Skyline Dx

The Markers That Matter.
The Decision That Counts.

Prognostic And Predictive Signatures In Oncology: Bridging From Bench To Bedside

Martin van Vliet, MSc, PhD
EVP Bioinformatics and Product Development

4th International Systems Biomedicine Symposium
Oct 5, 2017

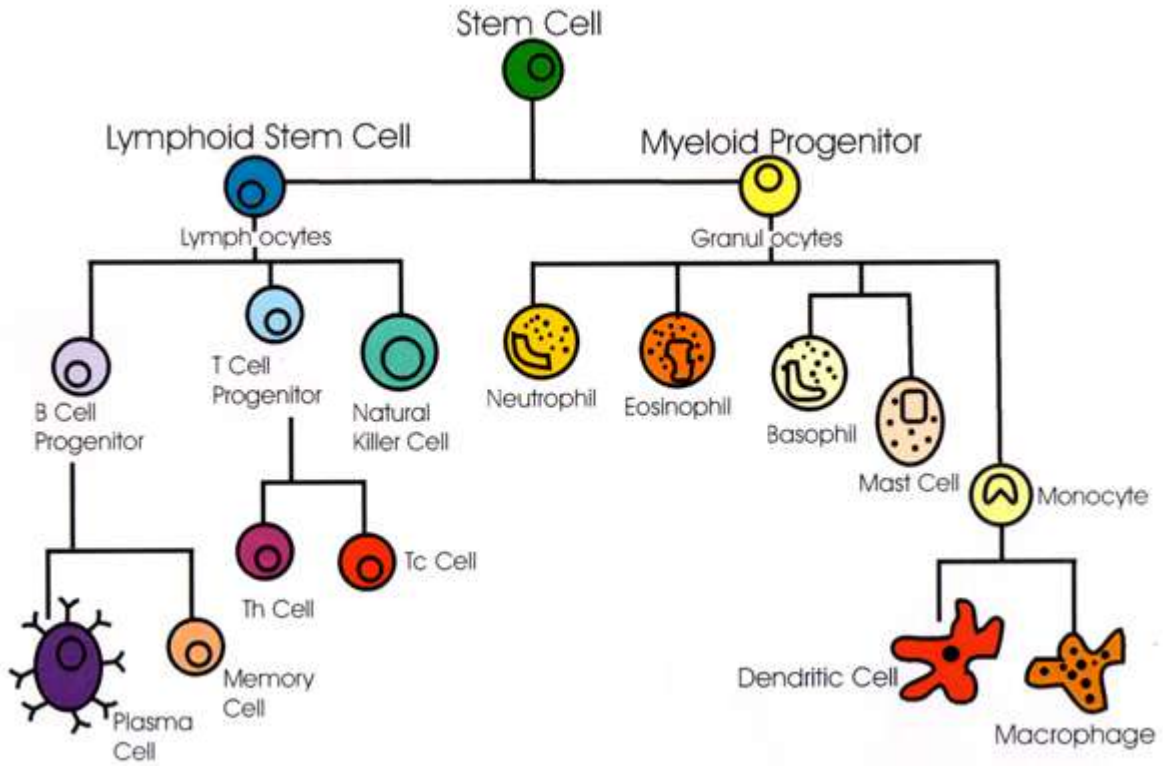
Multiple Myeloma Characteristics

- Second most common hematologic malignancy in the world
- 65% of patients older than 65
- Approximately 114,000 new cases occur annually[1]
- Characterized by a malignant proliferation of plasma cells
- Clinical features:
  - Hyper**C**alcemia
  - **R**enal dysfunction
  - **A**nemia
  - **B**one loss / fractures
  - Infections: neutropenia / hypogammaglobulinemia
  - Neurologic dysfunction

Despite improvement in outcomes, the disease is still incurable for most patients

1. Ferlay, et al. *International Journal of Cancer.* 2014.

Hematopoiesis (c.q. formation of immune cells)



Multiple Myeloma

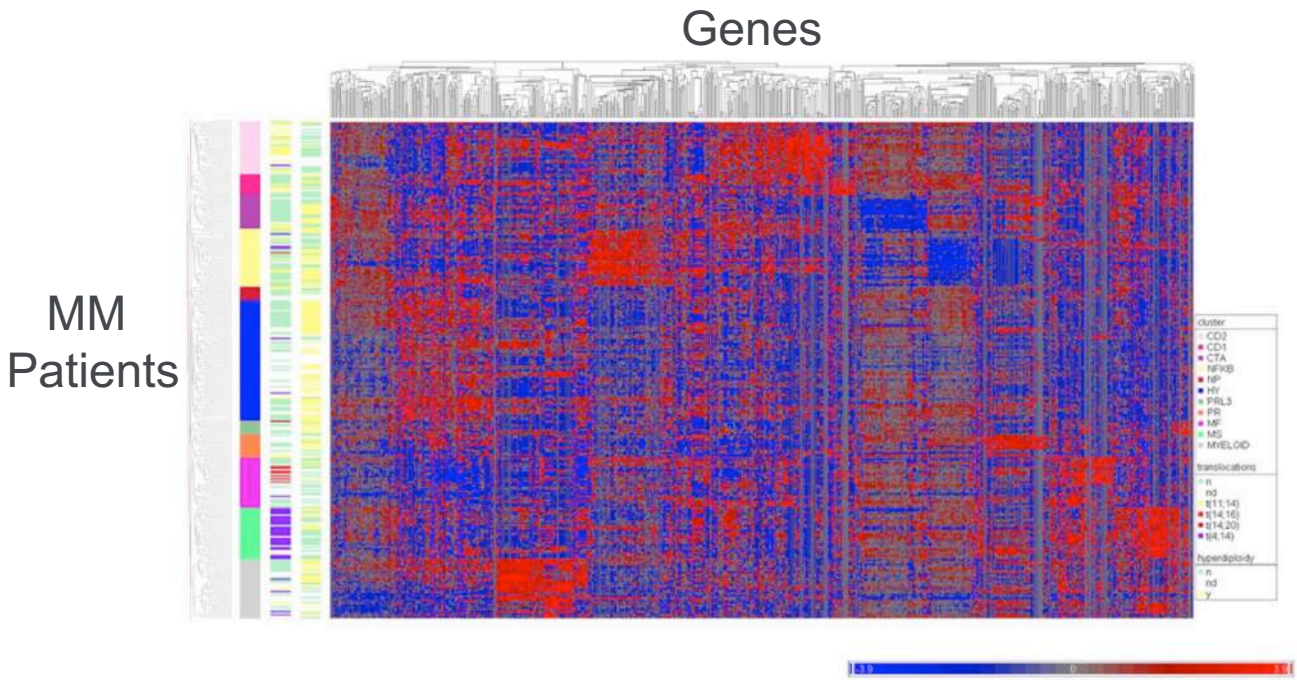
Microarray Data From MM Patients

- HOVON: Stichting Hemato-Oncologie voor Volwassenen Nederland
(Dutch-Belgian cooperative Trial Group for Hematology Oncology)

HOVON-65/GMMG-HD4 trial
- Phase 3 trial in NDMM (Newly Diagnosed Multiple Myeloma)
- PAD vs VAD treatments
- n = 329 have been analyzed using Affymetrix Microarrays

Hierarchical Clustering identified 10 clusters:

MS	NP
MF	HY
CD2	PRL3
CD1	PR
CTA	LB
NFKB	Myeloid



Broyl et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. Blood. 2010; 116:2543-2553

Kuiper et al. Leukemia 2012

Leukemia (2012) 26, 2406–2413

www.nature.com/leu

ORIGINAL ARTICLE

A gene expression signature for high-risk multiple myeloma

R Kuiper[1,9], A Broyl[1,9], Y de Knegt[1], MH van Vliet[2], EH van Beers[2], B van der Holt[3], L el Jarari[3], G Mulligan[4], W Gregory[5], G Morgan[6], H Goldschmidt[7], HM Lokhorst[8], M van Duin[1] and P Sonneveld[1]

There is a strong need to better predict the survival of patients with newly diagnosed multiple myeloma (MM). As gene expression profiles (GEPs) reflect the biology of MM in individual patients, we built a prognostic signature based on GEPs. GEPs obtained from newly diagnosed MM patients included in the HOVON65/GMMG-HD4 trial ($n = 290$) were used as training data. Using this set, a prognostic signature of 92 genes (EMC-92-gene signature) was generated by supervised principal component analysis combined with simulated annealing. Performance of the EMC-92-gene signature was confirmed in independent validation sets of newly diagnosed (total therapy (TT)2, $n = 351$; TT3, $n = 142$; MRC-IX, $n = 247$) and relapsed patients (APEX, $n = 264$). In all the sets, patients defined as high-risk by the EMC-92-gene signature show a clearly reduced overall survival (OS) with a hazard ratio (HR) of 3.40 (95% confidence interval (CI): 2.19–5.29) for the TT2 study, 5.23 (95% CI: 2.46–11.13) for the TT3 study, 2.38 (95% CI: 1.65–3.43) for the MRC-IX study and 3.01 (95% CI: 2.06–4.39) for the APEX study ($P < 0.0001$ in all studies). In multivariate analyses this signature was proven to be independent of the currently used prognostic factors. The EMC-92-gene signature is better or comparable to previously published signatures. This signature contributes to risk assessment in clinical trials and could provide a tool for treatment choices in high-risk MM patients.

