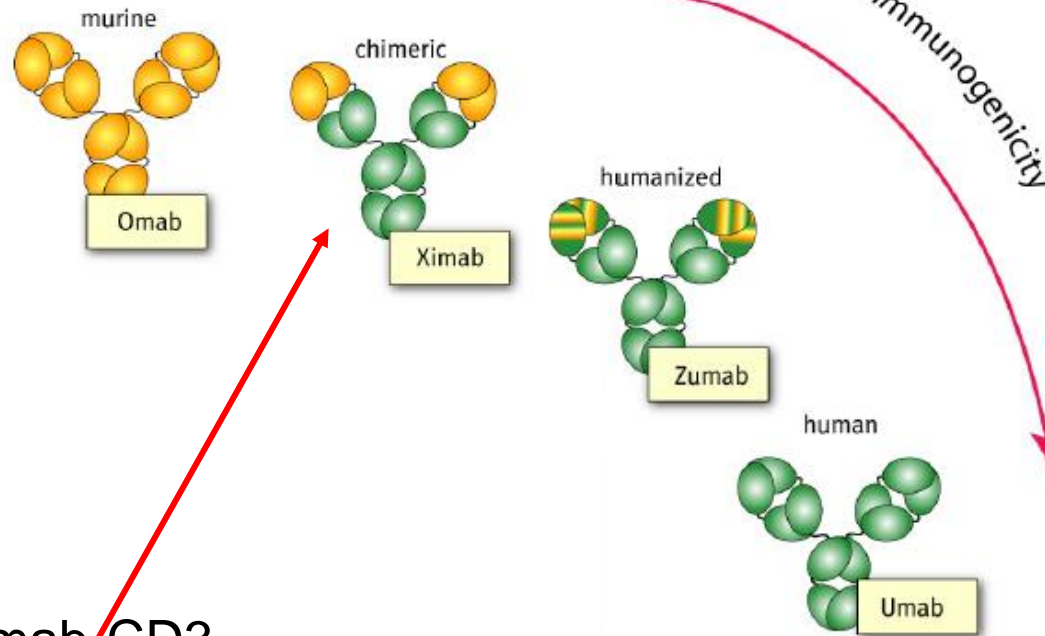
Therapeutic antibodies: From murine to human



Decreased immunogenicity

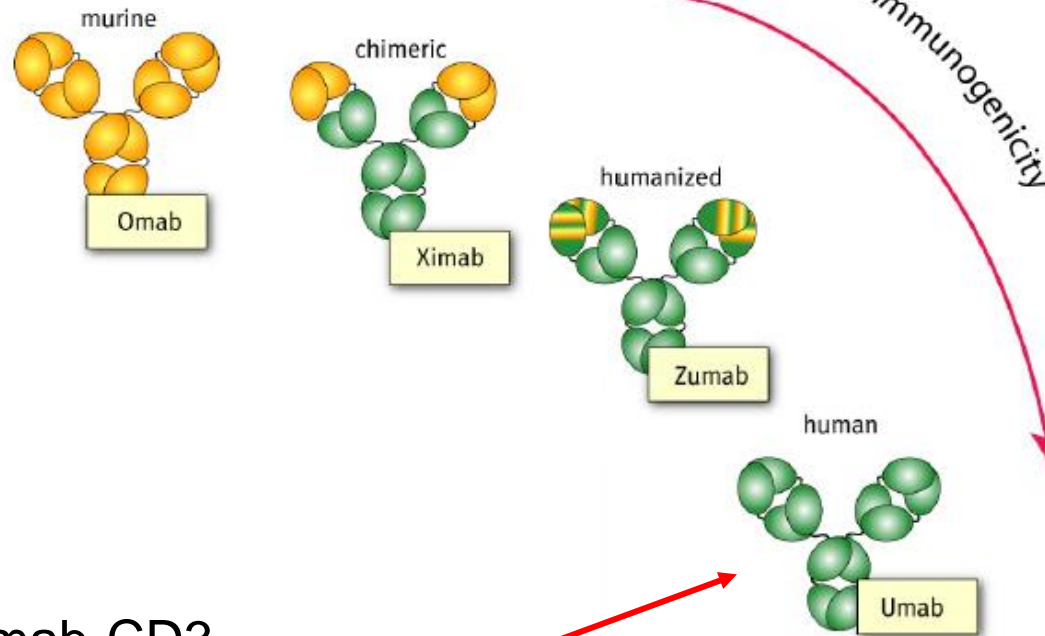
Muronomab-CD3
Rituximab
Daratumumab

Therapeutic antibodies: From murine to human



Muronomab-CD3
Rituximab
Daratumumab

Therapeutic antibodies: From murine to human



Muronomab-CD3

Rituximab

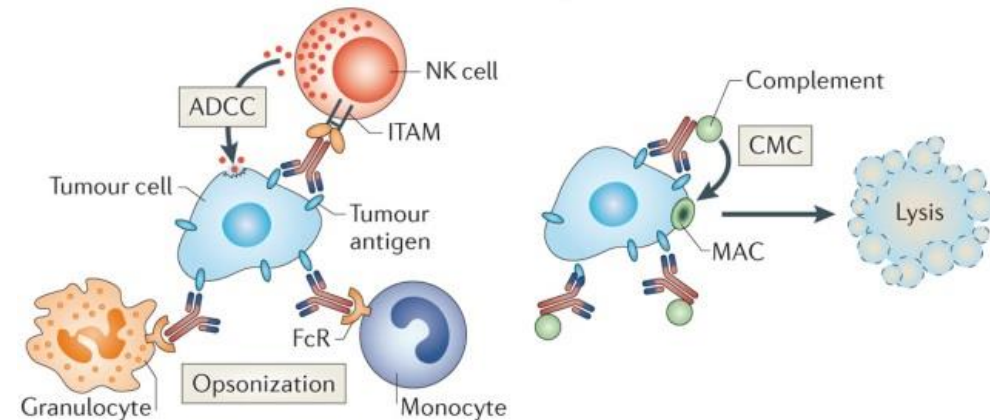
Daratumumab

Ruuls et al (2008) Biotech. J. 3: 1157

werkingsmechanismen

- Complement mediated cytotoxicity (CMC)
- Antibody dependent cell-mediated cytotoxicity (ADCC)
- Antibody dependent phagocytosis
- Inductie apoptose
- Receptor blocking
- Cross-priming tumorantigenen: anti-tumor antistoffen
- Labeling antistoffen

a Immune-mediated effects of tumour-specific IgG



b Direct effects of tumour-specific IgG

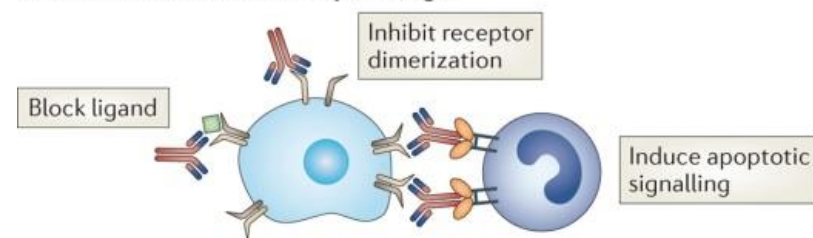


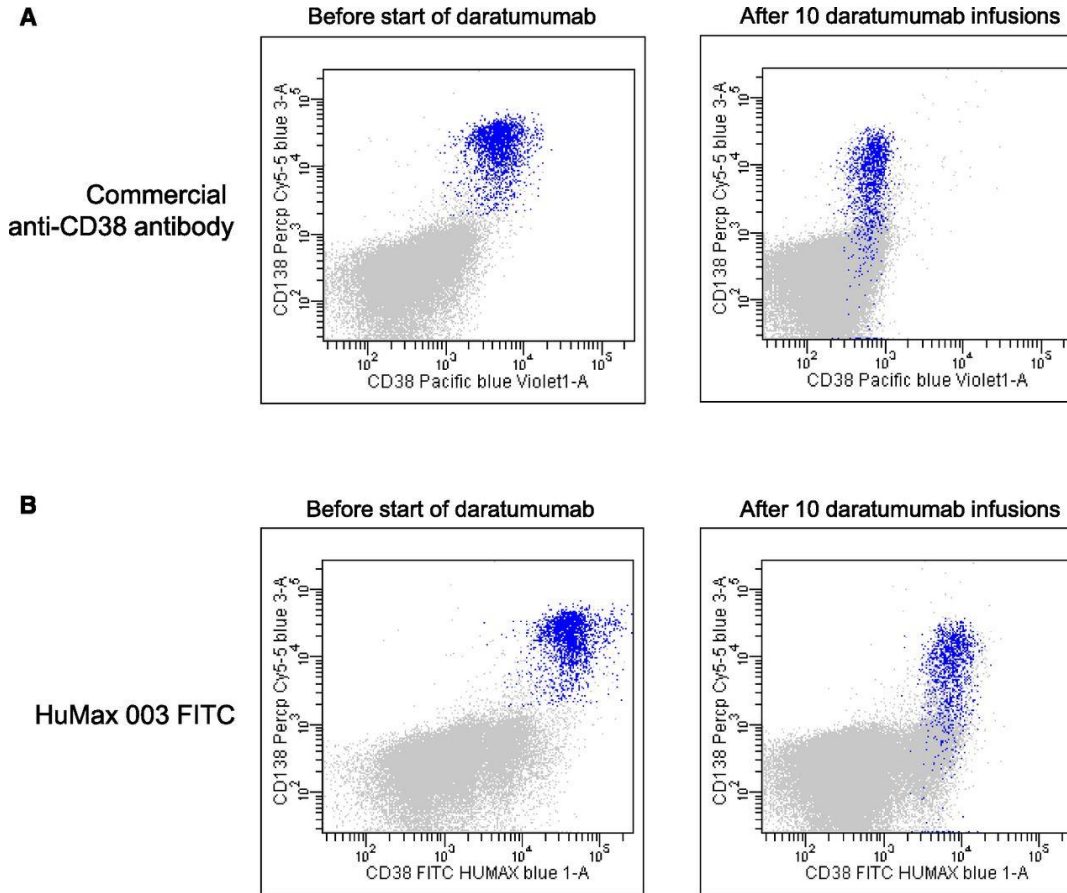
Table 6. Management of laboratory interference associated with elotuzumab and CD38-targeting antibodies

Laboratory test	Antibody	Management
Interference with serum protein electrophoresis and immunofixation assays	Several therapeutic antibodies	DIRA should be performed when daratumumab-treated patients with IgG-κ M-protein have achieved deep response (M-protein <2 g/L) New assays are in development for elotuzumab, isatuximab and MOR202
Interference with multiparametric flow cytometry	Daratumumab, isatuximab, MOR202, and possibly other therapeutic antibodies	Use of newly developed antibodies for flow cytometry, which bind to different epitopes compared with the therapeutic antibody Application of alternative plasma cell identification markers
Interference with blood compatibility testing	CD38-targeting antibodies (also observed with anti-CD44 antibodies)	Denaturation of CD38 from reagent RBCs by dithiothreitol Neutralization of therapeutic antibody with neutralizing antibodies or recombinant soluble CD38 Extensive RBC antigen phenotyping before the patient receives the first infusion of the CD38-targeting antibody or RBC antigen genotyping when the patient has already received treatment with an anti-CD38 antibody or a recent blood transfusion (<3 mo) A wallet card that informs physicians and blood banks of the interference with blood compatibility testing should be provided to all patients treated with CD38-binding antibodies

DIRA, daratumumab interference reflex assay.

