Personalized blood-based Mass Spectrometry methods introduce a new era in Multiple Myeloma diagnostics
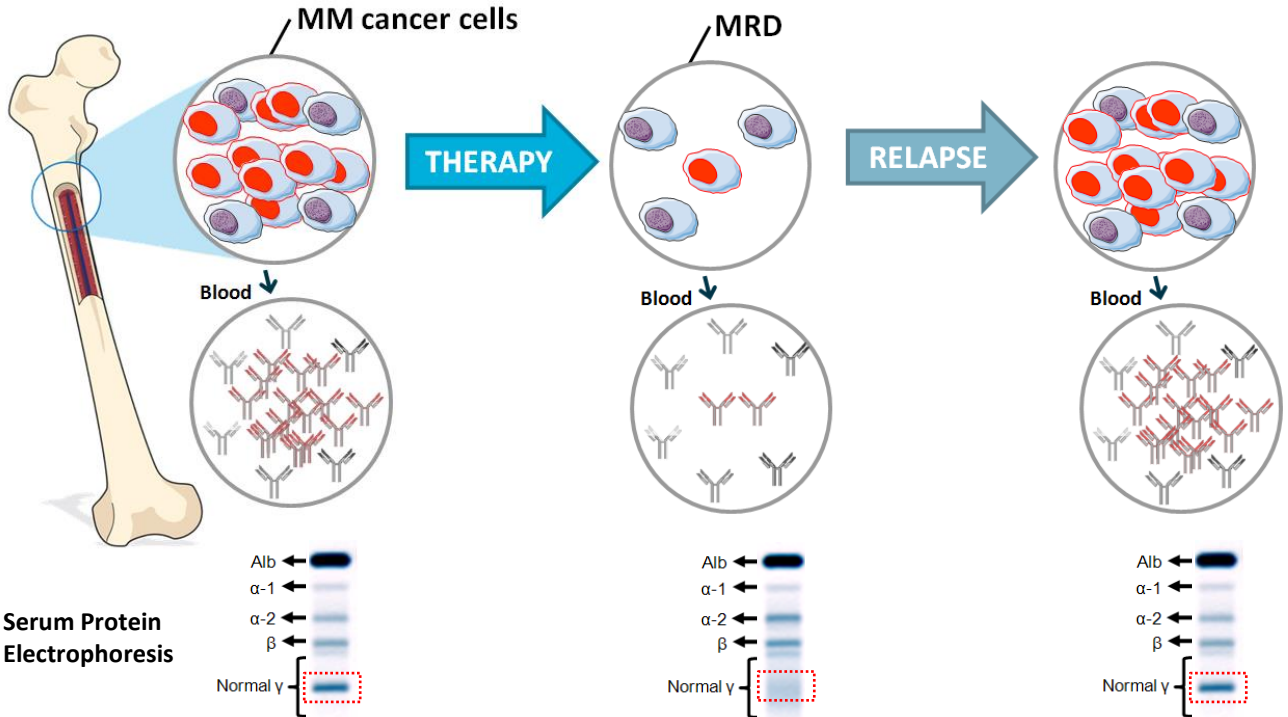
Pieter Langerhorst
X-omics festival
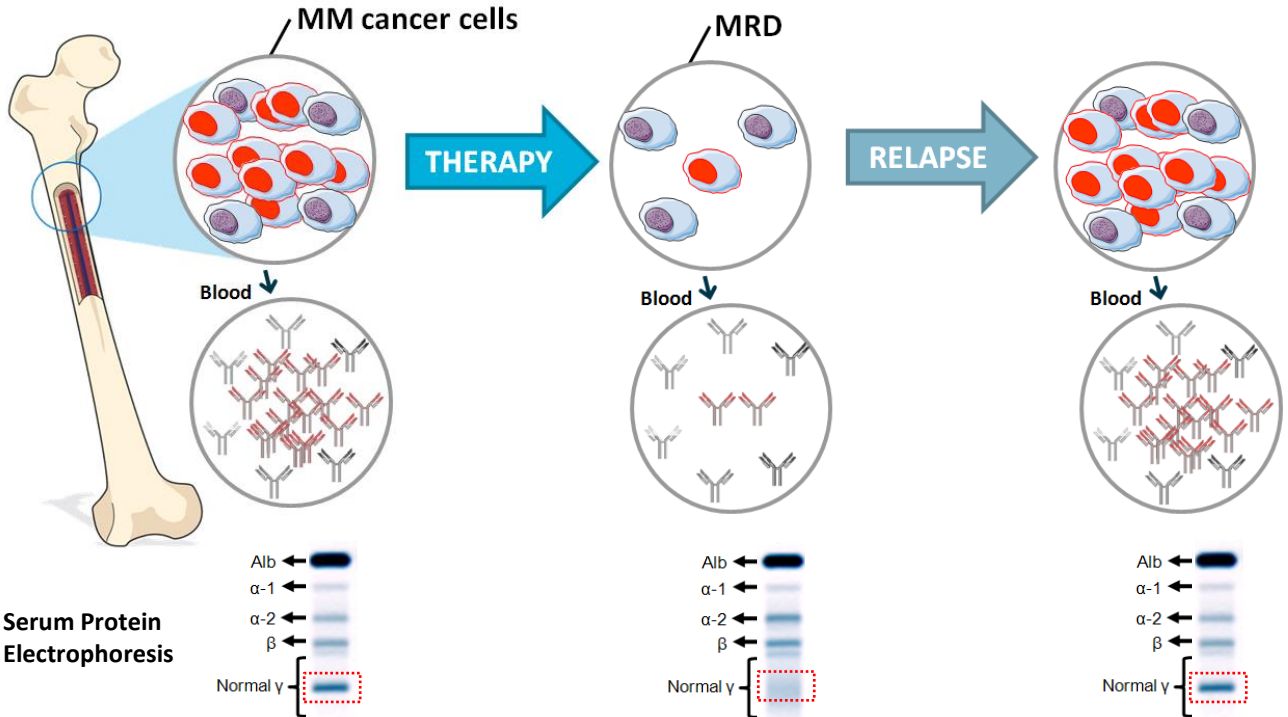**11-04-2022**