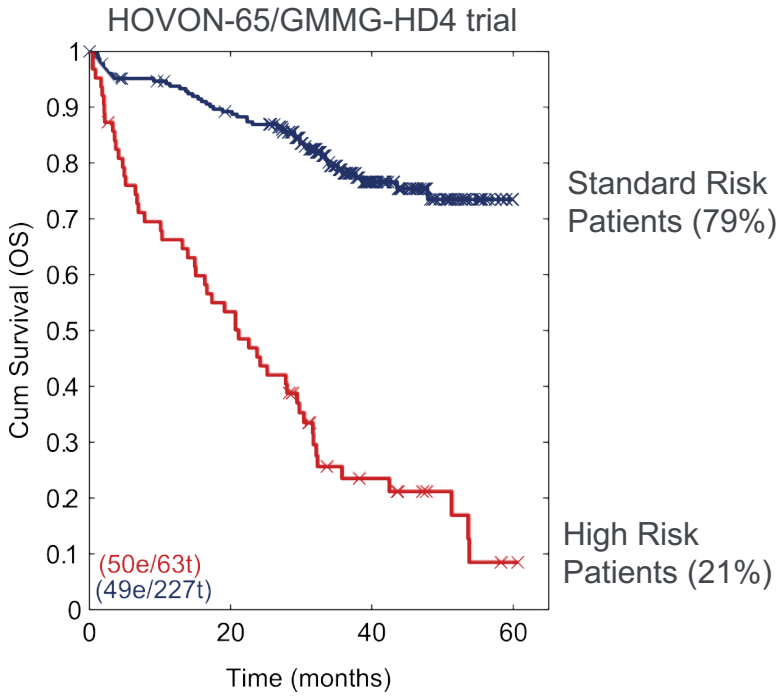
Leukemia (2012) **26**, 2406–2413; doi:10.1038/leu.2012.127

Keywords: multiple myeloma; gene expression; signature; prognosis; survival; comparison

Discovery of A New Prognostic Gene Signature: SKY92

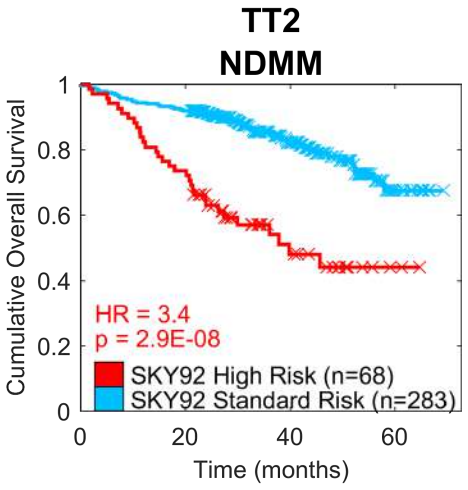
- SKY92 gene signature
- Discovered and published by EMC in Leukemia*
- Prognostic biomarker using the expression from 92 genes in bone marrow sample
- High risk cases have a more than two times higher chance to die than standard risk cases

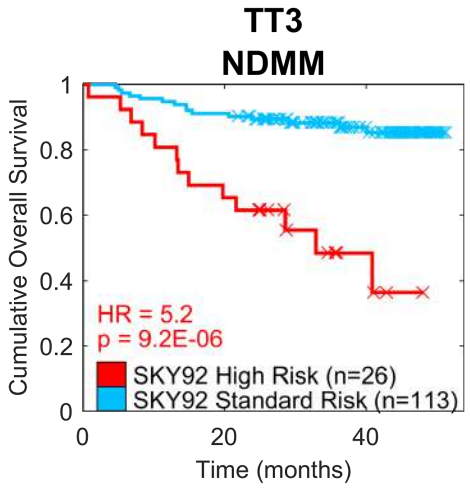
$$\text{SKY92 Score} = \sum_{i=1}^{92} w_i g_i$$

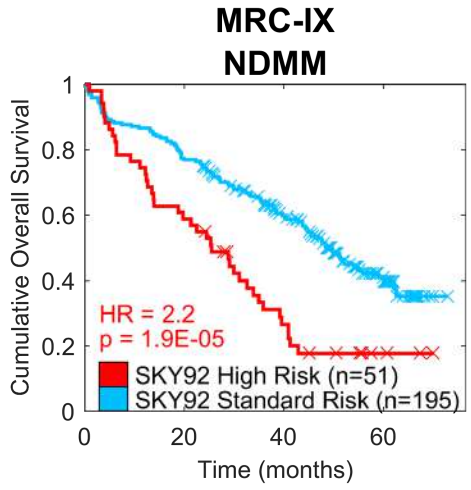


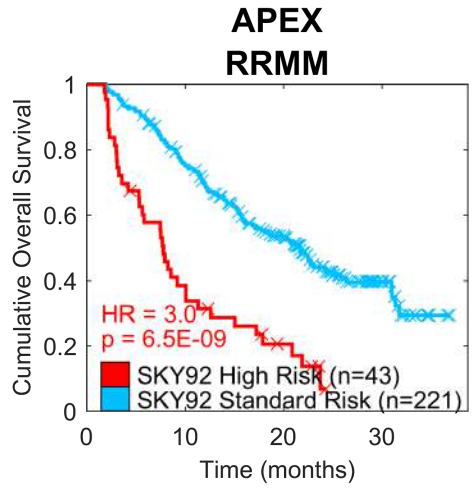
* "A gene expression signature for high-risk multiple myeloma" - Leukemia (2012) 26, 2406–2413

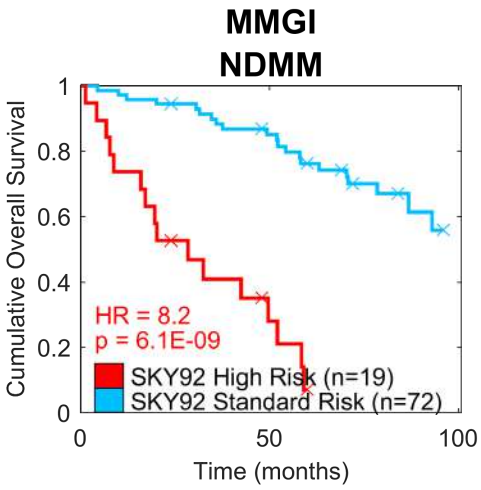
SKY92 Clinical Validation on 8 Independent Cohorts

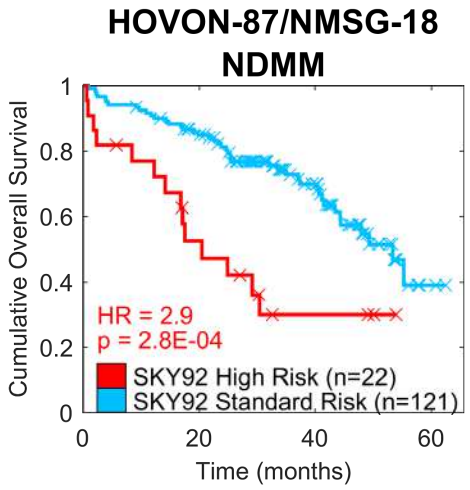


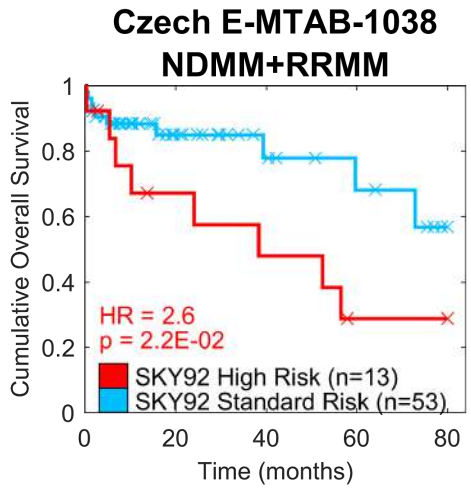


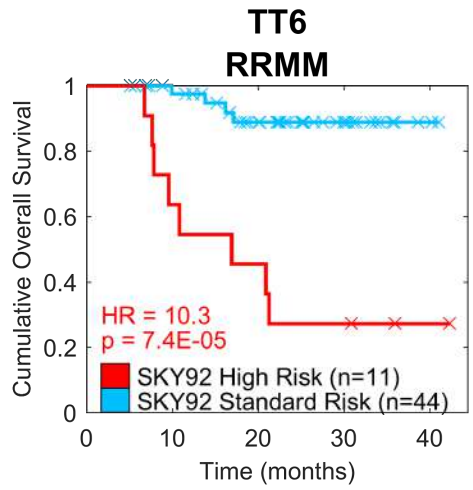












1. Kuiper et al. Leukemia 2012
2. Van Beers et al ASH 2013
3. Van Duin et al. ASH 2015
4. Van Vliet et al ASH 2015
5. Van Vliet et al. EHA 2016