Niels W. C. J. van de Donk et al. *Blood* 2016;127:681-695

Daratumumab maskeert de detectie van CD38 met commercieel verkrijgbare antistoffen.



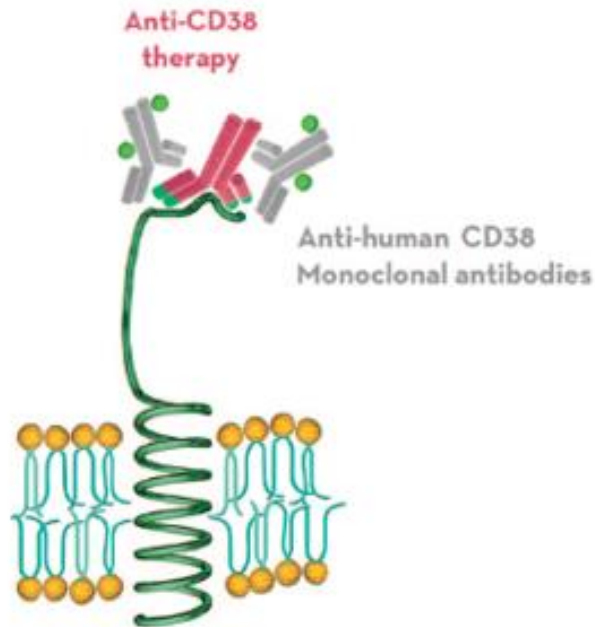
Niels W. C. J. van de Donk et al. Blood 2016;127:681-695

Consequenties voor flowcytometrie: afscherming (masking)



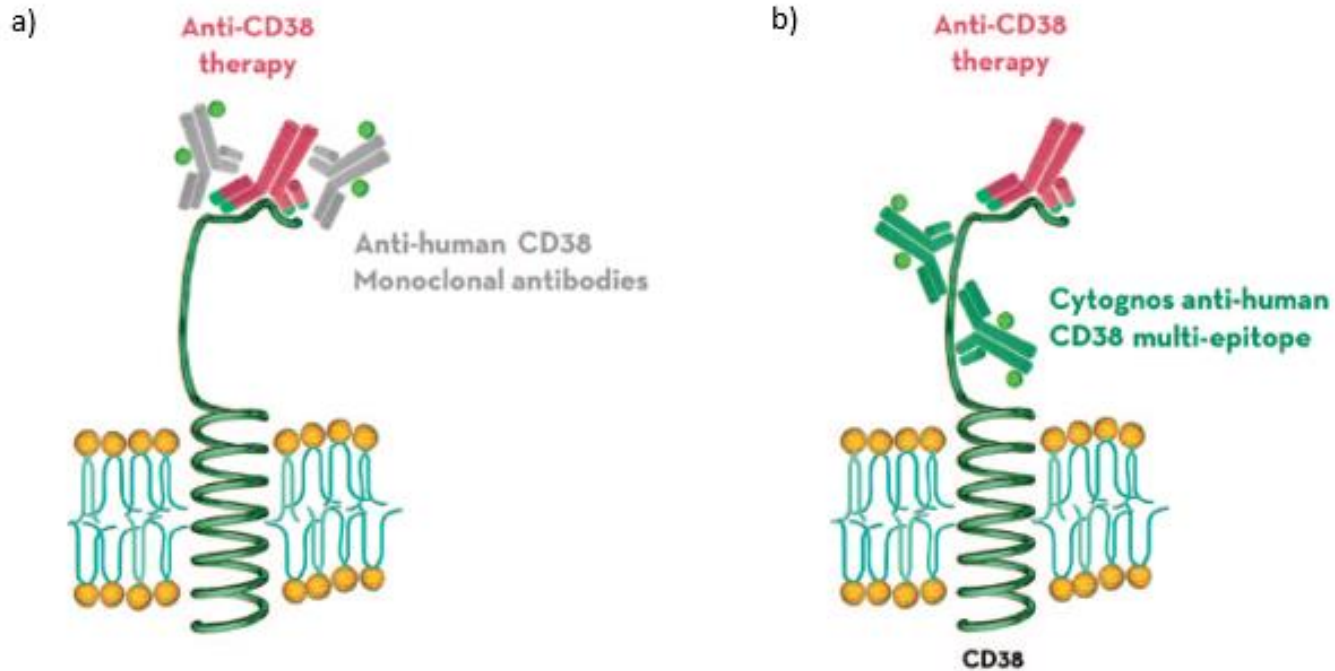
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Utrecht

a)



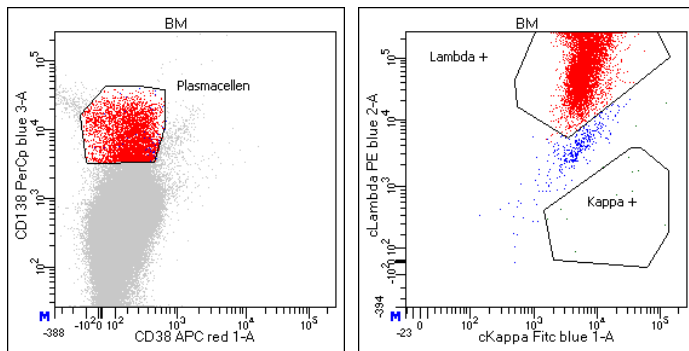
Therapeutische antistof en flowcytometrie antistof binden aan hetzelfde epitoom

Consequenties voor flowcytometrie: afscherming (shielding)

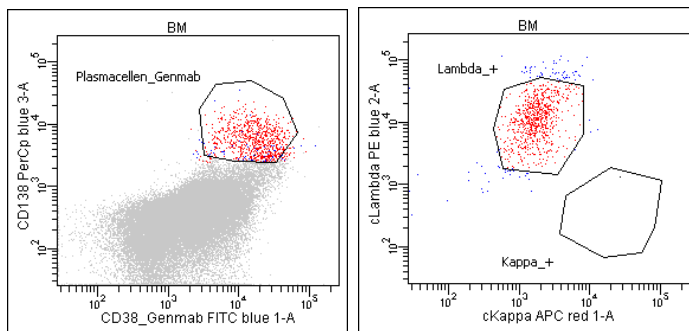


After Dara

Standard protocol

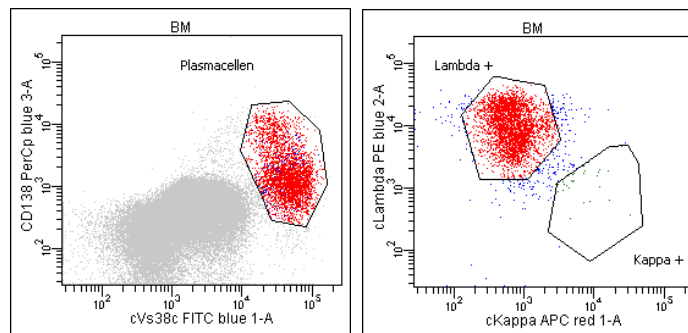


Genmab protocol
HuMax003

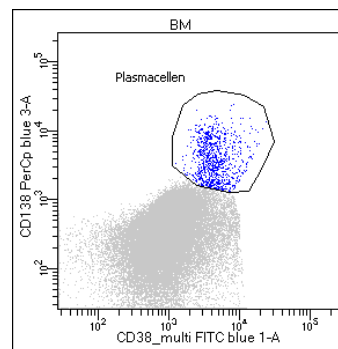


After Dara

Vs38c protocol

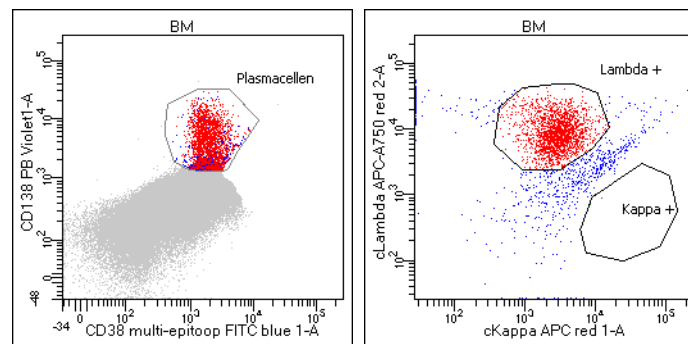


CD38 multi-epitope



Met dank aan Nina Wissing-Blokland ☺

Cytognos MM
MRD



- Antistof therapie kan interfereren met de diagnostische test (serologie of flowcytometrie)
- Voor aantonen van restziekte met behulp van flowcytometrie is het essentieel om uit te sluiten of een negatief resultaat wellicht wordt veroorzaakt door het maskeren van je antistof target
- Als dit is uitgesloten, hoeft een negatief resultaat niet te betekenen dat er geen restziekte meer is. Tumorcel kan het targetantigeen hebben ge-downreguleerd of op andere wijze kwijt zijn geraakt (bv trogocytose).



Met dank aan:

- Met dank aan medewerkers van het lab Celdiagnostiek LTI
- Met dank aan:
 - María Ochoa González, Cytognos
 - Patrick van der Meijden, Emelca Bioscience



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Naast maskeren van CD38 kan CD38 ook downgereguleerd worden.