Radboud umc

Multiple Myeloma

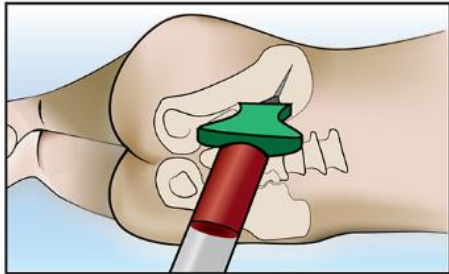- 2nd most common hematological malignancy

Multiple Myeloma

- 2nd most common hematological malignancy

50 % of newly diagnosed patients
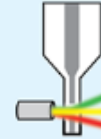
Bone marrow based MRD in MM

Myeloma cell based

NGS/aso-qPCR

V(D)J sequences

NGS-MRD
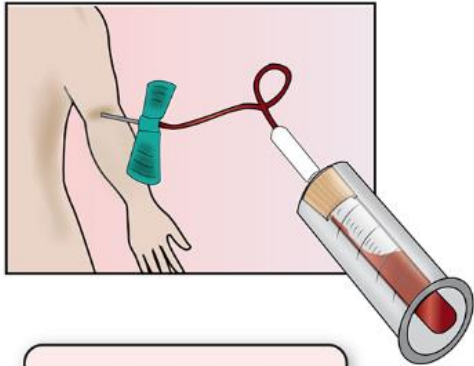
Flowcytometry

% Myeloma cells

MCF-MRD

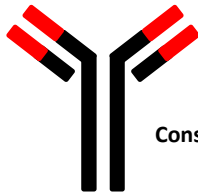**Good prognostic MRD biomarker**

Challenges
- Patchy disease
- Dry tap
- Repetitive sampling
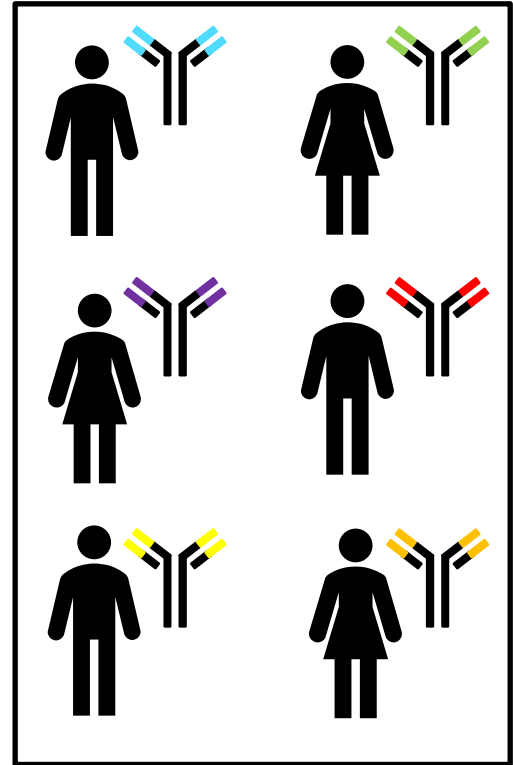
M-protein MRD in MM

M-protein based

Variable region
Patient & tumor specific

Constant region

MS-MRD approach

Qualitative assessment : MRD +/-
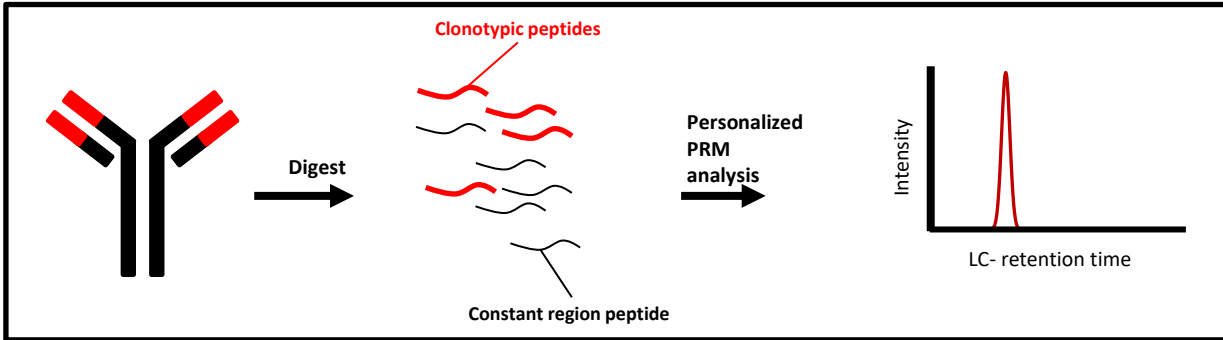
Quantitative assessment: [M-protein] g/L