Overall Survival

Prognostic Markers in MM

- Karyotyping

  Oldest method, still used in some labs

- International Staging System (ISS)[1]

  Based on β2-microglobulin and albumin

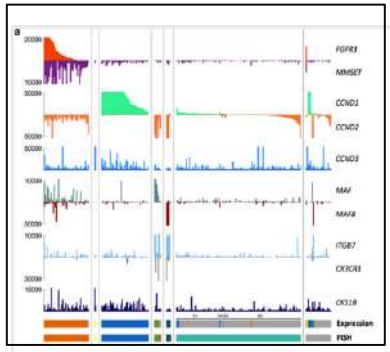
- FISH[4]

  t(4;14), t(11;14), t(14;16), t(14;20), gain1q, del13q, del17p, hyperdiploidy

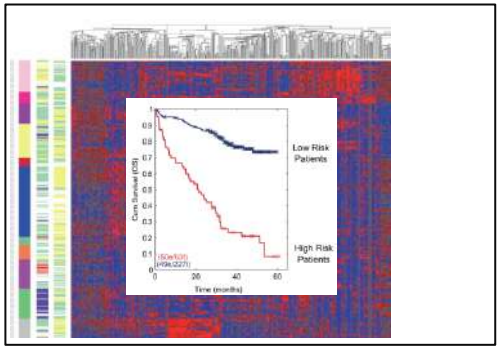
- GEP[5-9]

  - Risk signatures: UAMS-70, UAMS-17, MRCIX-6, UAMS-80, EMC-92
  - TC/classification system clusters [3,7]

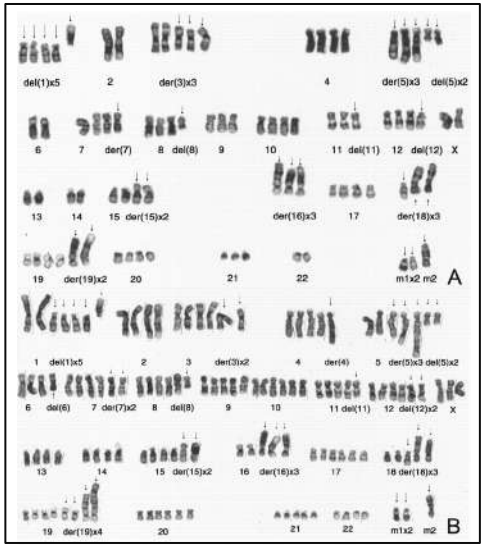
GEP / TC-clusters



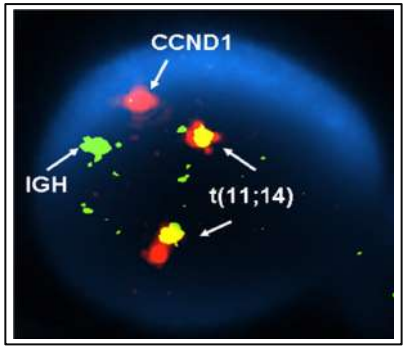
GEP / Risk Signatures



Karyotyping



FISH



1 Greipp *et al.*, JCO 2005
2. Fedele PL et al. Br J Haematol 2014
3. Kaiser M *et al.* Leukemia 2013
4. Avet-Loiseau, Best Pract Res Clin Haem 2007
5 Shaughnessy *et al.*, Blood 2007
6 Dickens *et al.*, Clin Can Res 2010
7 Shaughnessy *et al.*, Blood 2011
8. Broyl *et al.* Blood 2010
9 Kuiper *et al.*, Leukemia 2012

Univariate Hazard Ratios

	SKY92	SKY92 + ISS	ISS	i/v FISH t(4;14)	i/v FISH t(11;14)	i/v FISH t(14;16)/t(14;20)	i/v FISH del(13q)	i/v FISH gain(1q)	i/v FISH gain(9q)	i FISH del(17p)
HOVON-65/GMMG-HD4	4,7	12,2	4,6	1,5	0,8	2,8	1,7	1,3	0,7	3,4
HOVON-87/NMSG-18	2,9	3,8	2,2	1,3	0,8	2,5	1,6	2,0	0,6	2,5
MRC-IX	2,2	5,7	2,9	1,4	0,7	1,1	1,3	1,6	1,0	1,7
MMGI	8,2	10,1	3,4	0,0	2,5	13,4	1,2	3,9	0,9	NA
TT3	5,2	NA	NA	1,6	0,3	1,2	1,5	1,6	0,7	NA
TT6	10,3	NA	NA	4,3	0,2	62,7	4,2	9,6	0,8	NA
Czech E-MTAB-1038	2,6	inf	inf	0,3	NA	NA	NA	1,4	NA	1,7
TT2	3,4	NA	NA	NA	NA	NA	NA	NA	NA	NA
APEX	3,0	NA	NA	NA	NA	NA	NA	NA	NA	NA

HR: Hazard Ratio

p > 0.05

p < 0.05

Black font: iFISH
White font: vFISH

- Only SKY92 robust across all datasets
- SKY92 has higher Hazard Ratios

Kuiper et al. Blood 2015

Regular Article

LYMPHOID NEOPLASIA

Prediction of high- and low-risk multiple myeloma based on gene expression and the International Staging System

Rowan Kuiper,[1] Mark van Duin,[1] Martin H. van Vliet,[2] Annemiek Broijl,[1] Bronno van der Holt,[3] Laila el Jarari,[3] Erik H. van Beers,[2] George Mulligan,[4] Hervé Avet-Loiseau,[5] Walter M. Gregory,[6] Gareth Morgan,[7] Hartmut Goldschmidt,[8] Henk M. Lokhorst,[9] and Pieter Sonneveld[1]

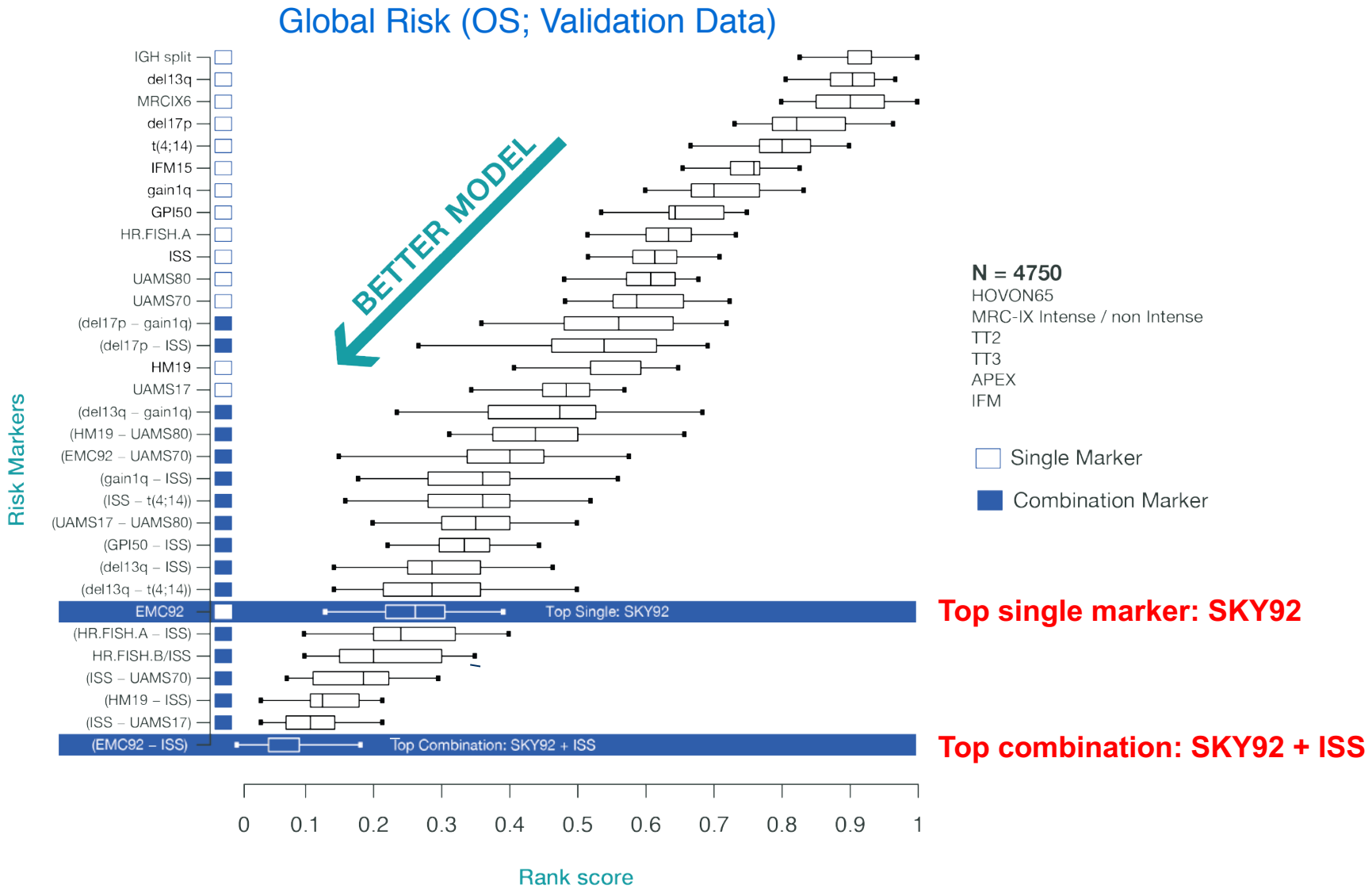
[1]Department of Hematology, Erasmus Medical Center Cancer Institute, Rotterdam, The Netherlands; [2]SkylineDx, Erasmus Medical Center Cancer Institute, Rotterdam, The Netherlands; [3]Hemato-Oncologie voor Volwassenen Nederland Data Center, Erasmus Medical Center Cancer Institute-Clinical Trial Center, Rotterdam, The Netherlands; [4]Millennium Pharmaceuticals, Cambridge, MA; [5]Unité de Génomique du Myélome, Centre Hospitalier Universitaire Rangueil, Toulouse, France; [6]Clinical Trials Research Unit, University of Leeds, Leeds, United Kingdom; [7]Royal Marsden Hospital, London, United Kingdom; [8]University of Heidelberg, Heidelberg, Germany; and [9]Department of Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

