Research Sample Lifecycle: From blood draw to publication

Stephen Spellman, MBS, Director, Immunobiology Research, CIBMTR

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November 9th, 2018

NATIONAL MARROW DONOR PROGRAM®

BE THE MATCH®

Disclosures

The following faculty and planning committee staff have the following financial disclosures:

Name	Institution	Disclosure
Misty Evans	Vanderbilt University	Jazz Pharmaceuticals, Monetary, Speakers Bureau
Shernan Holtan, MD	University of Minnesota	Incyte, Consulting Fee, Consulting
Wael Saber	Medical College of Wisconsin, CIBMTR	None
Stephen Spellman, MBS	CIBMTR	None
Stephanie Waldvogel	CIBMTR	None

Learning objectives

- At the conclusion of this session, attendees will be able to:
  - Describe the research sample lifecycle
  - Recognize the scientific value of NMDP research samples and their impact on transplant studies
  - Identify recent CIBMTR and BMT CTN studies where research samples contributed to the science

Research Sample Life Cycle: From Blood Draw to Publication

Council Meeting

Stephen Spellman

Director, Immunobiology Research

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The CIBMTR® (Center for International Blood and Marrow Transplant Research®) is a research collaboration between the National Marrow Donor Program® (NMDP)/Be The Match® and the Medical College of Wisconsin (MCW).

CIBMTR Research Repository

- Unrelated Donor Repository (Est.1987)
  - >200 Centers Participating
  - >40,500 Adult Recipient/Donor pairs
  - >6,600 Recipient/Cord pairs
- Related Donor Repository (Est. 2007)
  - 52 Centers Participating
  - >7,800 Adult Recipient/Donor pairs
- More than 2.6 million aliquots stored

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Clinical Trial Support

- CIBMTR Research Biorepository
  - Began supporting clinical sample processing and long-term storage of Blood and Marrow Transplant Clinical Trials Network (BMT CTN) research biospecimen collections in 2007.
- BMT CTN Biospecimen Collections
  - Currently total more than 400,000 biospecimens
  - Clinical samples provided by more than 6,480 subjects, associated with 21 clinical studies.

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CIBMTR Research Biospecimen Processing

Peripheral Blood Research Specimen Types	
Whole Blood	Buffy Coat WBC
Dried Blood Spots – Filter Cards	PAXgene RNA Lysates
Serum / Plasma	PAXgene DNA Lysates
Granulocytes	Viable Peripheral Blood Mononuclear Cells (PBMC)
Additional Research Specimen Types	
Buccal Swabs	PAXgene Marrow Aspirate DNA Lysates
Viable Bone Marrow Aspirate	Viable PBSC/Bone Marrow Product Mononuclear Cells (BMMC)
Protocol Development	
Urine	Stool

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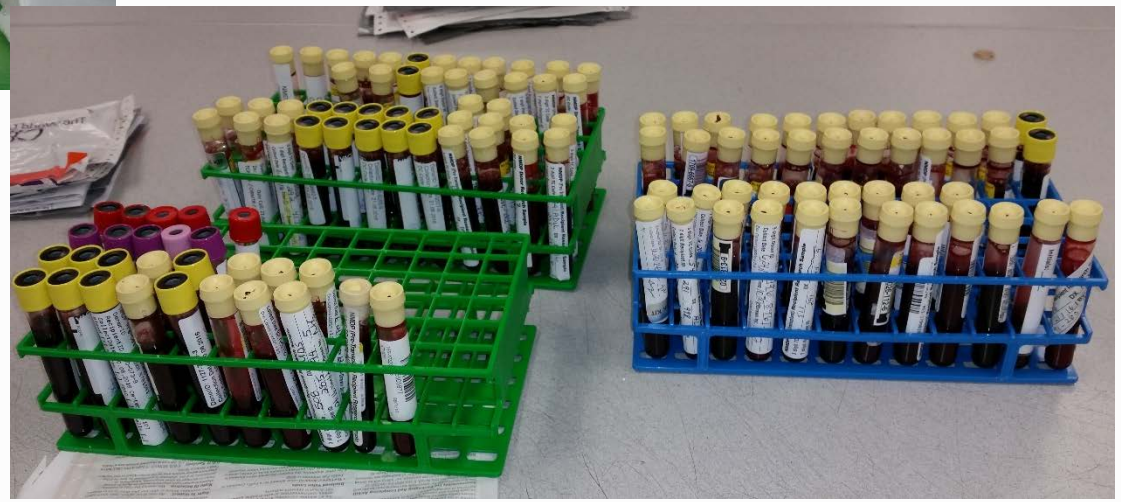
Receiving Samples