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- Consequentie antistof therapie therapie
- Resistentie (complement regulerende eiwitten)
- Downregulatie target molecuul (vb CD20)
- Trogocytose; target cells evade opsonization when immune effector cells may evade opsonization by internalization of Ag/Ab/FcgRIIB
- Verhoogde effectiviteit in combinaties met andere stoffen; bv signalering via IL-2
signalering of TNF- α signalering + lenalidomide (andere antistoffen, chemotherapie, groeifactoren)



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8-kleur MFC

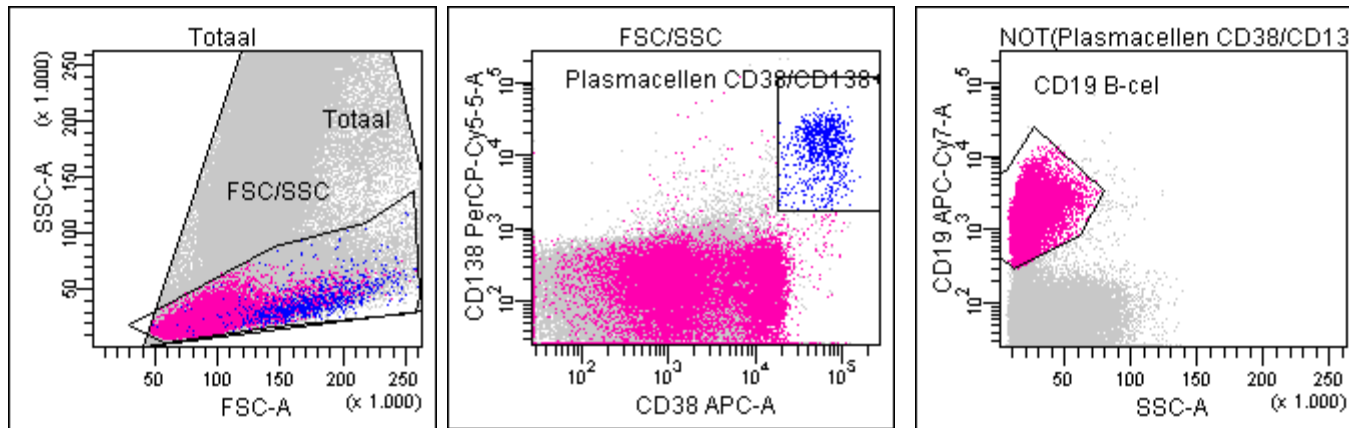


Tabel 6. Voorbeelden van achtkleurenpanels.

Pac. Blue/ V450	Pac. Org/ V500/ AmCyan	FITC	PE	PerCP/ PerCP-Cy5.5	PE-Cy7	APC	APC-Cy7
CD56	CD45	cylg	cylg	CD19	CD138	CD38	CD20
CD45	CD138	CD38	CD56	β_2 -micro	CD19	cylg	cylg
CD45	CD27	cy κ	cyl λ	CD138	CD56	CD38	CD19

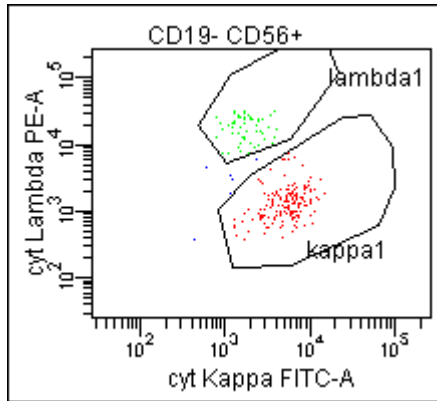
Pac. Blue='pacific blue', Pac. Org='pacific orange', AmCyan=anemoniamajanocyanine, FITC=fluoresceïne-isothiocyanaat, PE=phyco-erythrine, PerCP=peridininechlorofylproteïne, PE-Cy7=phyco-erythrine Cy7, APC=antigeenpresenterende cel, CD='cluster of differentiation', Ig=immuunglobuline.

Analyse PC compartiment in normaal beenmerg

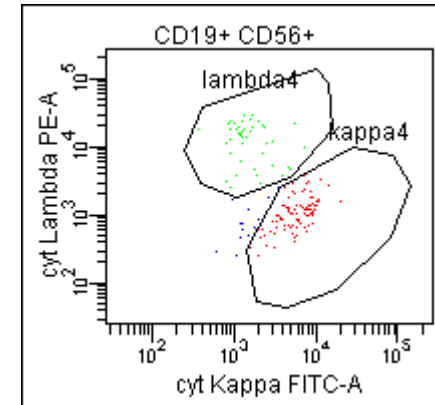
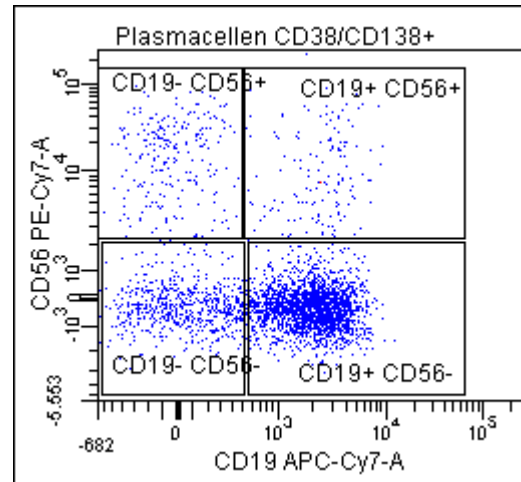


1 x 10⁶ cellen meten

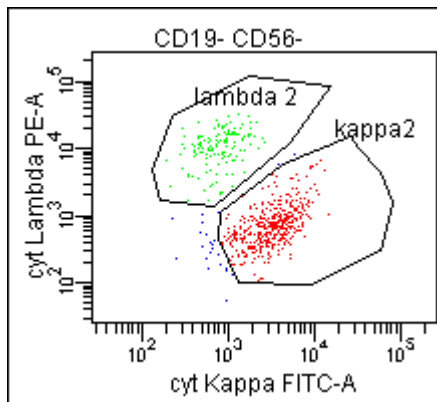
CD19/CD56 distributie op normale PC



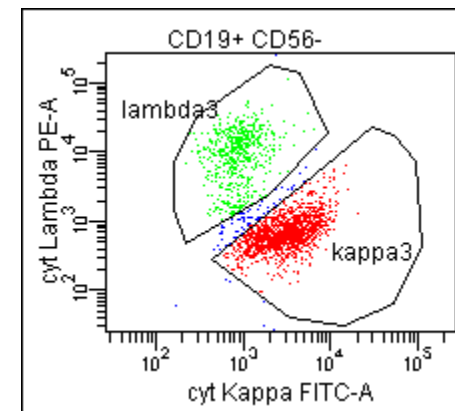
$\kappa/\lambda = 3,1$



$\kappa/\lambda = 2,1$

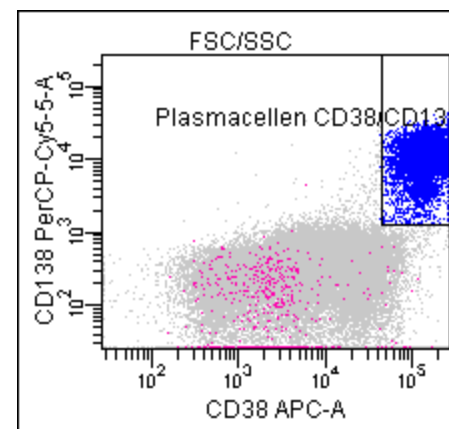
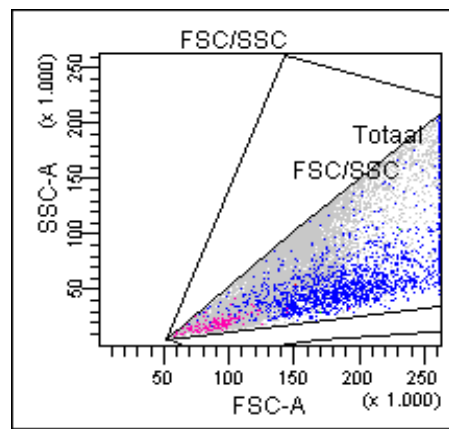
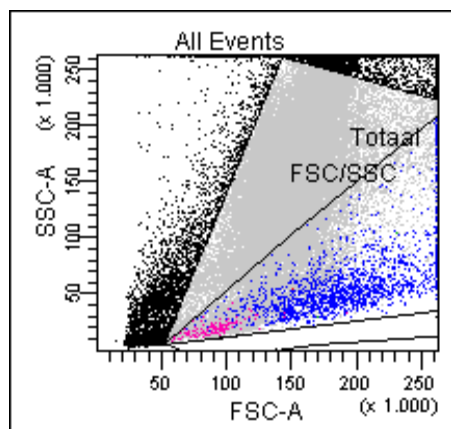