Key Points

- Combination of ISS and the EMC92 gene classifier is a novel clinically applicable risk classification for survival in multiple myeloma.
- ISS has clear independent additive prognostic value in combination with GEP classifiers or FISH markers.

Patients with multiple myeloma have variable survival and require reliable prognostic and predictive scoring systems. Currently, clinical and biological risk markers are used independently. Here, International Staging System (ISS), fluorescence in situ hybridization (FISH) markers, and gene expression (GEP) classifiers were combined to identify novel risk classifications in a discovery/validation setting. We used the datasets of the Dutch-Belgium Hemato-Oncology Group and German-speaking Myeloma Multicenter Group (HO65/GMMG-HD4), University of Arkansas for Medical Sciences-TT2 (UAMS-TT2), UAMS-TT3, Medical Research Council-IX, Assessment of Proteasome Inhibition for Extending Remissions, and Intergroupe Francophone du Myelome (IFM-G) (total number of patients: 4750). Twenty risk markers were evaluated, including t(4;14) and deletion of 17p (FISH), EMC92, and UAMS70 (GEP classifiers), and ISS. The novel risk classifications demonstrated that ISS is a valuable partner to GEP classifiers and FISH. Ranking all novel and existing risk classifications showed that the EMC92-ISS combination is the strongest predictor for overall survival, resulting in a 4-group risk classification. The median survival was 24 months for the highest risk group, 47 and 61 months for the intermediate risk groups, and the median was not reached after 96 months for the lowest risk group. The EMC92-ISS classification is a novel prognostic tool, based on biological and clinical parameters, which is superior to current markers and offers a robust, clinically relevant 4-group model. (*Blood*. 2015;126(17):1996-2004)