Langerhorst, Zajec et al, Clin Chem, 2020
Langerhorst et al, Clin Chem 2021a

Radboud umc
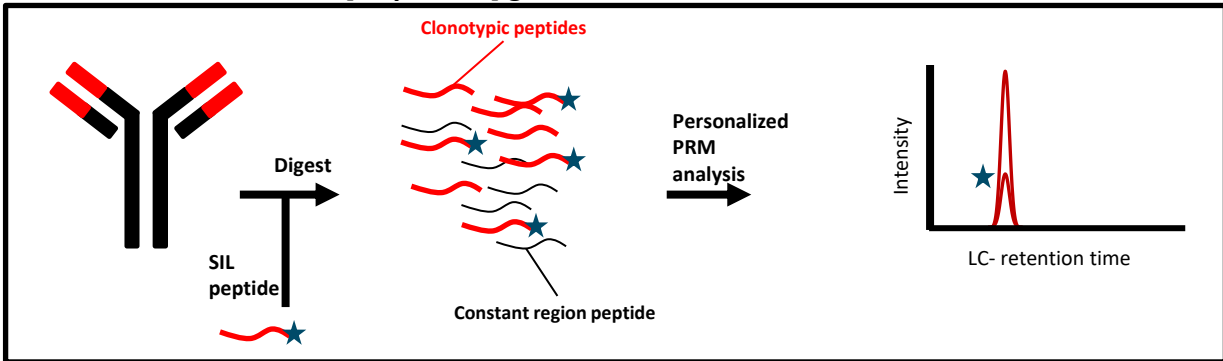
MS-MRD vs. NGS-MRD
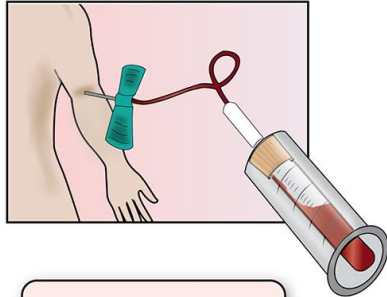
MS-MRD

NGS-MRD

VERSUS

M-protein based
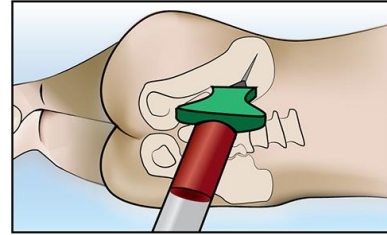
Myeloma cell based
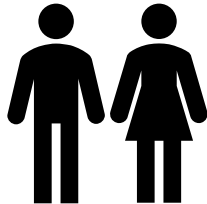
n=41

✓ Survival data
✓ NGS-MRD data
✓ Frozen sera for MS-MRD
✓ Sera available at 3 time points:
   Screening
   Pre-Maintenance therapy
   Post-Maintenance therapy