Receive and process ~55 samples daily, 6 days/week

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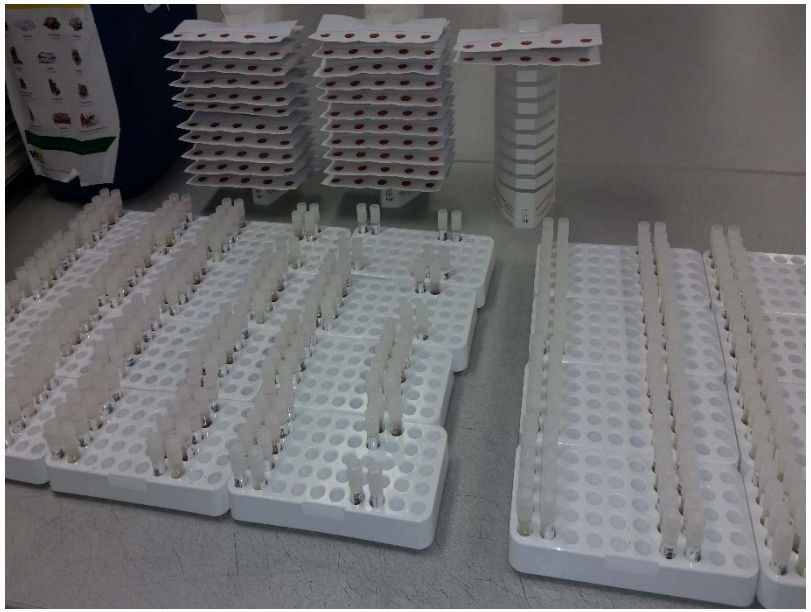
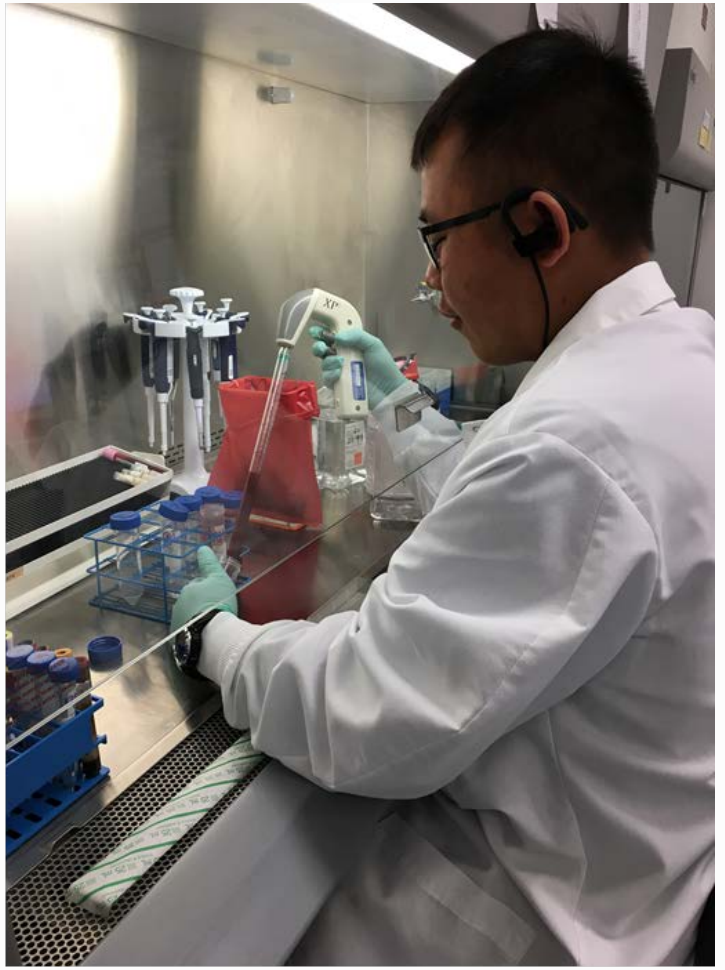
Processing