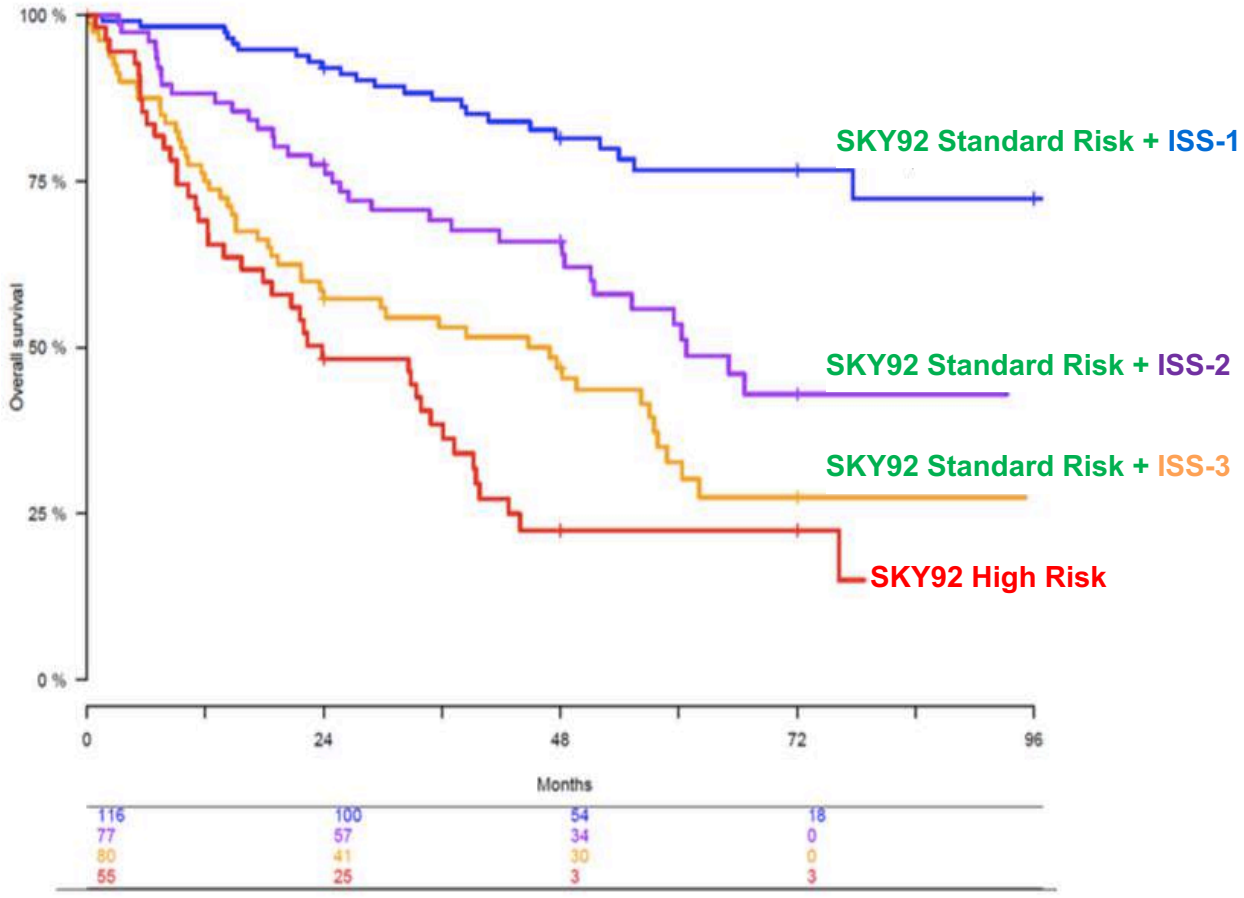
Comparison of Prognostic Markers in MM



Top single marker: SKY92

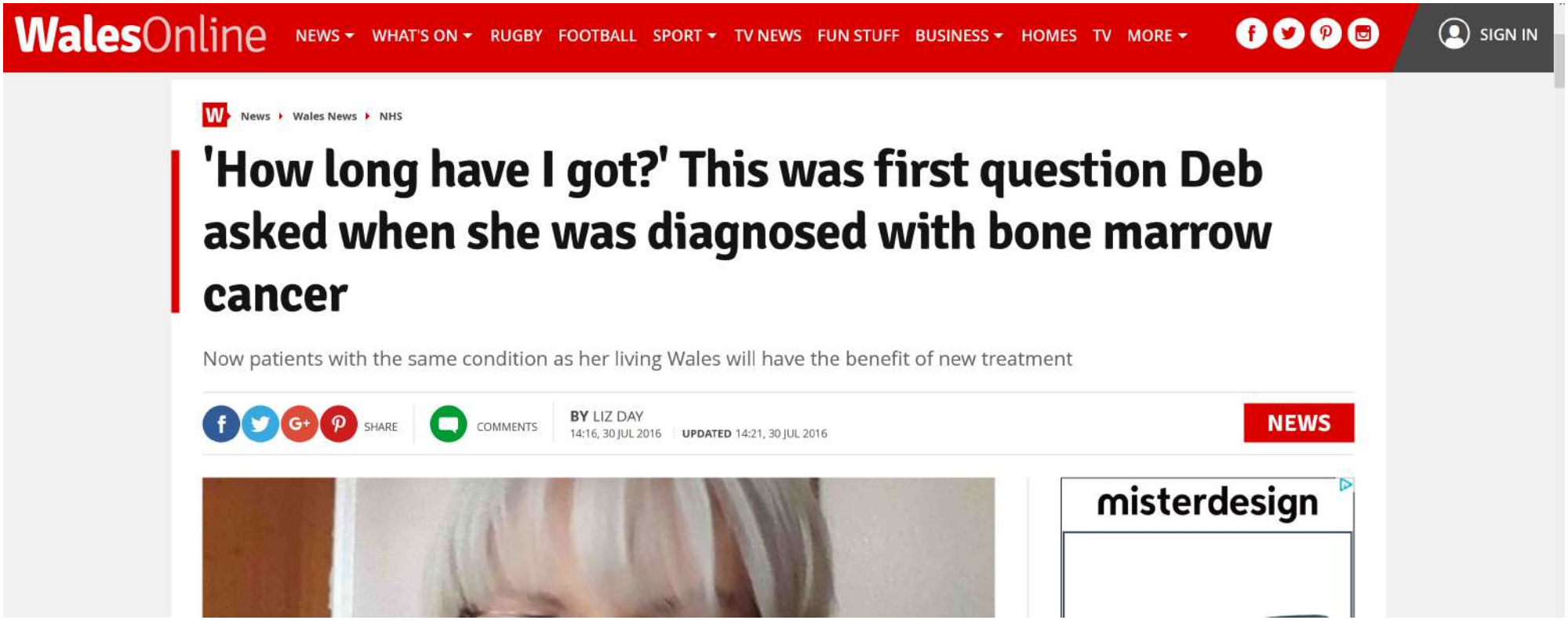
Top combination: SKY92 + ISS

MM stratification into 4 risk groups using SKY92 + ISS

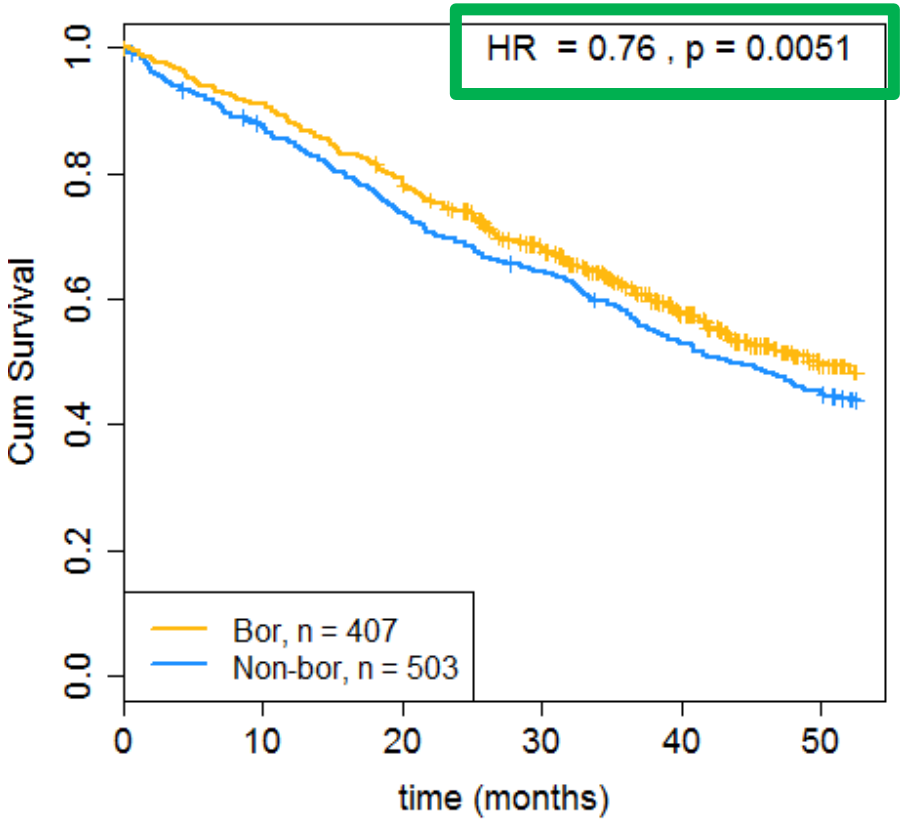


SKY92 + ISS detects both High Risk and Low Risk MM patients

Clinical Utility of Prognostic Signatures



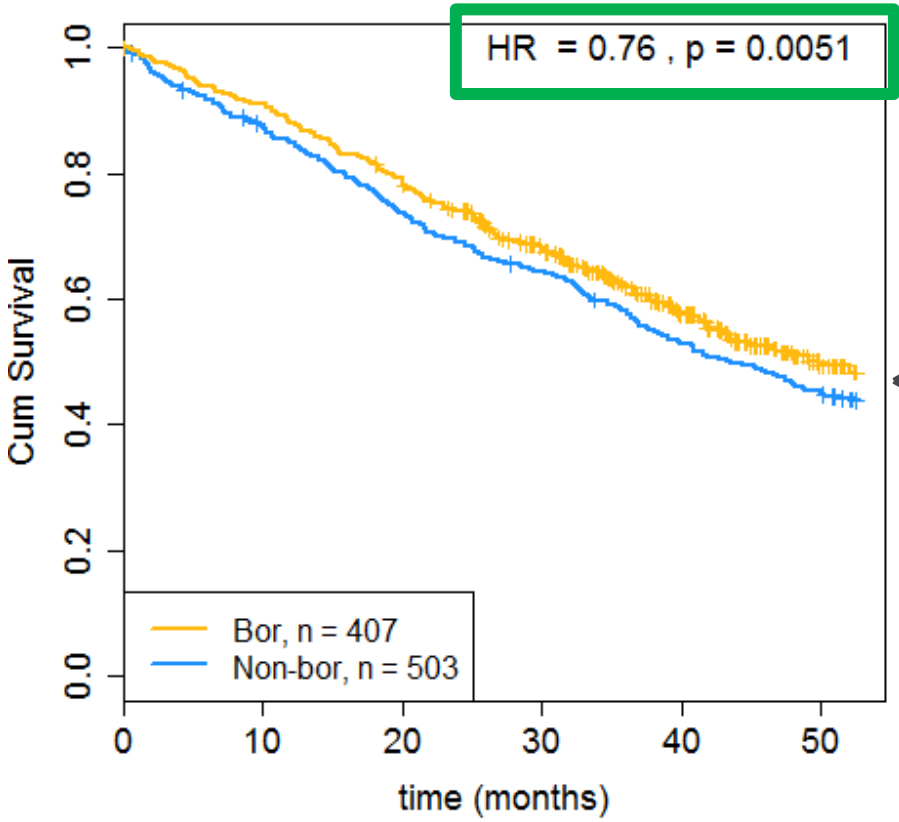
How do we Treat those Patients?



Phase 3 trial results indicate longer survival with the orange treatment regimen (Bortezomib)

Result: all patients get the orange treatment

Who benefits from treatment?

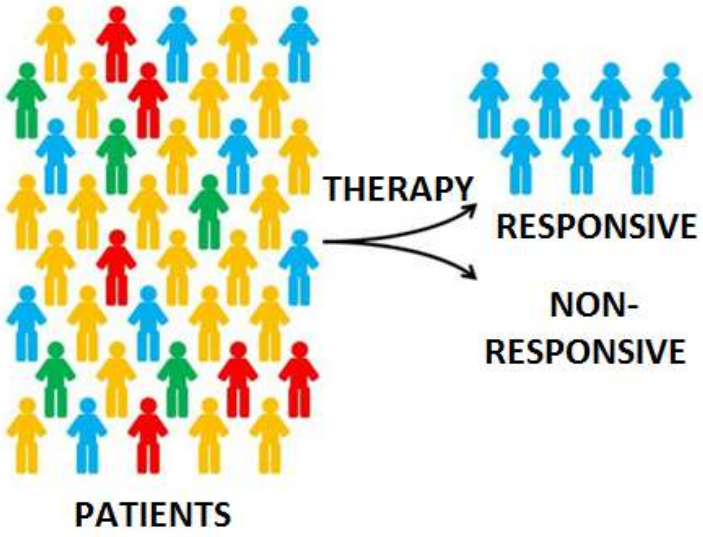


Small benefit for all?