$\kappa/\lambda = 2,9$

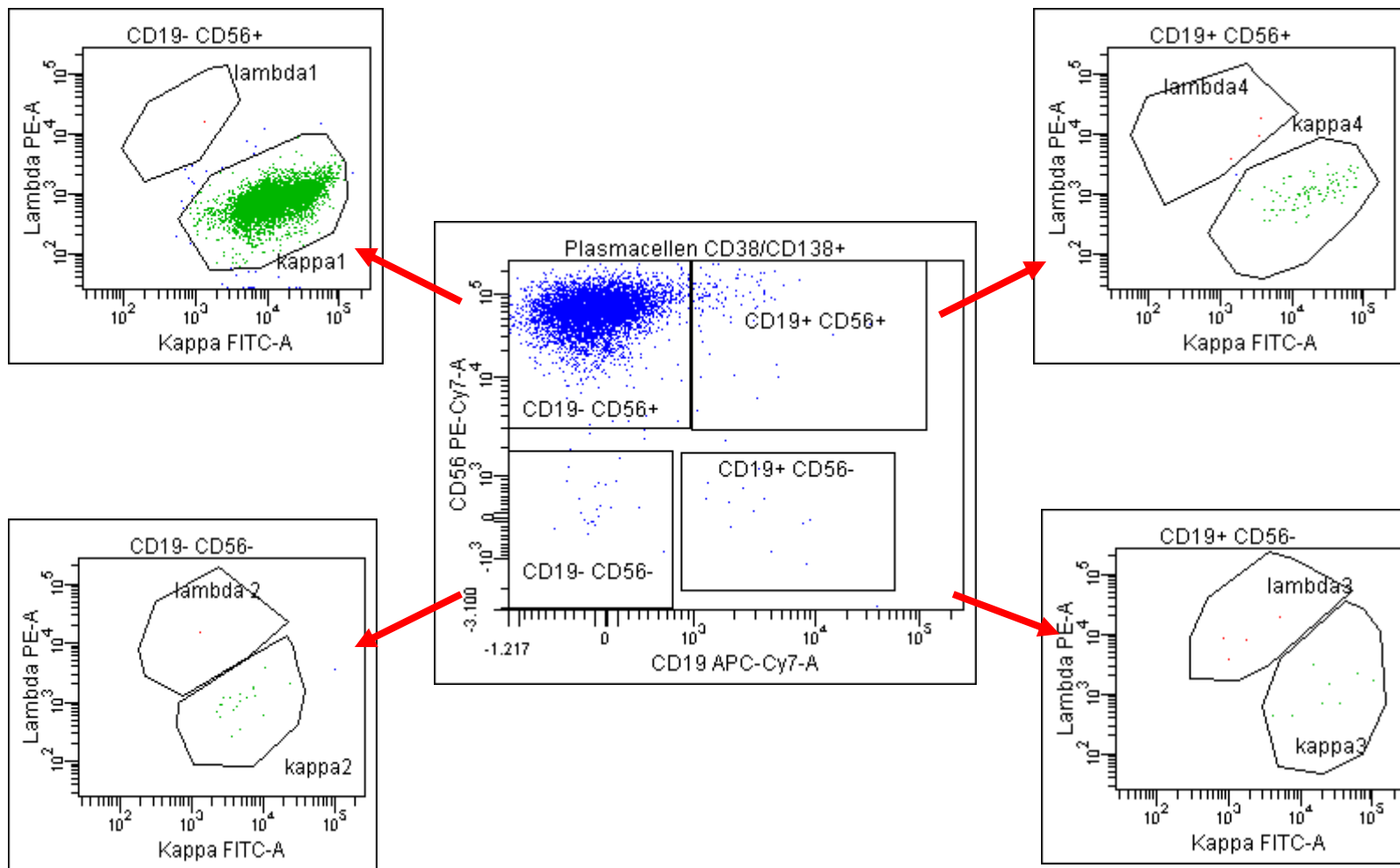


$\kappa/\lambda = 2,3$

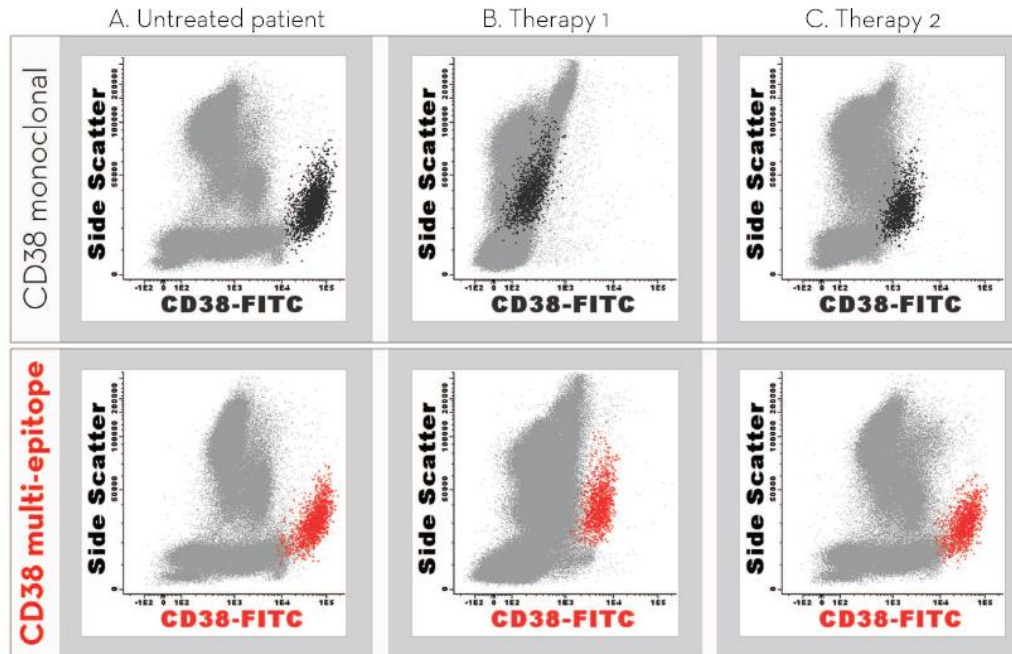
Voorbeeld myeloom



Conclusie: klonaal kapp^a+CD19⁻CD56⁺CD45⁻

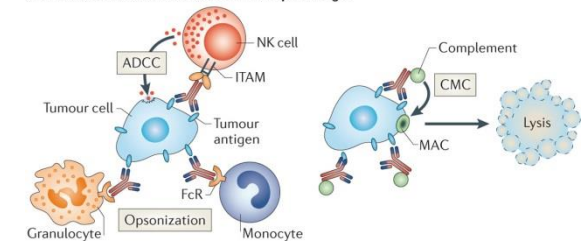


Consequenties voor flowcytometrie: afscherming (shielding)

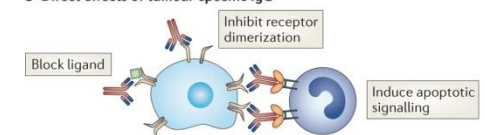


- Mechanismen
 - CDC
 - ADCC
 - Signaling mediated Apoptosis
 - Non-apoptotic cell death via actin dependent lysosomal pathway (type II anti-CD20)
 - ADCC en CDC (anti-CD20) indirectly contributes tot tumor destruction by cross-priming antigen-presenting cells (APC). Tumor peptides are presented telefonisch overleg CD8 and CD4 T cellen; CTL and Ab
 - Antilichaam gemedieerde fagocytosis
 - Ligand blockade (EGFR blocks ligand binding and receptor dimerization)
- IgG1 (FcRI – III) ADCC en CDC
- IgG2 (FcRII) wel ADCC geen CDC
- IgG3 (FcRI - III) wel ADCC en CDC
- IgG4 (FcRI) geen ADCC, geen CDC

a Immune-mediated effects of tumour-specific IgG



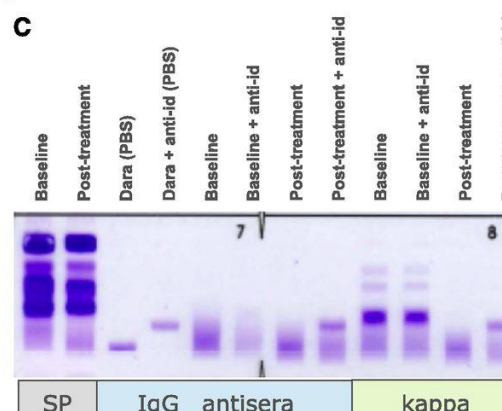
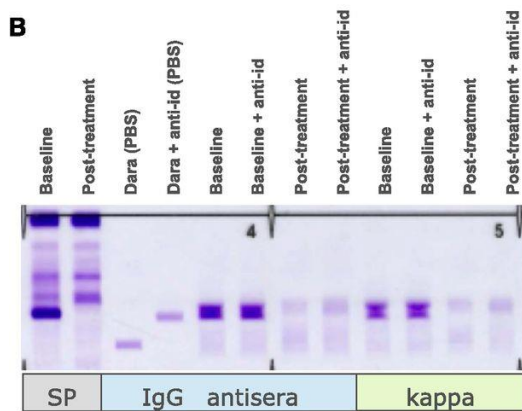
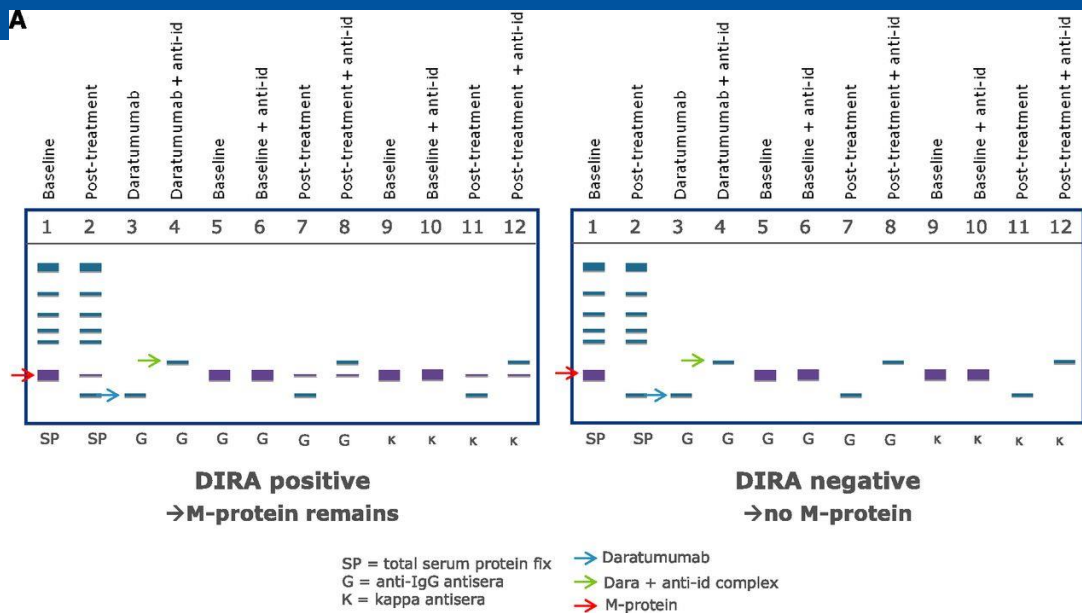
b Direct effects of tumour-specific IgG





- Consequentie antistof therapie voor flowcytometrische diagnostiek

Management of interference of daratumumab with SPEP and IFE assays by using DIRA. (A) Schematic representation of DIRA for a DIRA-positive (left) and DIRA-negative case (right).