Langerhorst, Noori et al, Clin Chem 2021b

NGS-MRD vs. MS-MRD

- PFS comparison between NGS-MRD and MS-MRD during therapy

Langerhorst, Noori et al, Clin Chem 2021b

NGS-MRD vs. MS-MRD

- PFS comparison between NGS-MRD and MS-MRD during therapy

PFS NGS-MRD

Legend: NGS-MRD pos, NGS-MRD neg

NGS-MRD vs. MS-MRD

- PFS comparison between NGS-MRD and MS-MRD during therapy

PFS NGS-MRD versus MS-MRD

Langerhorst, Noori et al, Clin Chem 2021b

Radboud umc

NGS-MRD vs. MS-MRD

- PFS comparison between NGS-MRD and MS-MRD during therapy

PFS NGS-MRD versus MS-MRD
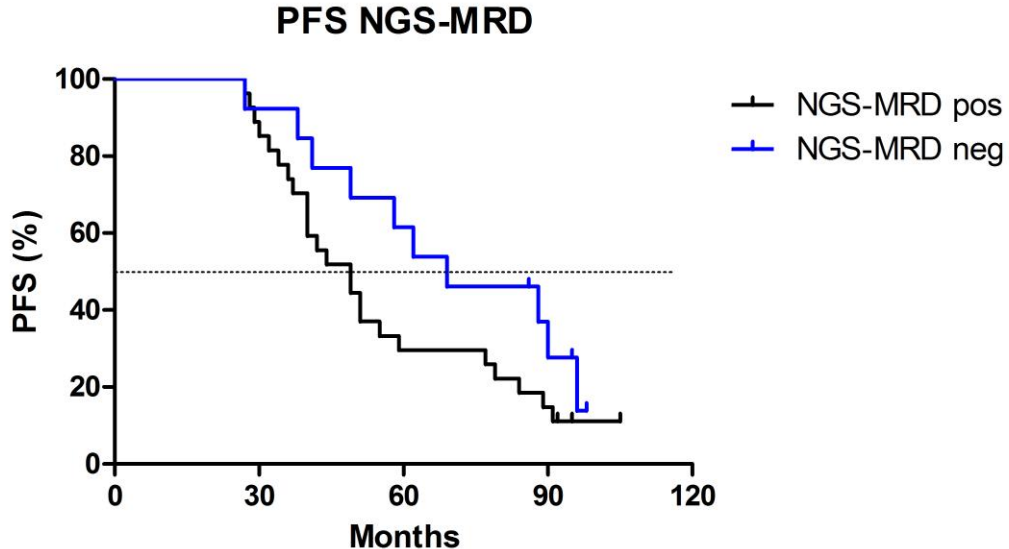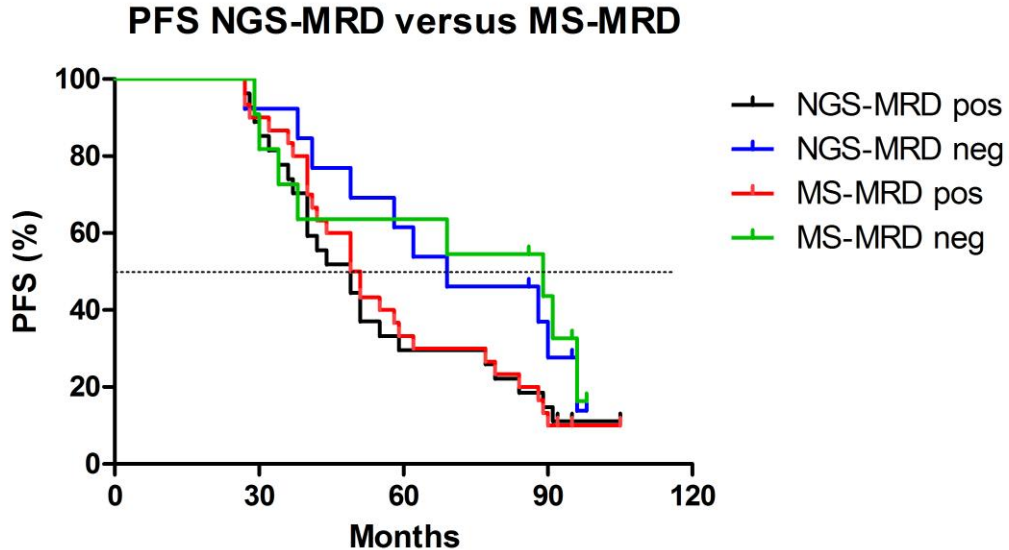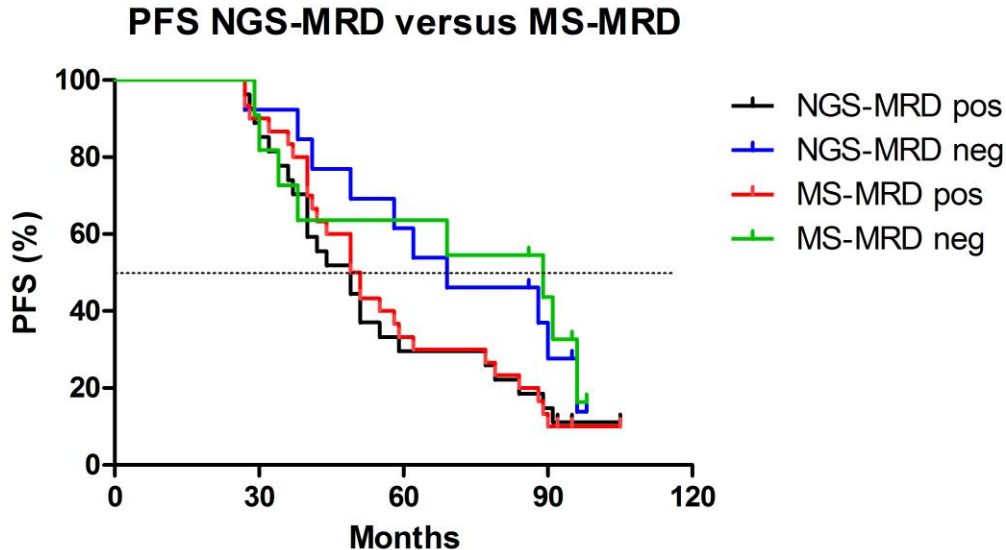
NGS-MRD in bone marrow and MS-MRD in blood
have similar prognostic value