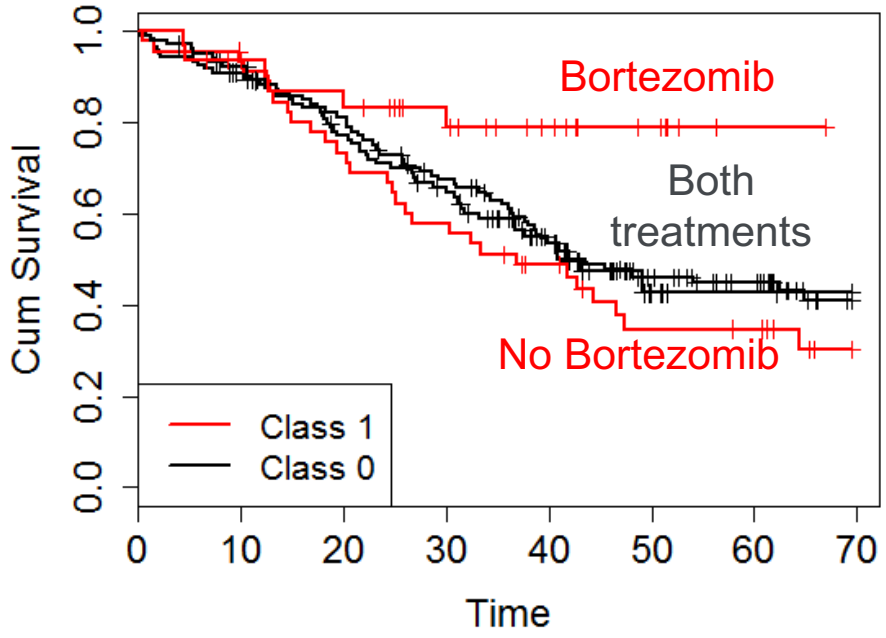
Large benefit for some?

Predictive Markers

- How can we identify the responders?
- Which medicine for which patient?



Identify the patients that will benefit from bortezomib



‘Simple’ approach

Bortezomib + long survival

Non-bortezomib + short survival

Class 1

- Identify differentially expressed genes and build classifier

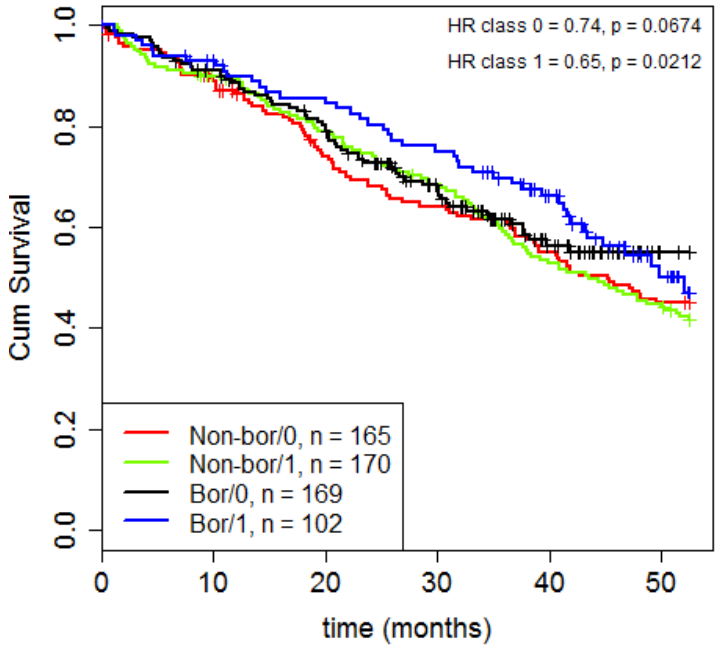
‘Simple’ approach

Bortezomib + long survival

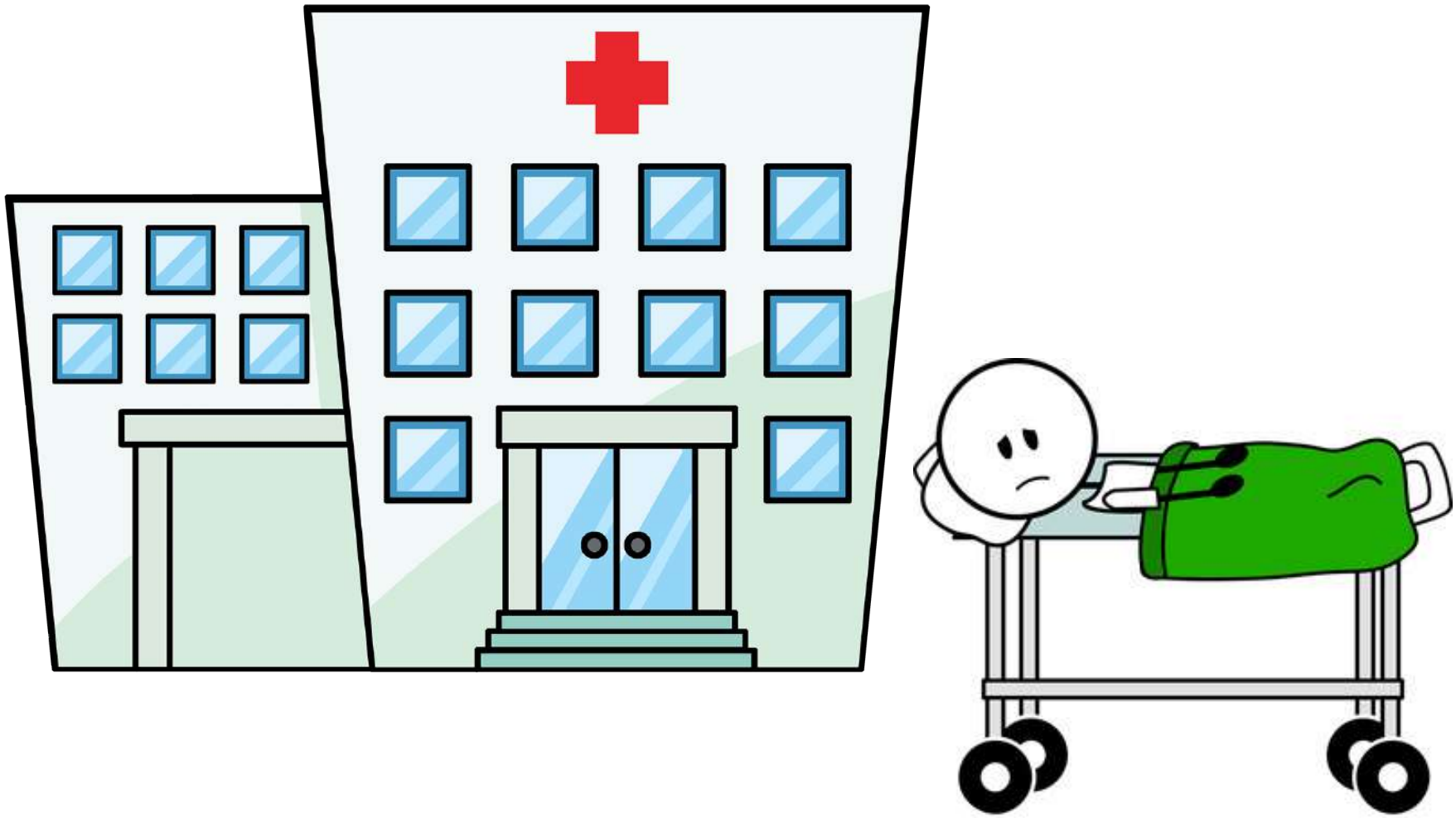
Non-bortezomib + short survival

Class 1

- Identify differentially expressed genes and build classifier

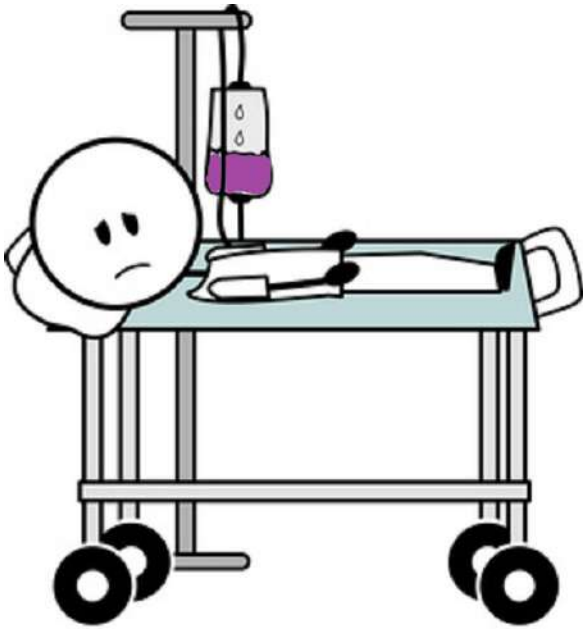


Doesn’t work!