Niels W. C. J. van de Donk et al. Blood 2016;127:681-695

Interferentie van therapeutische antistoffen met laboratorium assays

- Respons evaluatie
 - Plasmacel analyse met multiparamter flowcytometrie
 - M-proteïne analyse in serum
- Kruisproeven tbv bloedtransfusies

Tragocytosis als een mechanisme voor verlaagde CD38 expressie op plasmacellen



C

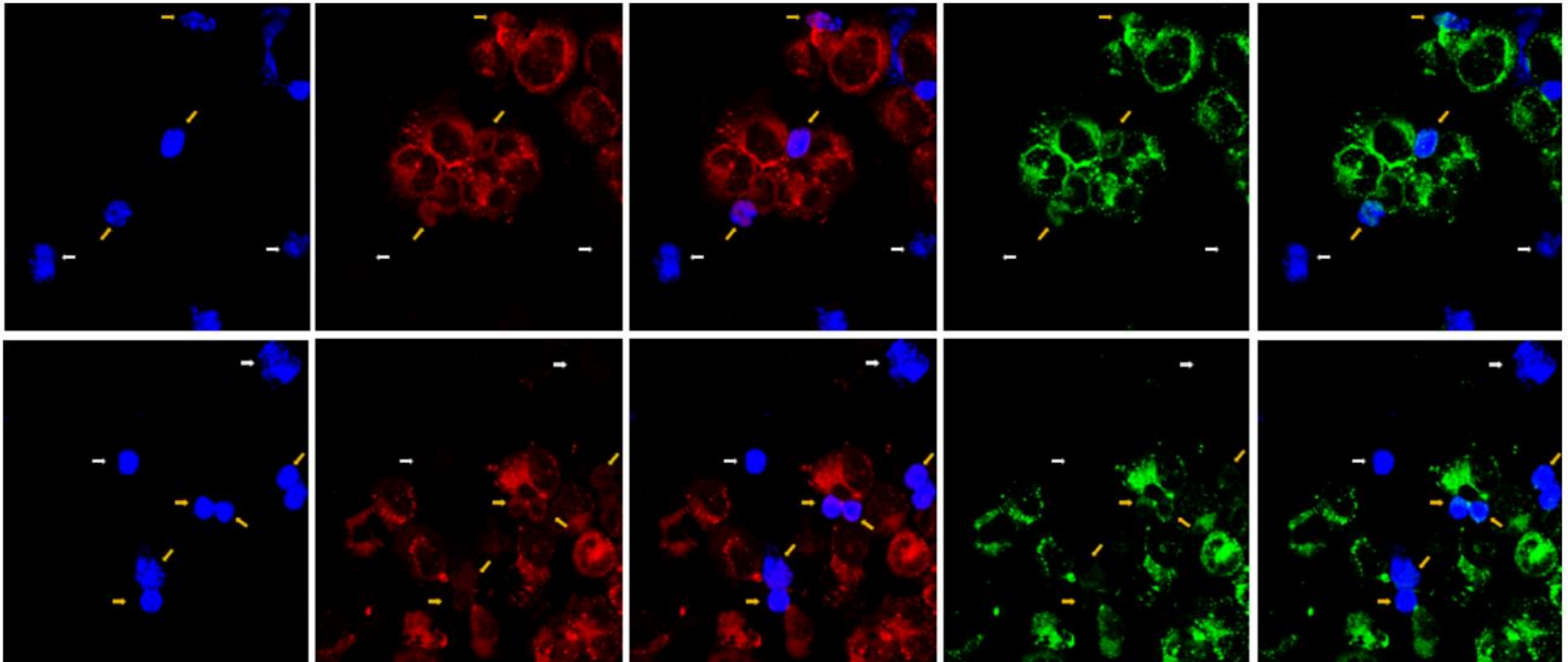
CellTrace Violet

PKH 26

PKH 26
+ CellTrace Violet
merged

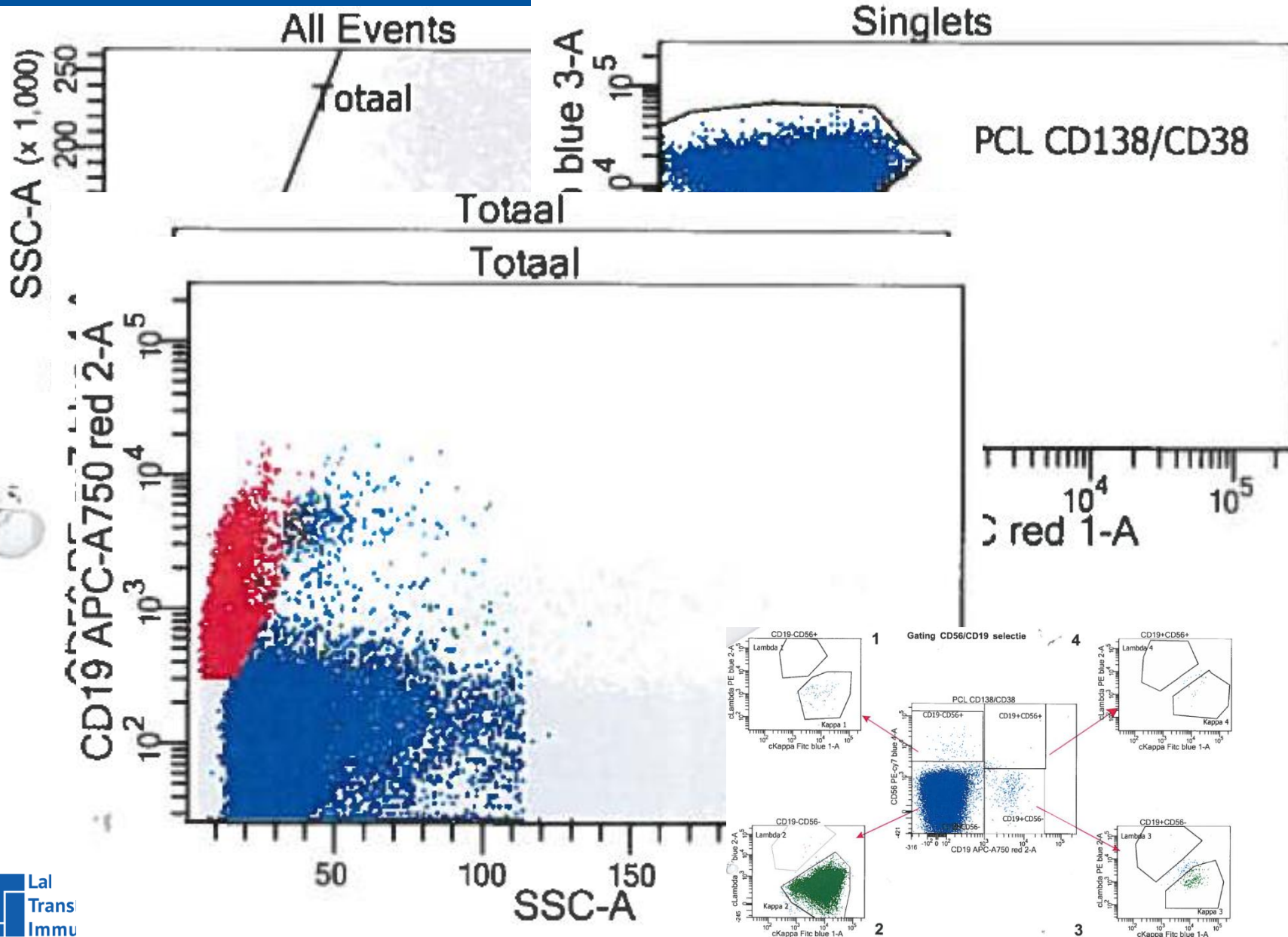
dara - AF488

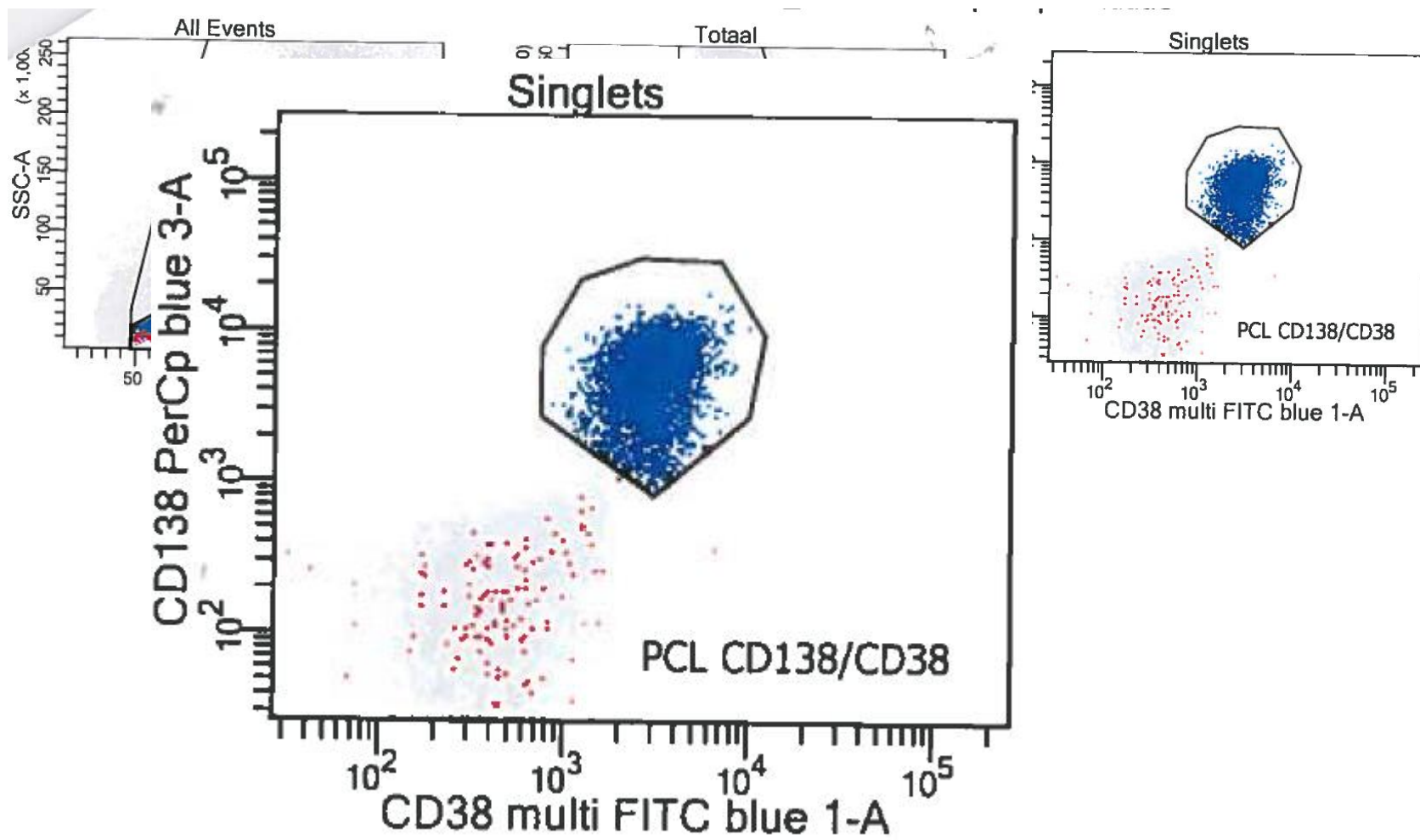
dara - AF488
+ CellTrace Violet
merged

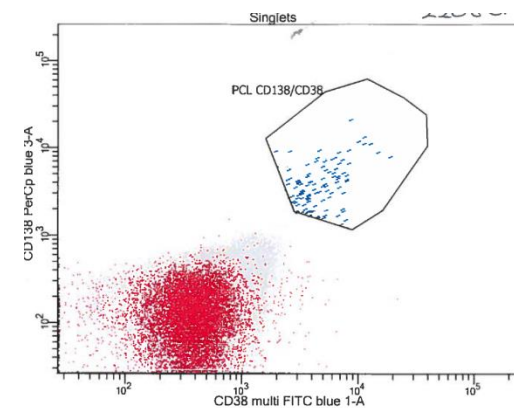
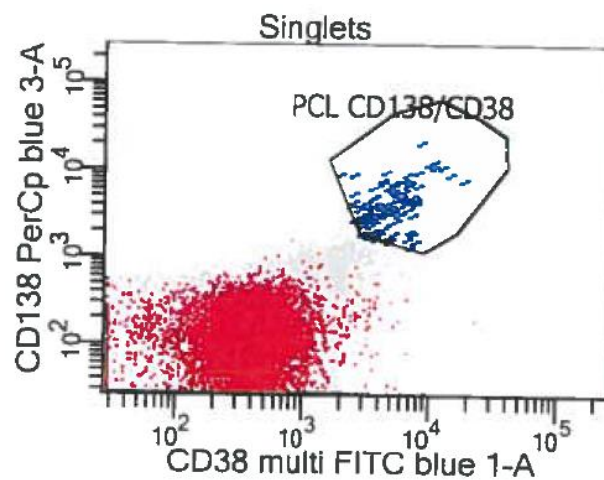