Samples processed, aliquoted and placed in storage per study specific SOPs



Frozen and Room Temperature Dry Storage

Ultralow freezers (-80°C)

Liquid Nitrogen (LN_2)

Room temp/
humidity controlled

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Research Sample Life Cycle and Impact on Role of AlloHCT for Patients with MDS

Wael Saber, MD, MS
CIBMTR, Medical College of Wisconsin
November, 2018

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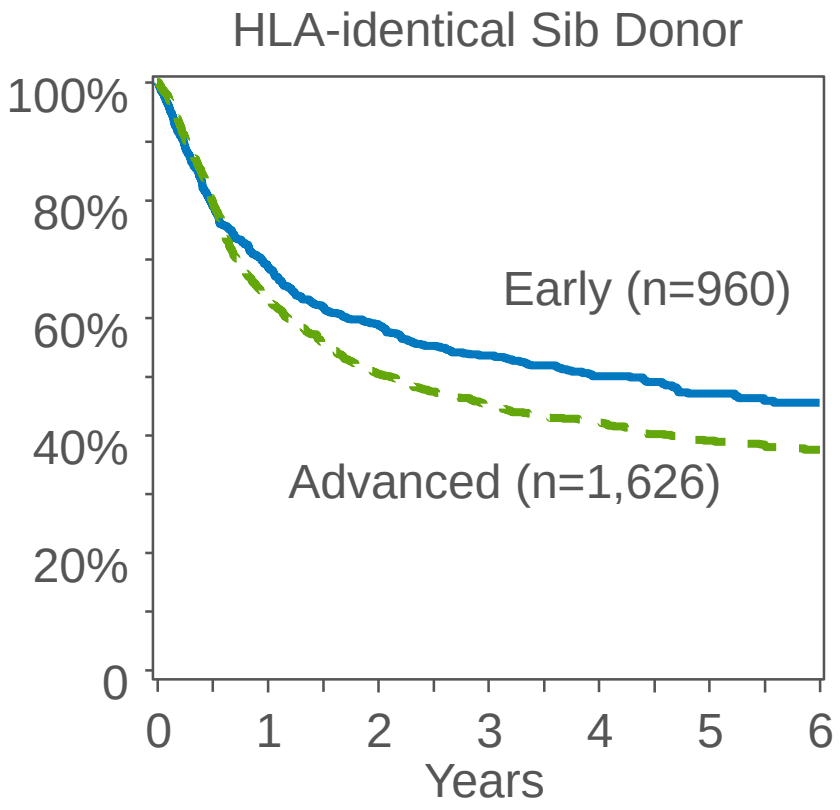
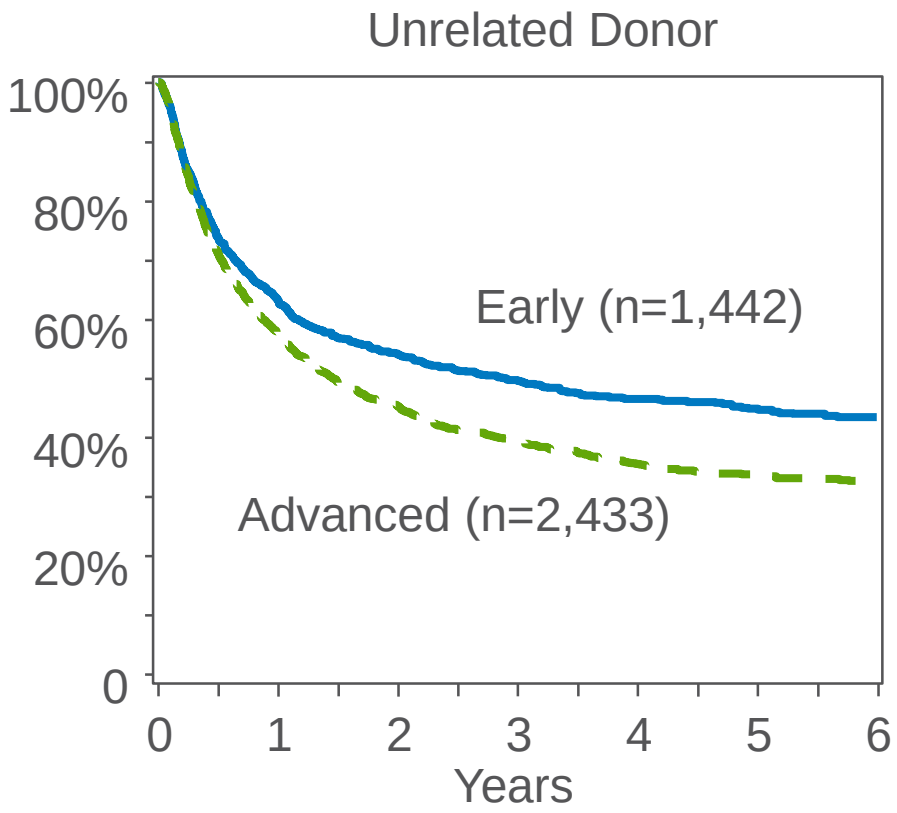
A research collaboration between the National Marrow Donor Program (NMDP)/Be The Match and the Medical College of Wisconsin