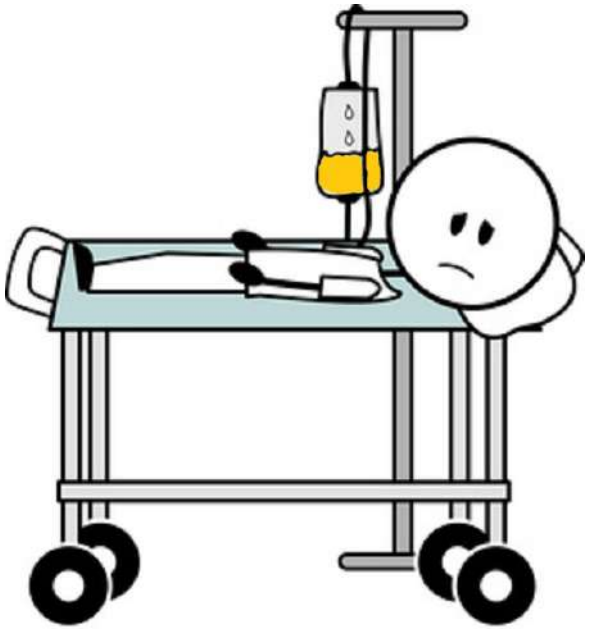


TREATMENT A

TREATMENT A

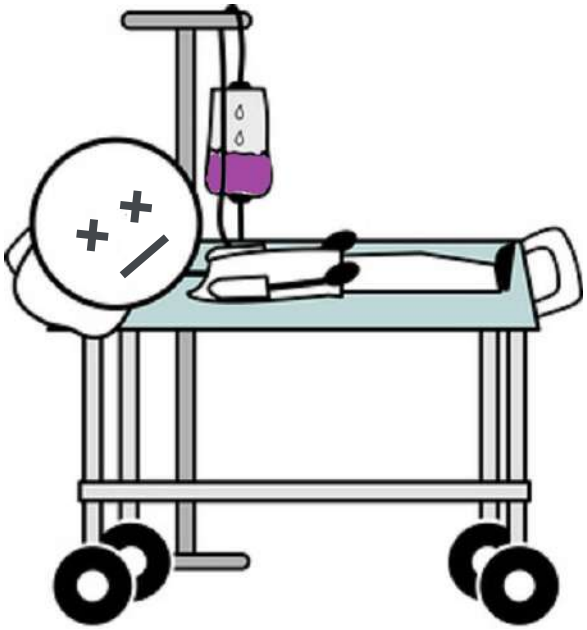


TREATMENT B



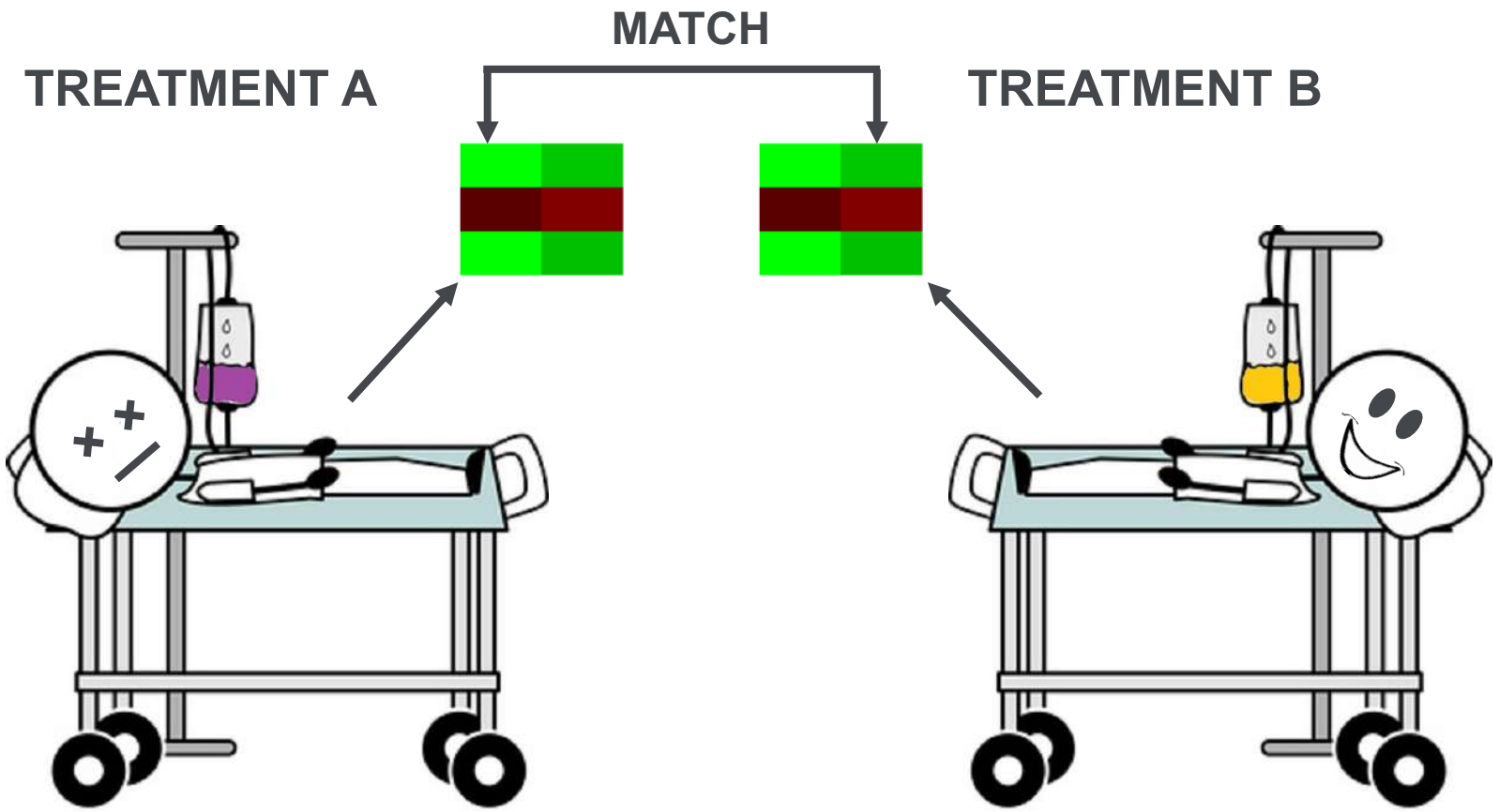
Parallel universe

TREATMENT A



TREATMENT B

Parallel universe


MATCH
TREATMENT A
TREATMENT B

Main Idea of Simulated Treatment Learning

- Take a few genetically similar patients
- That were treated differently
- See who survives longer

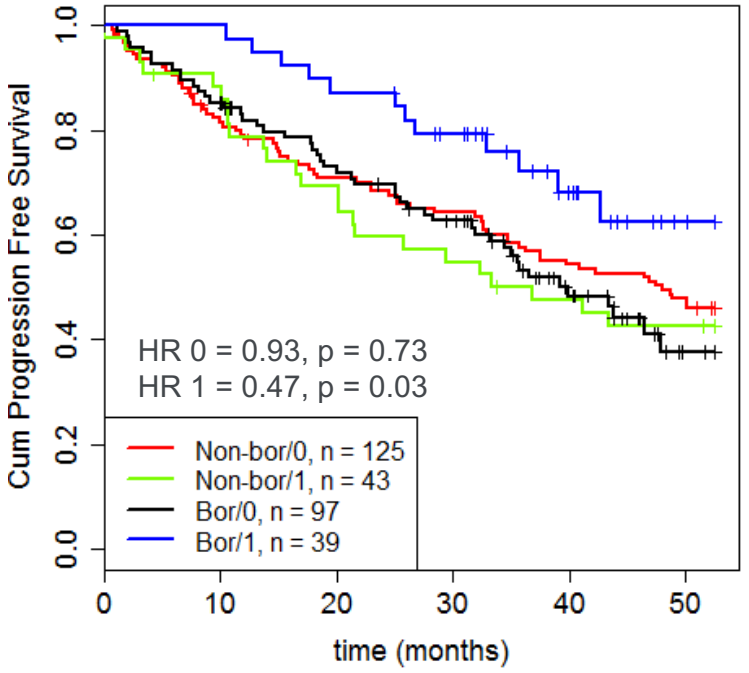
Simulated Treatment Learning result in MM

STL algorithm
- Enables discovery of predictive biomarkers
- Uses genomics datasets

Example
- Microarray data from 910 MM patients:
  - 407 received Bort
  - 503 received non-Bort
- Part of the data used to train
- Part of the data used to validate (see KM)

STL found a predictive biomarker, which was successfully validated:
- 27% of patients, with more than twofold PFS advantage when given Bort
  Class 1, blue/green lines
- 73% patients for which Bort didn't provide an PFS advantage
  Class 0, red/black lines

Kaplan Meier of Validation result



Let’s start using those signatures in the clinic!

We can do that now, right?