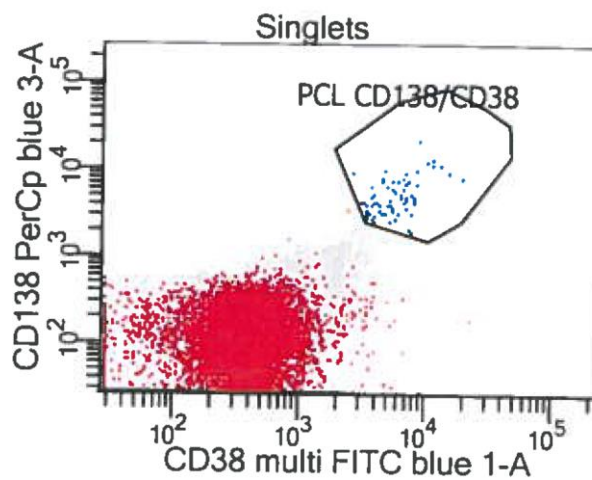
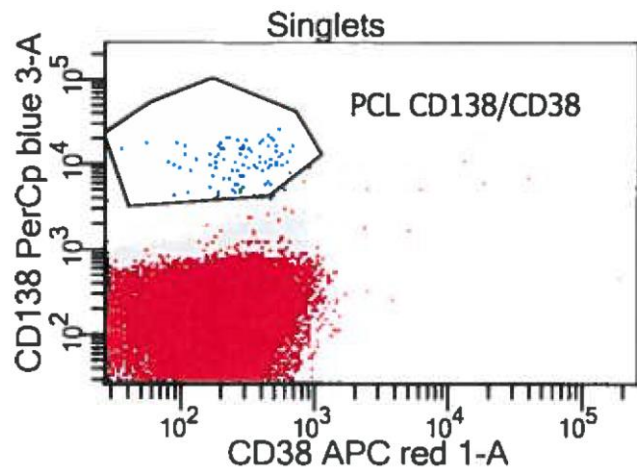


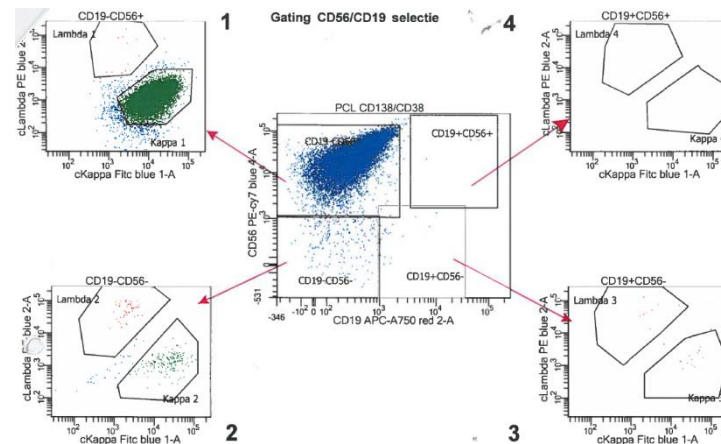
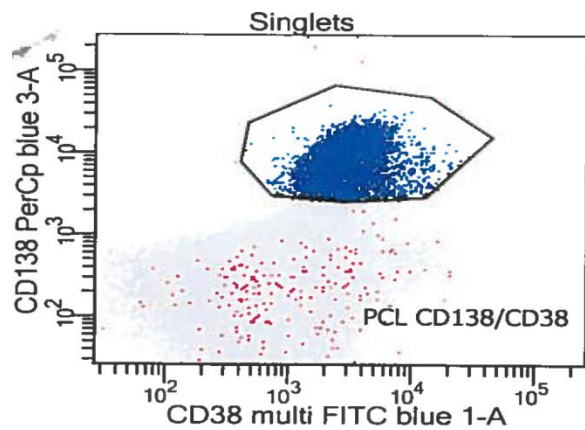
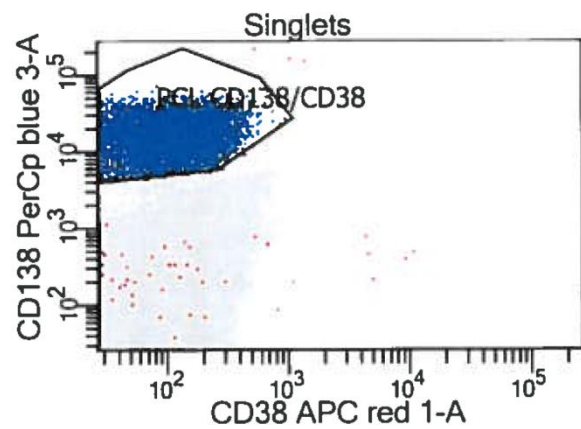
→ monocytes with uptake

→ monocytes without uptake





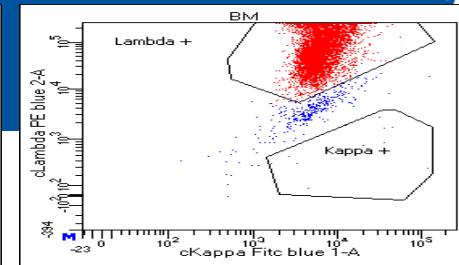
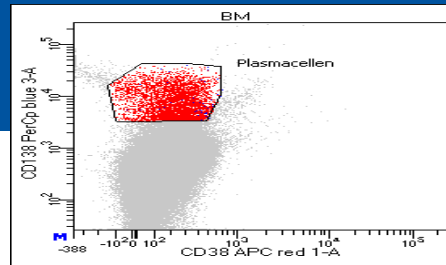




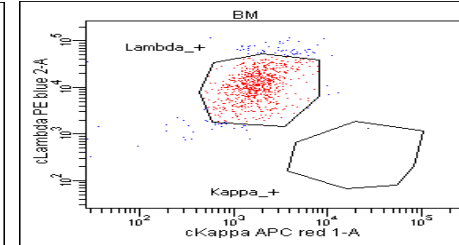
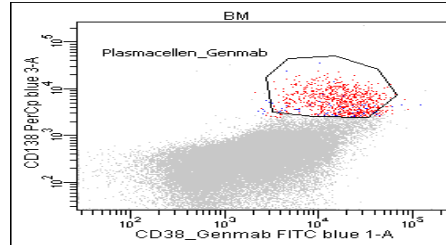
Daratumumab



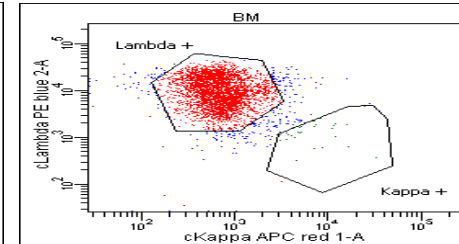
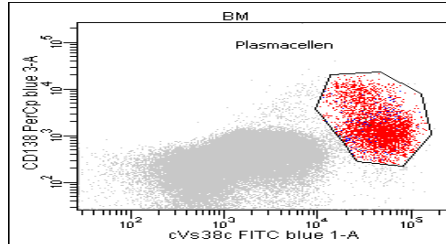
Standard protocol



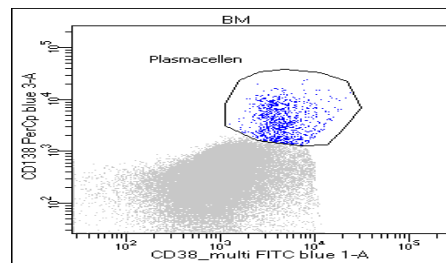
Genmab protocol



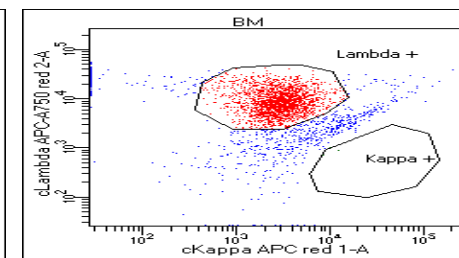
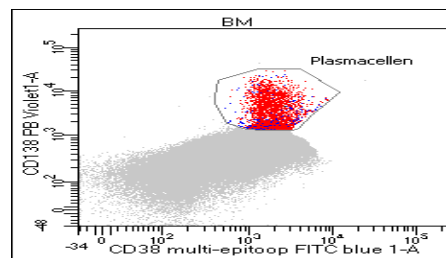
Vs38c protocol