Langerhorst, Noori et al, Clin Chem 2021b

Radboudumc

MS-MRD for dynamic monitoring

- Quantitative MS-MRD to monitor disease progression
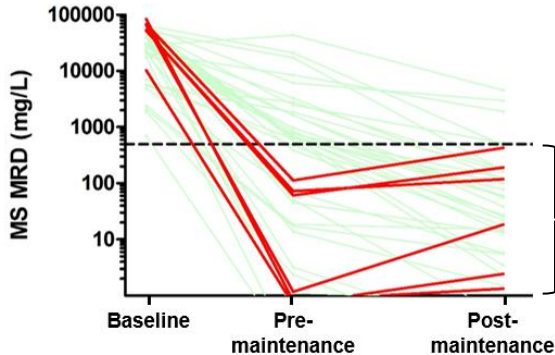
Red lines: (early) relapse detected
during maintenance therapy

- Early relapse detected in 6 patients
- Strongly correlated with poor-prognosis
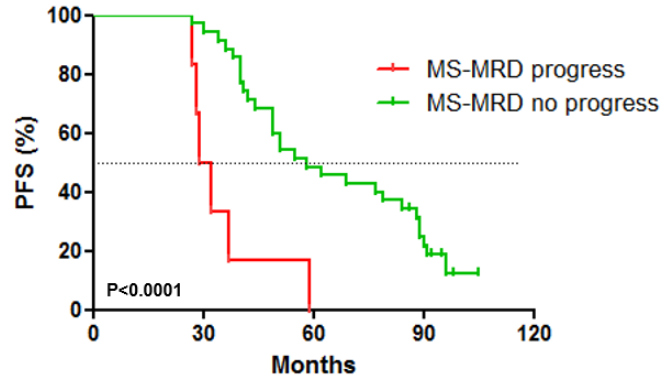
Langerhorst, Noori et al, Clin Chem 2021b