SKY92 has been independently validated

SKY92 outperforms other prognostic markers

Regulatory Approval

- RUO: Research Use Only
  - –Not to be used in a diagnostic setting

- IVD: In Vitro Diagnostic
  - –Allowed to be used in a diagnostic setting

CE

DEPARTMENT OF HEALTH & HUMAN SERVICES • USA FDA

New IVD Regulation from the EU

Official Journal of the European Union

L 117

English edition

Legislation

Volume 60

5 May 2017

Contents

Relevant ISO Norms, and Regulations

- Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

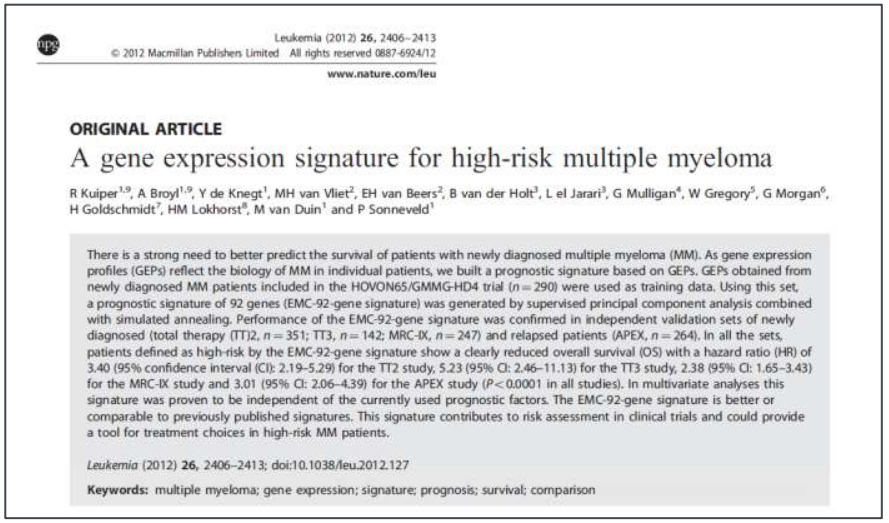
  – Old Directive: most assays are “self-declare”
  – New Regulation: 80% will need to go through a Notified Body

- 21 CFR part 820
  Code of Federal Regulations: US law (FDA)

- ISO 13485:2016 Medical devices (design, development, manufacturing)
- ISO 15189:2012 Medical laboratories – Particular Requirements for quality and competence

What's Needed for Regulatory Approval?

- Clinical Validation



Leukemia (2012) **26**, 2406–2413

www.nature.com/leu

ORIGINAL ARTICLE

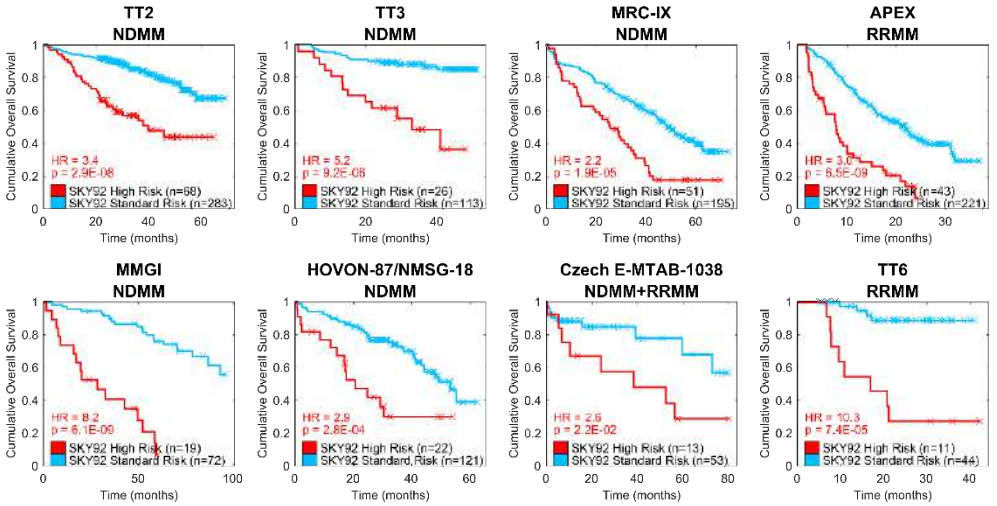
A gene expression signature for high-risk multiple myeloma

R Kuiper[1,9], A Broyl[1,9], Y de Knegt[1], MH van Vliet[2], EH van Beers[2], B van der Holt[3], L el Jarari[3], G Mulligan[4], W Gregory[5], G Morgan[6], H Goldschmidt[7], HM Lokhorst[8], M van Duin[1] and P Sonneveld[1]

There is a strong need to better predict the survival of patients with newly diagnosed multiple myeloma (MM). As gene expression profiles (GEPs) reflect the biology of MM in individual patients, we built a prognostic signature based on GEPs. GEPs obtained from newly diagnosed MM patients included in the HOVON65/GMMG-HD4 trial ($n = 290$) were used as training data. Using this set, a prognostic signature of 92 genes (EMC-92-gene signature) was generated by supervised principal component analysis combined with simulated annealing. Performance of the EMC-92-gene signature was confirmed in independent validation sets of newly diagnosed (total therapy (TT)2, $n = 351$; TT3, $n = 142$; MRC-IX, $n = 247$) and relapsed patients (APEX, $n = 264$). In all the sets, patients defined as high-risk by the EMC-92-gene signature show a clearly reduced overall survival (OS) with a hazard ratio (HR) of 3.40 (95% confidence interval (CI): 2.19–5.29) for the TT2 study, 5.23 (95% CI: 2.46–11.13) for the TT3 study, 2.38 (95% CI: 1.65–3.43) for the MRC-IX study and 3.01 (95% CI: 2.06–4.39) for the APEX study ($P < 0.0001$ in all studies). In multivariate analyses this signature was proven to be independent of the currently used prognostic factors. The EMC-92-gene signature is better or comparable to previously published signatures. This signature contributes to risk assessment in clinical trials and could provide a tool for treatment choices in high-risk MM patients.

Leukemia (2012) **26**, 2406–2413; doi:10.1038/leu.2012.127

Keywords: multiple myeloma; gene expression; signature; prognosis; survival; comparison

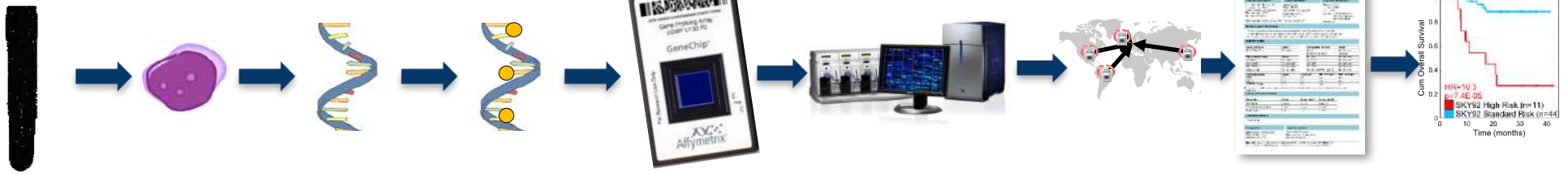


- Analytical Validation

Many studies required!

The MMprofiler Workflow

Bone Marrow	Purified Plasma Cells	RNA	Labelled cRNA	Micro Array	Hybridization, Wash & Stain and Scan (DX2 System)	GxP Compliant Secure Analysis	Report	Clinical relevance



Assay from tissue to report

Examples of Analytical Validation Studies

Bone Marrow	Purified Plasma Cells	RNA	Labelled cRNA	Micro Array	Hybridization, Wash & Stain and Scan (DX2 System)	GxP Compliant Secure Analysis	Report	Clinical relevance

Do you get the same result with different lots of reagents?
(RUO reagents get “improvements” from manufacturers)
And with different technicians?
And with different sites?

Bone Marrow as starting material:
We claim stability for 24 hours

Provide data from 0, 24, 25 hours
to supporting that claim

At what temperature?
During transportation?

Ballpark needed:
~1500 assays
Much patient material

Conclusions

- Prognostic signatures work, outperform other clinical parameters, and enable risk stratified treatment approaches

- Predictive biomarkers: smart algorithms needed to find them!

- RUO → IVD assays:
  - Can be used in clinical decision making
  - Standardized workflow
  - Comparability of data between labs

Acknowledgements

Joske Ubels

Rowan Kuiper

Belinda Dumee

Erik van Beers

Mark van Duin

Annemiek Broijl

Pieter Sonneveld

Jeroen de Ridder

Patients, participating hospitals, and staff from the trials:

HOVON-65/GMMG-HD4, HOVON-87/NMSG-18, TT2, TT3, TT6, APEX, MMGI, MRC-IX

QUESTIONS/CONTACT INFORMATION

To contact SkylineDx:

SkylineDx BV

Rotterdam Science Tower

Marconistraat 16

3029 AK Rotterdam

The Netherlands

+31 (0)10 7038410

SkylineDx USA

23046 Avenida De La Carlota

Laguna Hills

California 92653

USA

+1 619 654 0629

www.skylinedx.com

Info@skylinedx.com

@skylinedx

/company/skylinedx-bv

/facebook.com/SkylineDx92