Why Allogeneic HCT for MDS?





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By Disease Status for Unrelated Donor

How Much More Life Might Perfectly Safe Curative Therapy Provide?

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Life Expectancy of Patients with MDS by IPSS, years

AGE	IPSS-R Very Low	IPSS-R Low	IPSS-R Intermediate	IPSS-R High	IPSS-R Very High
50y	>13	9	5	2	1
55y	>13	9	5	2	1
60y	10	6	3	2	1
65y	10	6	3	2	1
70y	7	5	3	2	1
75y	7	5	3	2	1

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How Much More Life Might Perfectly Safe Curative Therapy Provide?

- Average life expectancy for:
  - Newborn ~ 76 years
  - 50 year old ~ 30 years (80)
  - 55 year old ~ 25 years (80)
  - 60 year old ~ 22 years (82)
  - 65 year old ~ 18 years (83)
  - 70 year old ~ 14 years (84)
  - 75 year old ~ 10 years (85)

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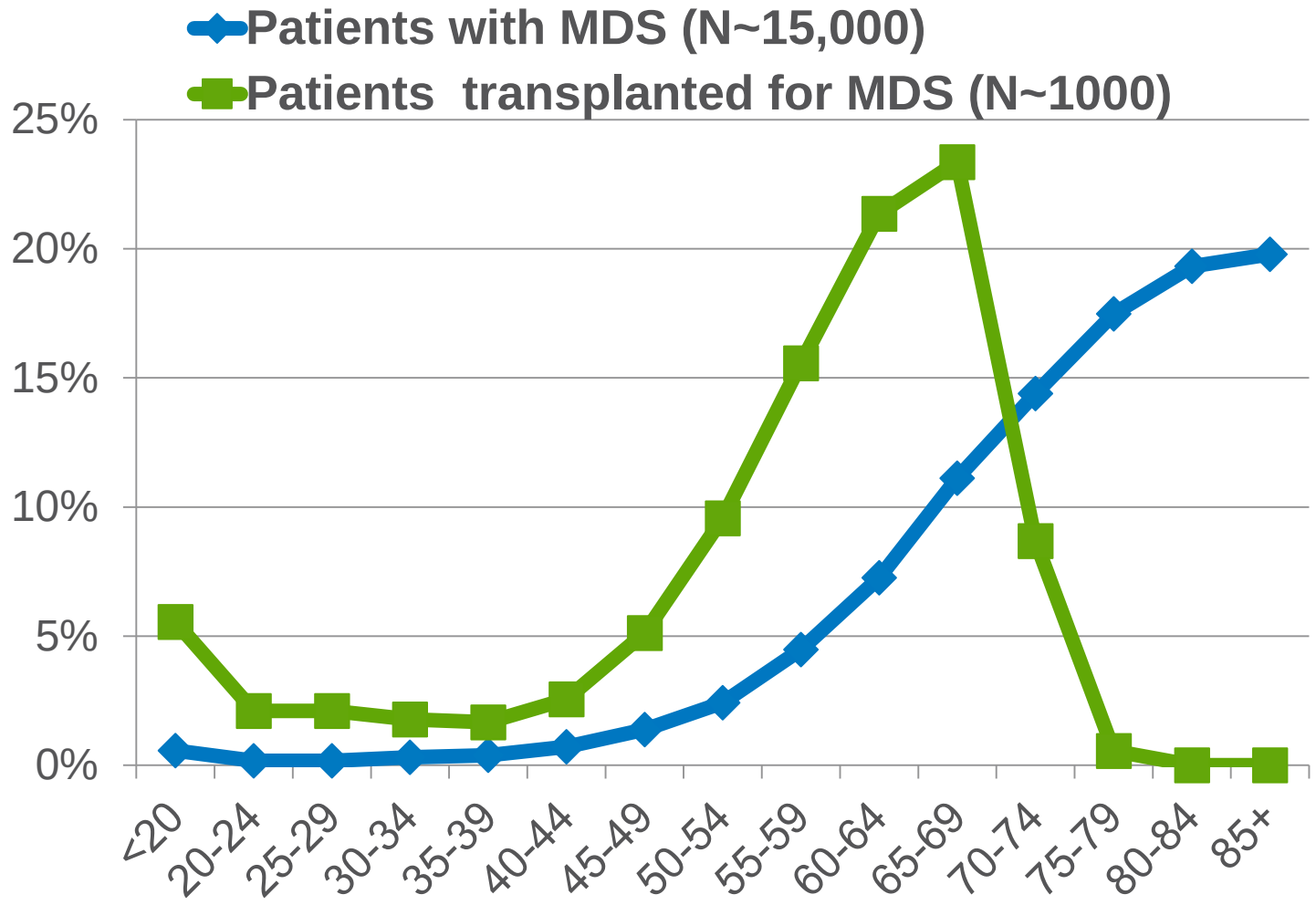
Allogeneic HCT for MDS – Why Not?