Radboud**umc**

MS-MRD for dynamic monitoring

MS-MRD for dynamic monitoring

MS-MRD for dynamic monitoring

Radboudumc
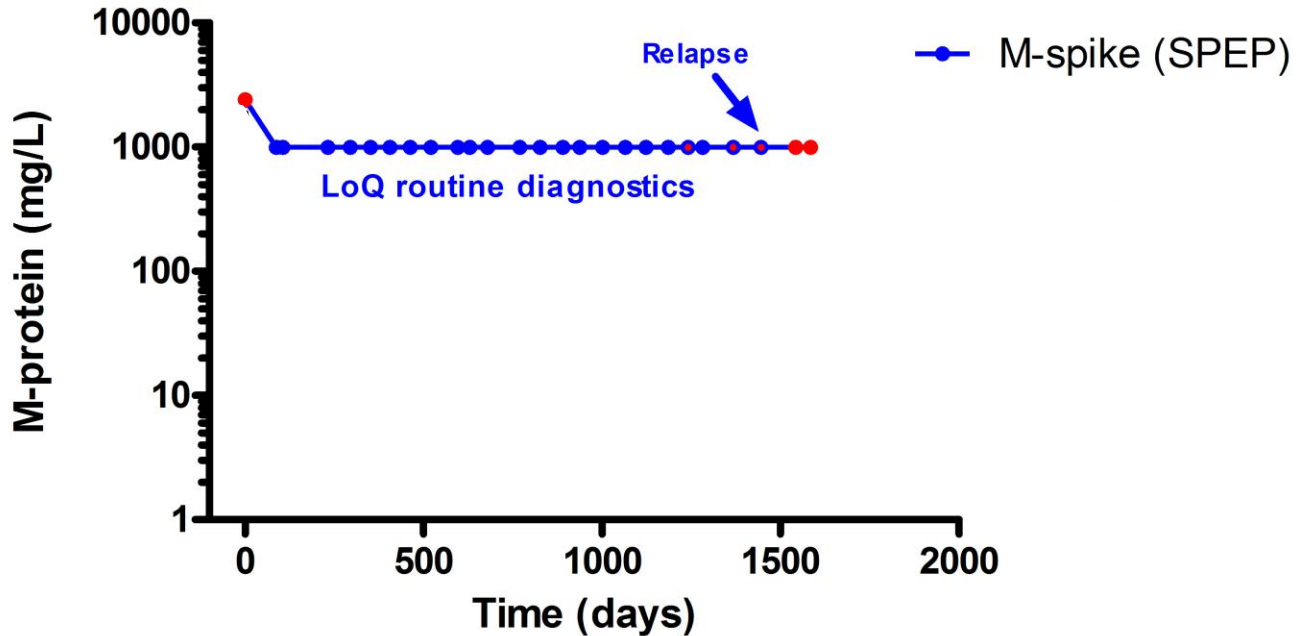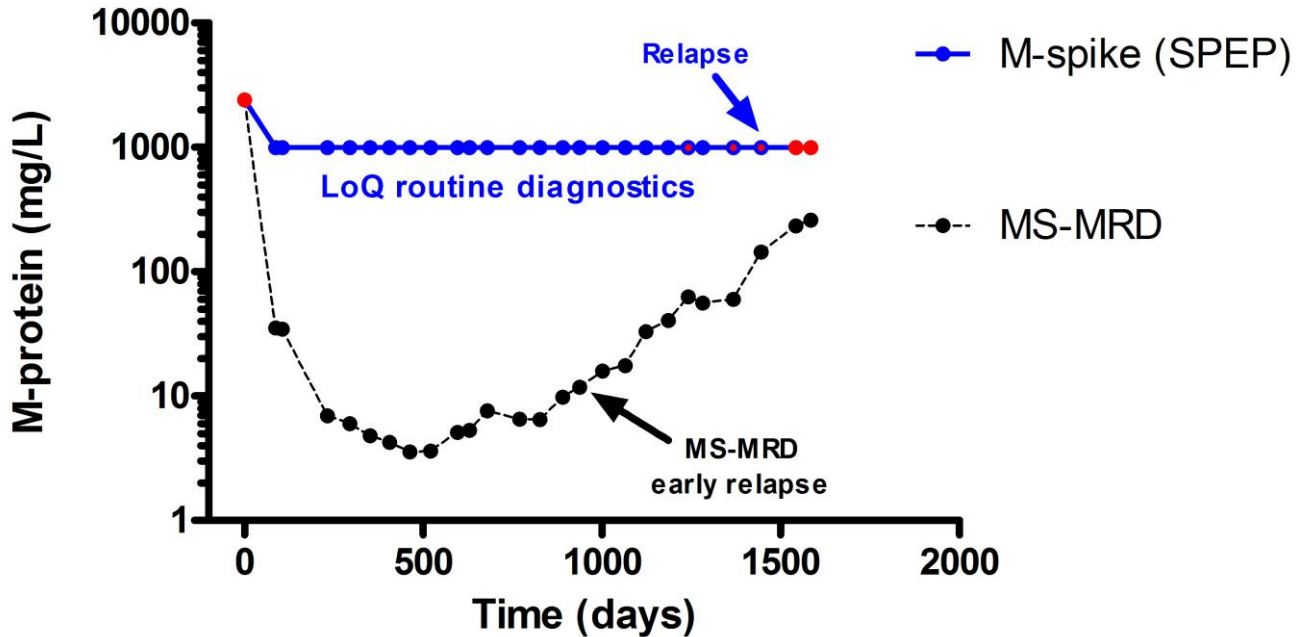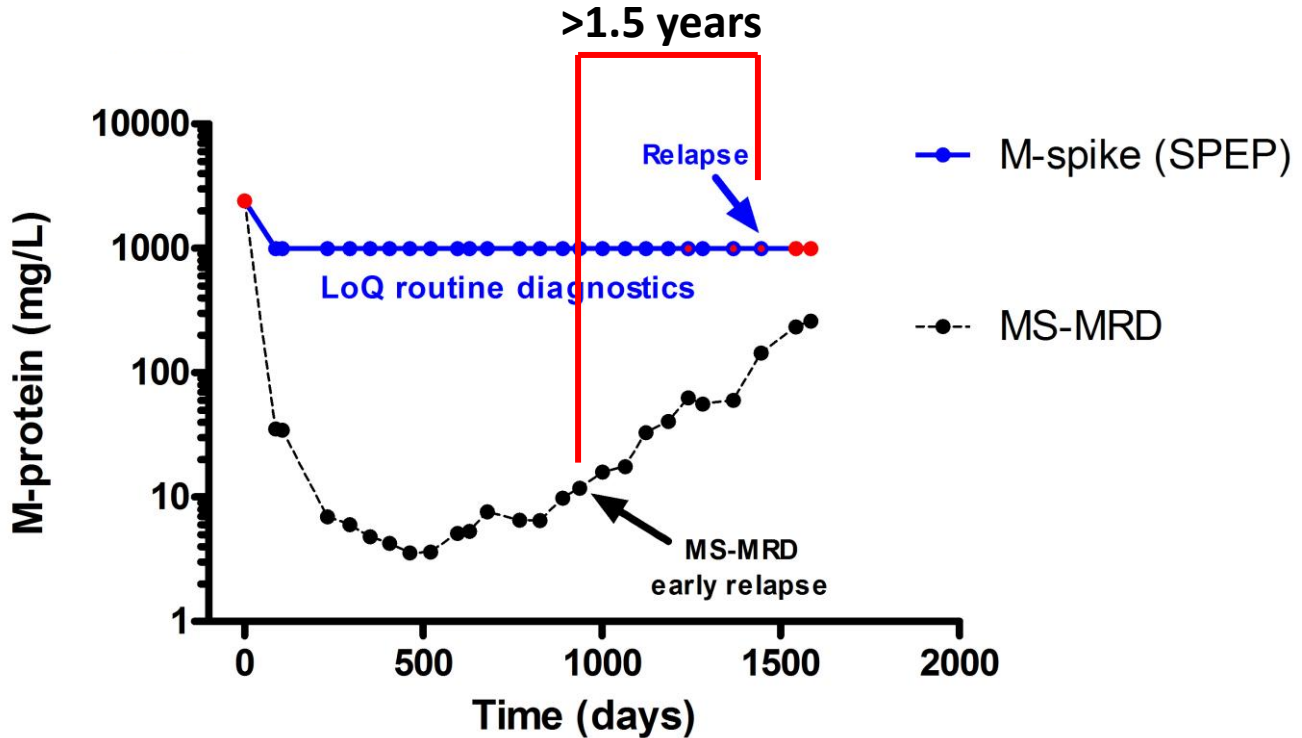
M-protein Fab N-glycosylation