CD38 multi-epitope



Cytognos MM MRD



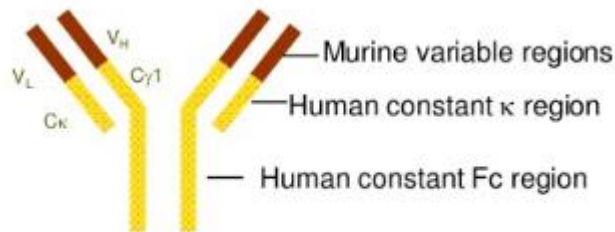


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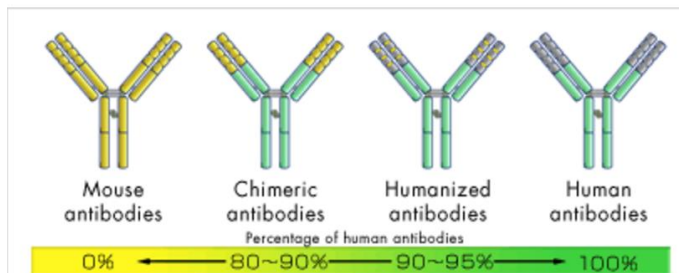
Antistof therapie



- Januari 2017: 68 door FDA goedgekeurde therapeutische monoklonale antistoffen
- Targets
 - membraangebonden moleculen, cytokinen, cytokine receptoren etc
- Muronomab-CD3: OKT3 (muis) in 1986
- Rituximab: CD20 (chimeer IgG1)

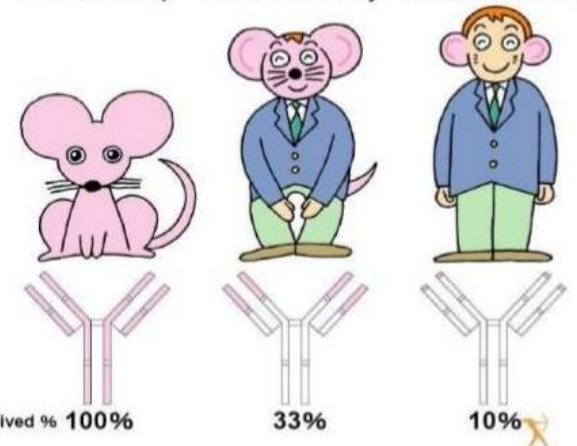


- Variable regions from murine anti-CD20 antibody IDEC-2B8^{1,2}
- Linked to human IgG1 and kappa constant regions¹



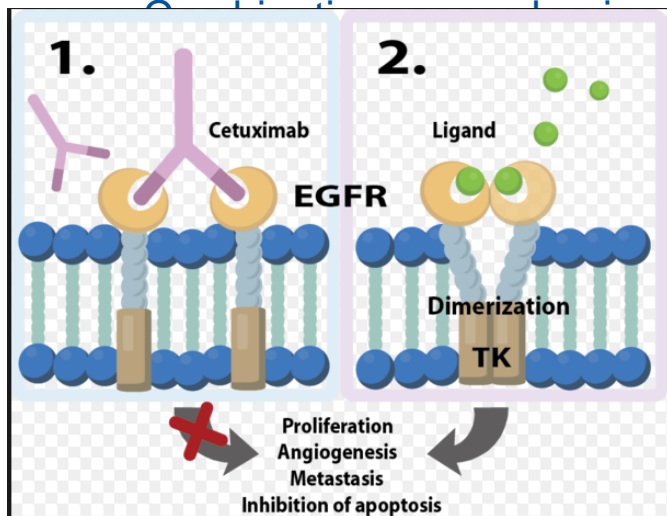
Differences in antibodies

Murine antibody Chimeric antibody Humanized antibody

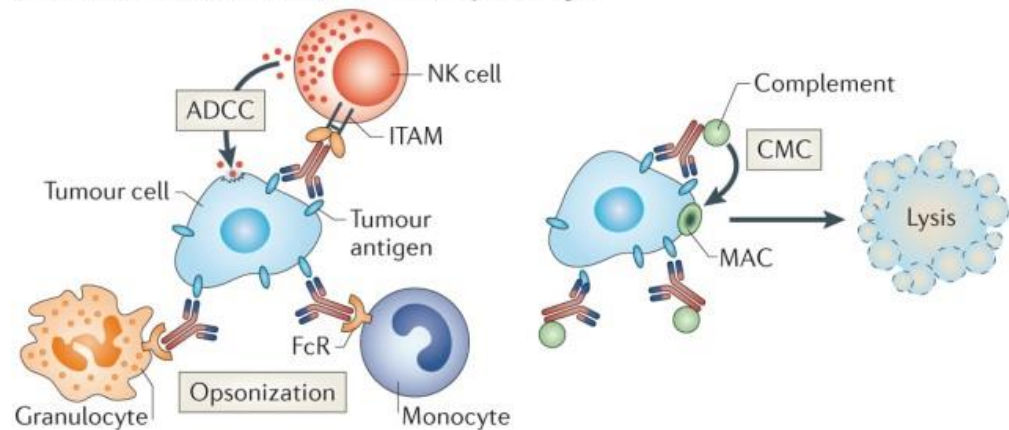


werkingsmechanismes

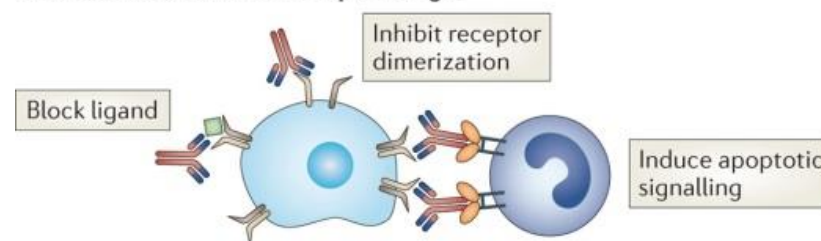
- Antigeen bindende deel + Fc deel (effector functie)
 - Fc deel: half waarde tijd, functie
- Inhibitie van binding van een signaalstof aan zijn receptor (epidermale groei factor receptor. Remming signalering
- Inductie apoptose
- CDC
- ADCC
- ADPh



a Immune-mediated effects of tumour-specific IgG



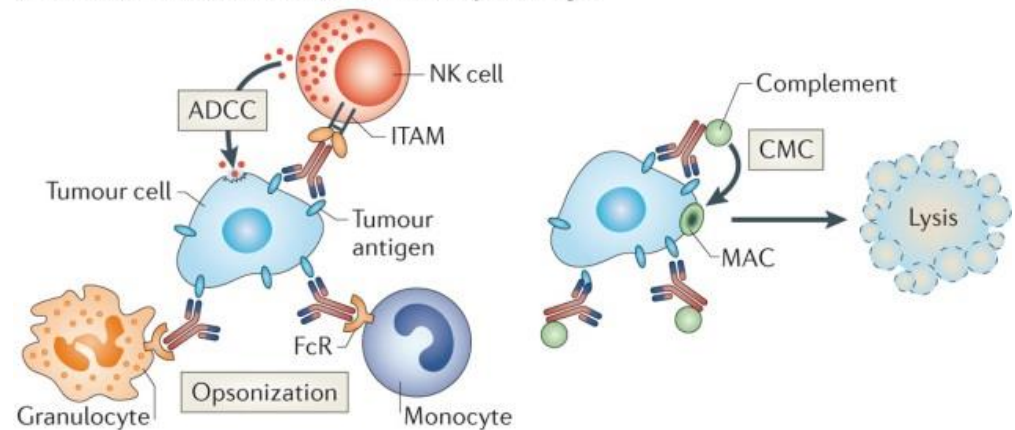
b Direct effects of tumour-specific IgG



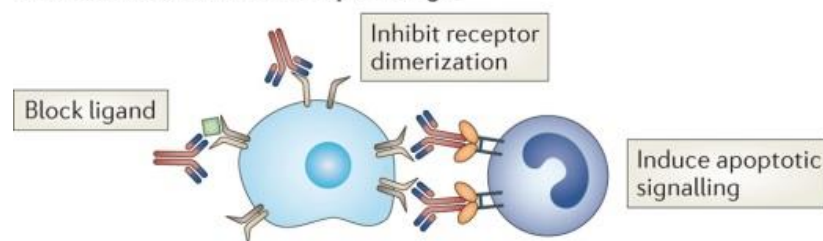
werkingsmechanismes

- Antigeen bindende deel + Fc deel (effector functie)
 - Fc deel: half waarde tijd, functie
- Inhibitie van binding van een signaalstof aan zijn receptor (epidermale groei factor receptor. Remming signalering
- Inductie apoptose
- CDC
- ADCC
- ADPh
- Combinatie van mechanismen