Age Distribution of Patients with MDS



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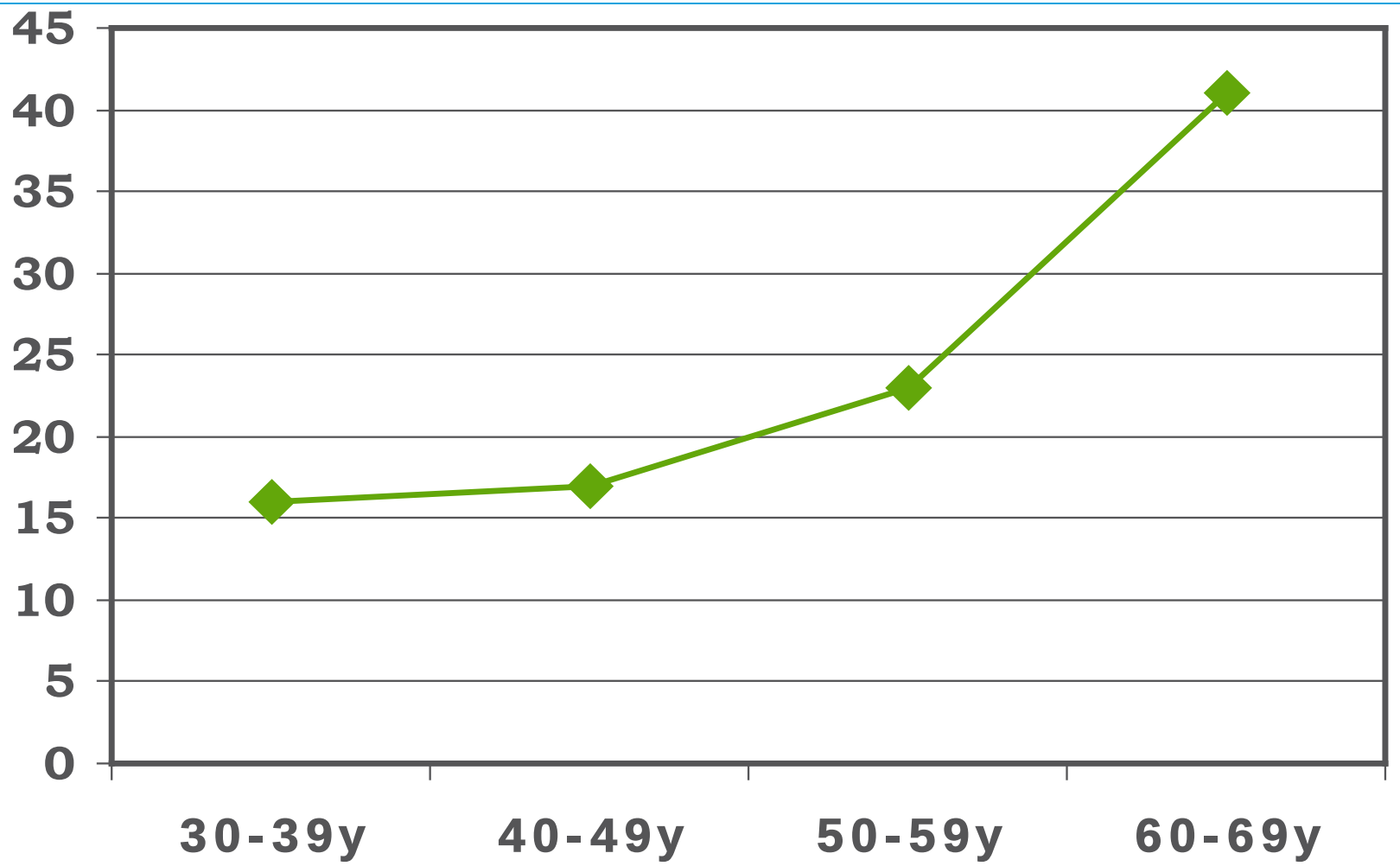
Transplant-Related Mortality by Age-Standard Intensity Conditioning