N-linked glycosylation motif

X= any amino acid except proline

...ASQNISFYLTWYQQNPTK...
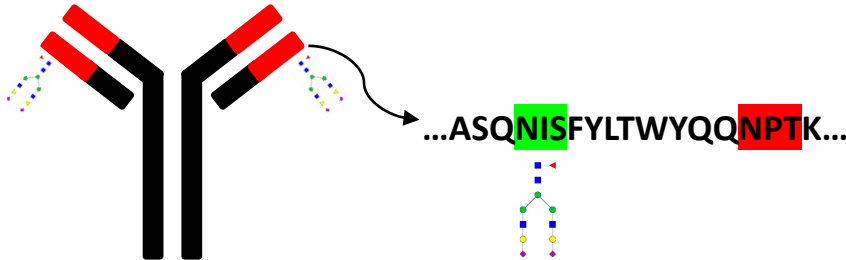
- Somatic hypermutations in the M-protein variable region can lead to *de novo* N-glycosylation sites
- Possibly clinically relevant

Organ damage, disease progression

- Extra layer of personalized biomarker

Langerhorst et al, *in prep*

Increase in M-protein Fab N-glyco sites

- Genomic screening for *de novo* Fab N-glycosylation sites

MM patients

THE MMRF COMMPASS STUDY℠
The largest genomics data set in all of cancer

1150 patients from **90** sites worldwide

Langerhorst et al, *in prep*

Radboudumc
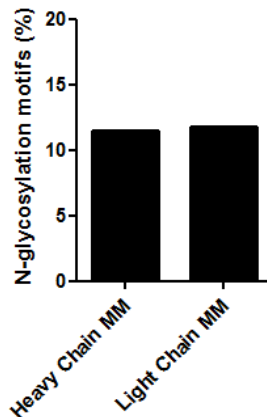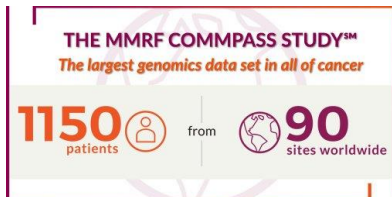
Increase in M-protein Fab N-glyco sites

- Genomic screening for *de novo* Fab N-glycosylation sites
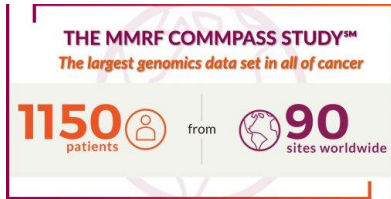
MM patients

Healthy individuals
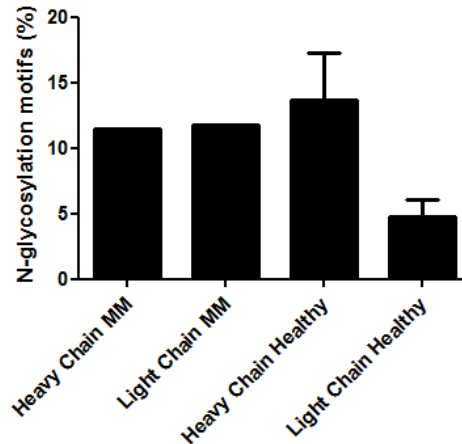
Increase in M-protein Fab N-glyco sites

- Genomic screening for *de novo* Fab N-glycosylation sites
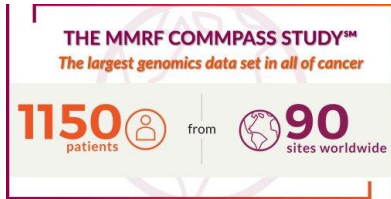
MM patients