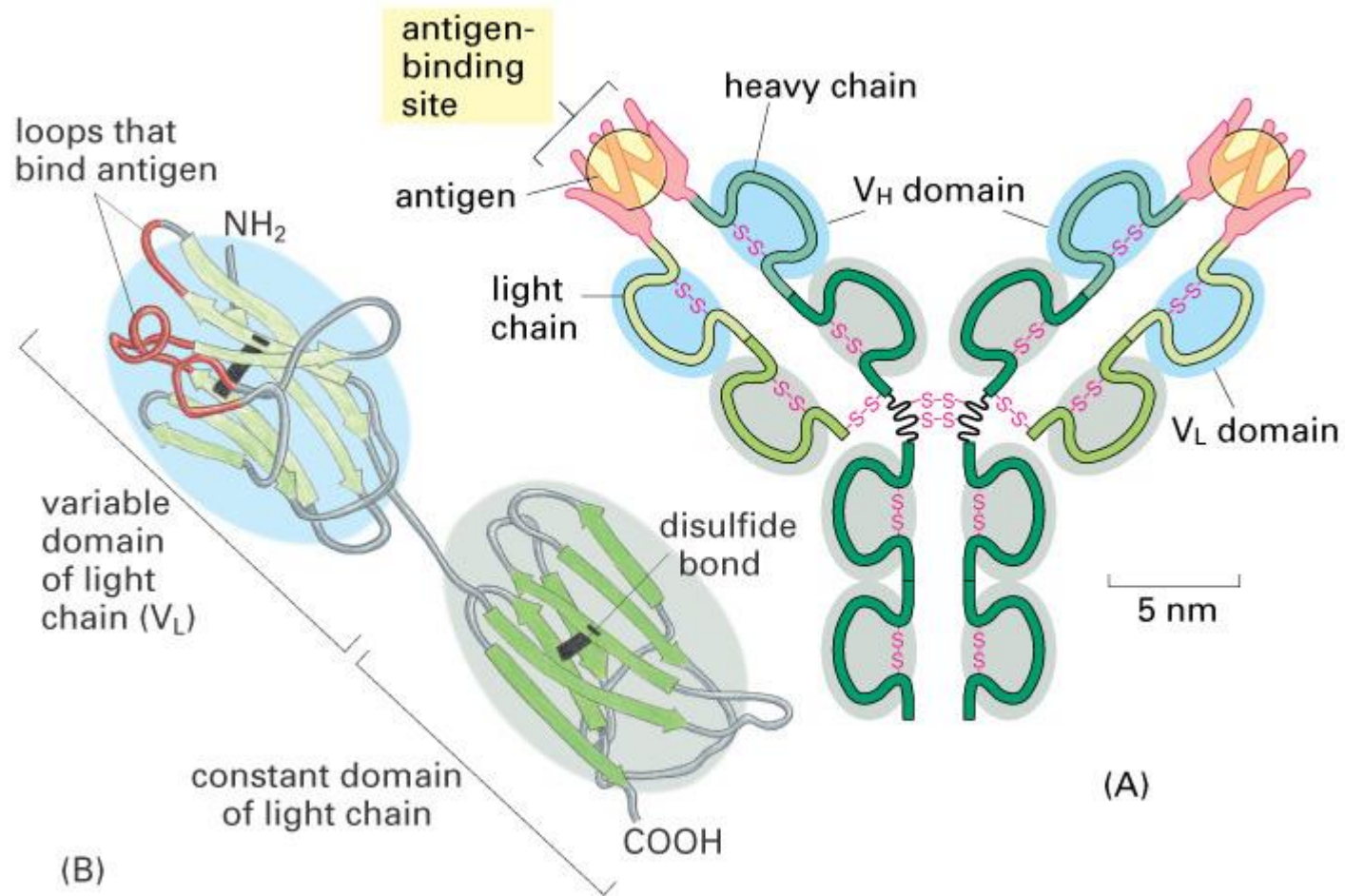
a Immune-mediated effects of tumour-specific IgG



b Direct effects of tumour-specific IgG



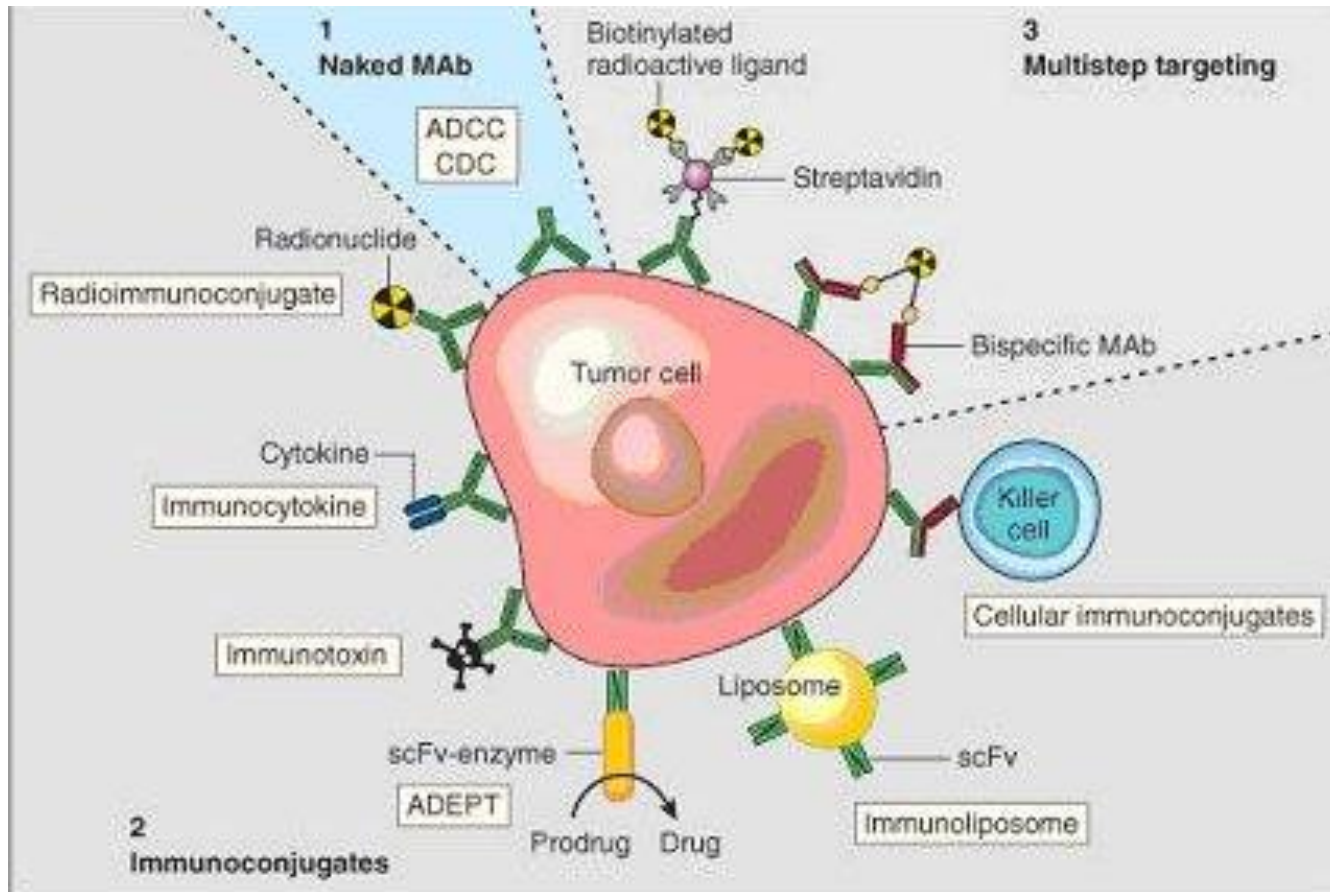
immunoglobulin



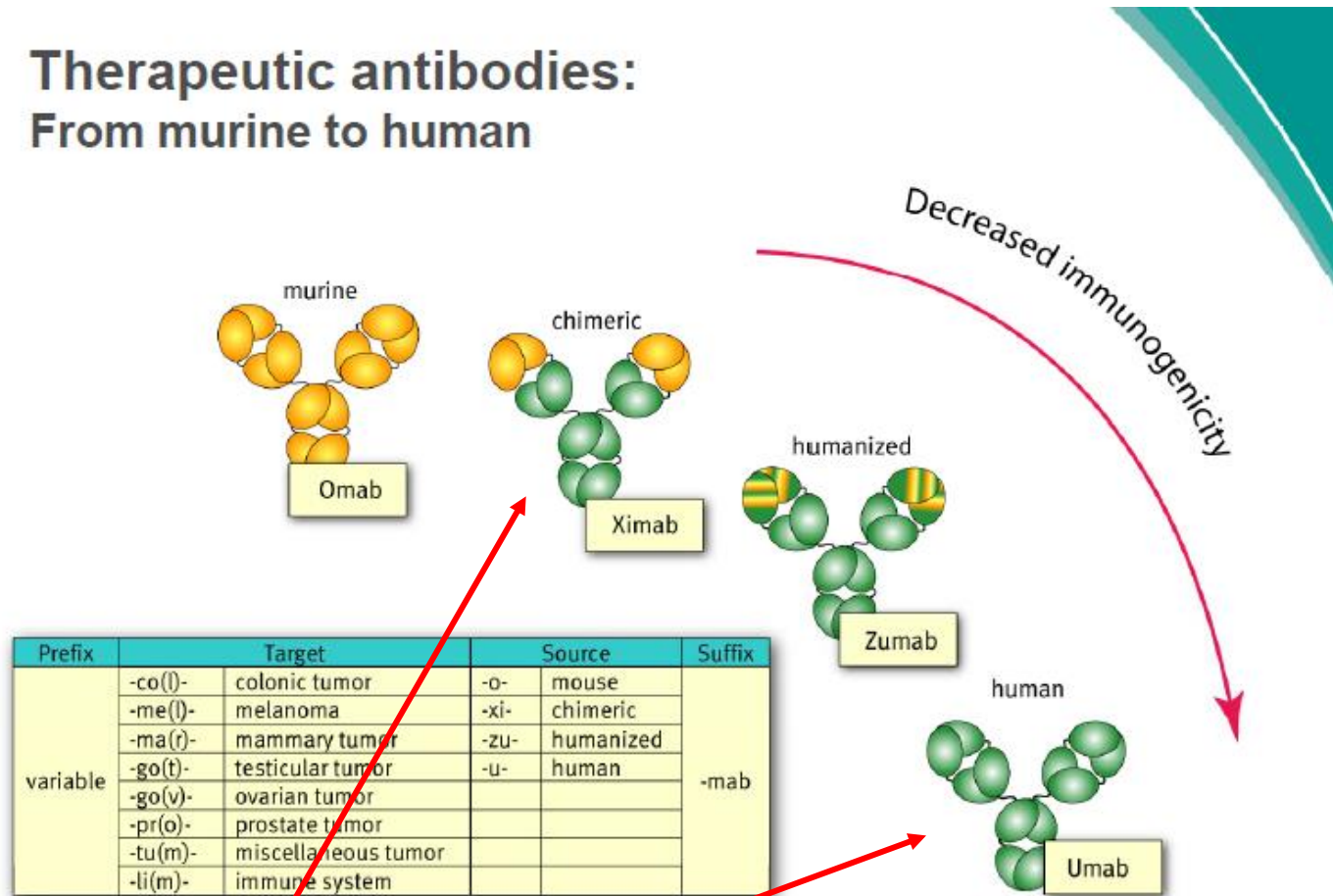


Nieuwe IMWG respons criteria

- Belang van flowcytometrie bij MM wordt belangrijker
- Serologie:
 - CR = afwezigheid van M-proteïne bepaald met immunofixatie en <5 % PC in het BM (morfologie/immunohistochemie)
 - sCR = als CR + normale sFLC ratio (IMWG vereist afwezigheid monoklonale PC in beenmerg (immunohistochemie))
- IR (immunophenotypic response) = geen monoklonale PC aantoonbaar in BM (10^{-4} – 10^{-5}) aangetoond met MFC



Therapeutic antibodies: From murine to human



Rituximab

Daratumumab