Healthy individuals
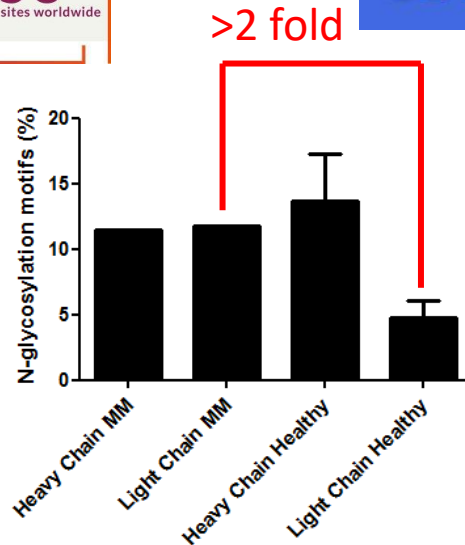
Glycoproteogenomics

Glycoproteomics
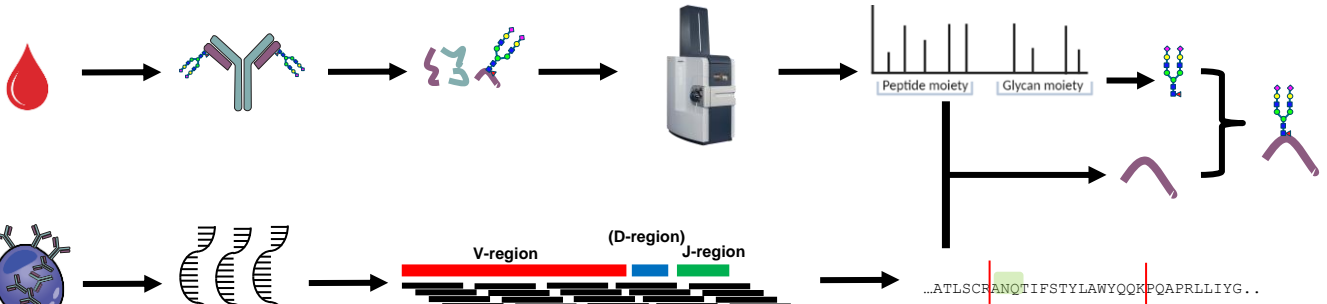
Genomics

Applied to a cohort of 41 MM patients
7/41 had a *de novo* N-glycosylation site in their M-protein

Radboud umc

Glycoproteogenomics

	V - region	J - region
Germline V3-20*01/J3*01	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP-FTFGPGTKVDIK	
Alterations Insertions	..L.....A...............N.TIF.T....................I....T.I.....A.........I...........F..H.NT..L...........	
PT1 - Kappa Light Chain	EIVLTQSPATLSLSPGERATLSCRA**NQT**IFSTYLAWYQQKPGQAPRLLIYGISSRAAGVPDRFSASGSGTDFTLIISRLEPEDFAVYFCQHY**NTS**PLFTFGPGTKVDIK	

N15

N128

Relative abundance (%)

50.0
40.0
30.0
20.0
10.0
0.0

Langerhorst et al, *in prep*

Radboud umc

Glycoproteogenomics

	V - region		J - region	Fc Region

Germline V3-20*01/J3*01 EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP-FTFGPGTKVDIK   KPREEQYNSTYRV

Alterations Insertions ..L....A..............N.TIF.T...................I....T.I.....A.........I...........F..H.NT..L...........   ....NST...

PT1 - Kappa Light Chain EIVLTQSPATLSLSPGERATLSCRANQTIFSTYLAWYQQKPGQAPRLLIYGISSRAAGVPDRFSASGSGTDFTLIISRLEPEDFAVYFCQHYNTSPLFTFGPGTKVDIK   KPREEQYNSTYRV

N15   N128   N297

Relative abundance (%)

50.0
40.0
30.0
20.0
10.0
0.0

Langerhorst et al, *in prep*

Radboud umc

Conclusions

Personalized MS methods in blood introduce a new era in M-protein diagnostics

MS-MRD in blood
- Personalized diagnostic to monitor MRD In the blood of patients with MM
- Similar prognostic value as NGS-MRD in bone marrow
- Enables dynamic monitoring for early relapse detection

Glycoproteogenomics
- Increased de novo N-glycosylation in M-proteins of patients with MM
- Enables detailed glycoprofiling of de novo glycosites
- Opens possibilities to study clinical value/pathogenicity of M-protein fab glycosylation
- Possible integration with diagnostic workflows for also other diseases
WES/WGS/RNA-seq → Glycosite-ID → Glycoproteomics

Acknoweledgements

Radboudumc

Dr. Sandra Croockewit (Hematology)
Dr. Jolein Gloerich (Laboratory Medicine)
Prof. Alain van Gool (Laboratory Medicine)
Dr. Patricia Groenen (Pathology)
Dr. Hans Jacobs (Laboratory Medicine)
Prof. Irma Joosten (Laboratory Medicine)
Jenneke Keizer-Garritsen (Laboratory Medicine)
Prof. Dirk Lefeber (Laboratory Medicine)
Merel Post (PhD student)
Dr. Blanca Scheijen (Pathology)
Dr. Hans Wessels (Laboratory Medicine)
Charissa Wijnands (PhD student)

Erasmus MC
Universitair Medisch Centrum Rotterdam
zafung

Dr. Annemiek Broijl (Hematology/HOVON)
Dr. Mark van Duin (Hematology)
Dr. Martijn van Duijn (Neurology)
Dr. Theo Luider (Neurology)
Somayya Noori (PhD student)
Dr. Henk Russcher (Clinical Chemistry)
Dr. Yolanda de Rijke (Clinical Chemistry)
Prof. Pieter Sonneveld (Hematology/HOVON)
Marina Zajec (PhD student)

Amsterdam UMC
University Medical Centers

Prof. Niels vd Donk (Hematology)
Kristine Frerichs (PhD student)
Christy Verkleij (PhD student)
Prof. Sonja Zweegman (Hematology)

ifm

Prof. Hervé Avet-Loiseau
Dr. Hélène Caillon
Dr. Jill Corre
Dr. Thomas Dejoie

VU

Dr. Melissa Bärenfänger

KWF

HOVON

Mrace

N*W*O
Netherlands Organisation
for Scientific Research

sebia

ifcc
International Federation
of Clinical Chemistry
and Laboratory Medicine

MP*e*
Myeloma
Patients
Europe

skml

EuroClonality

BRUKER

MULTIPLE MYELOMA
Research Foundation

NVKC

ThermoFisher
SCIENTIFIC

NL Health~Holland

ZonMw

Radboudumc