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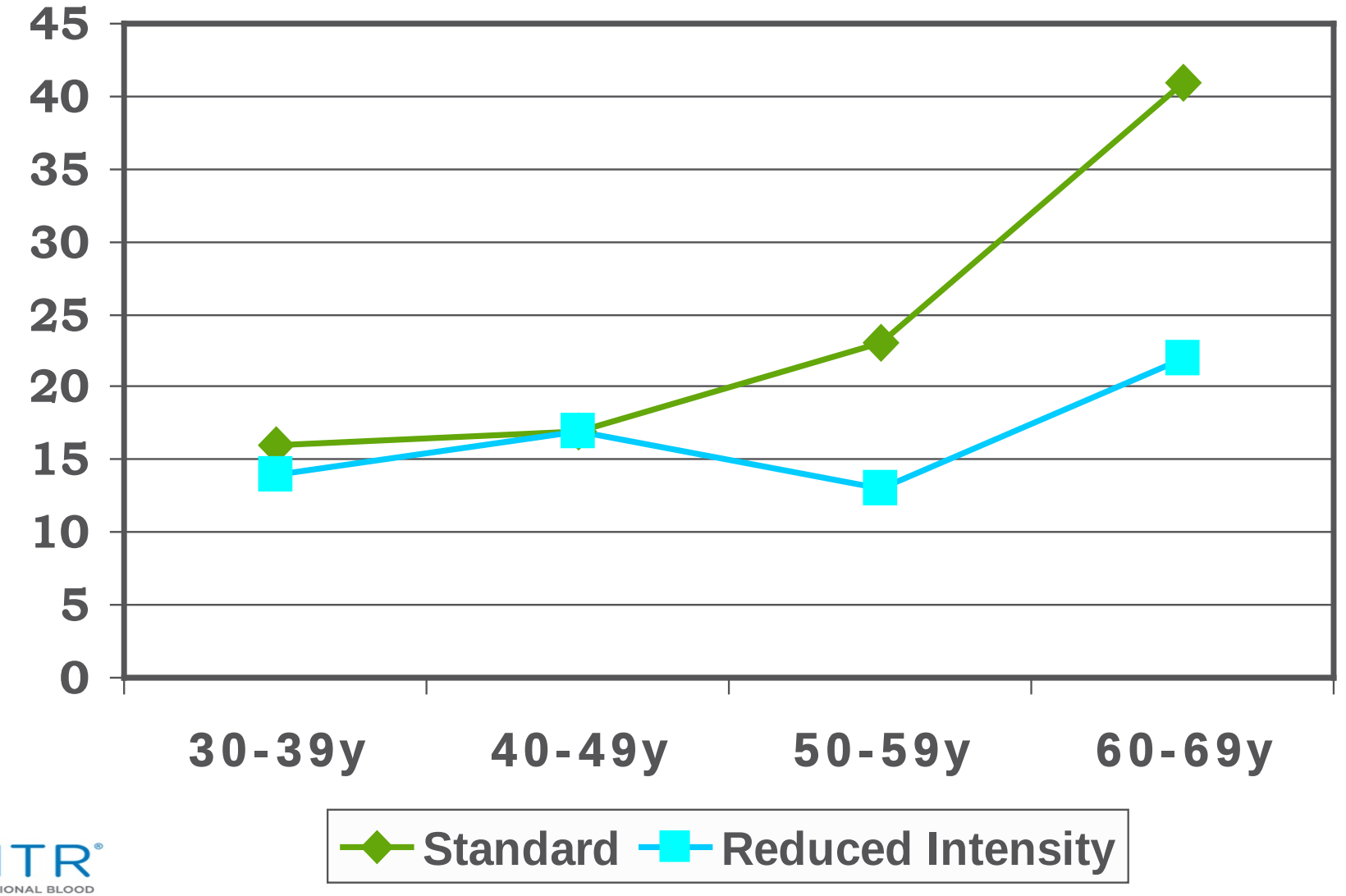
TRANSPLANT-RELATED MORTALITY BY AGE
Standard vs Reduced Intensity Conditioning



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So, Should Everyone Get a Transplant?

- Need to consider the expected survival with non-transplant therapy

But also

- Need to consider the likelihood of a successful transplant

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Shaffer et al. J Clin Oncol 2016: MDS Prognostic Score for HCT – 1728 patients transplanted in 2000-12

	Training Cohort	Validation Cohort
N	1151	577
Relapse (3-year)	**25** (22-28)%	**25** (22-29)%
TRM (3-year)	**34** (31-37)%	**31** (27-35)%
DFS (3-year)	**41** (38-44)%	**44** (39-48)%
OS (3-year)	**43** (40-46)%	**47** (42-51)%
Median follow-up, months (range)	**52** (3-169)	**48** (3-145)

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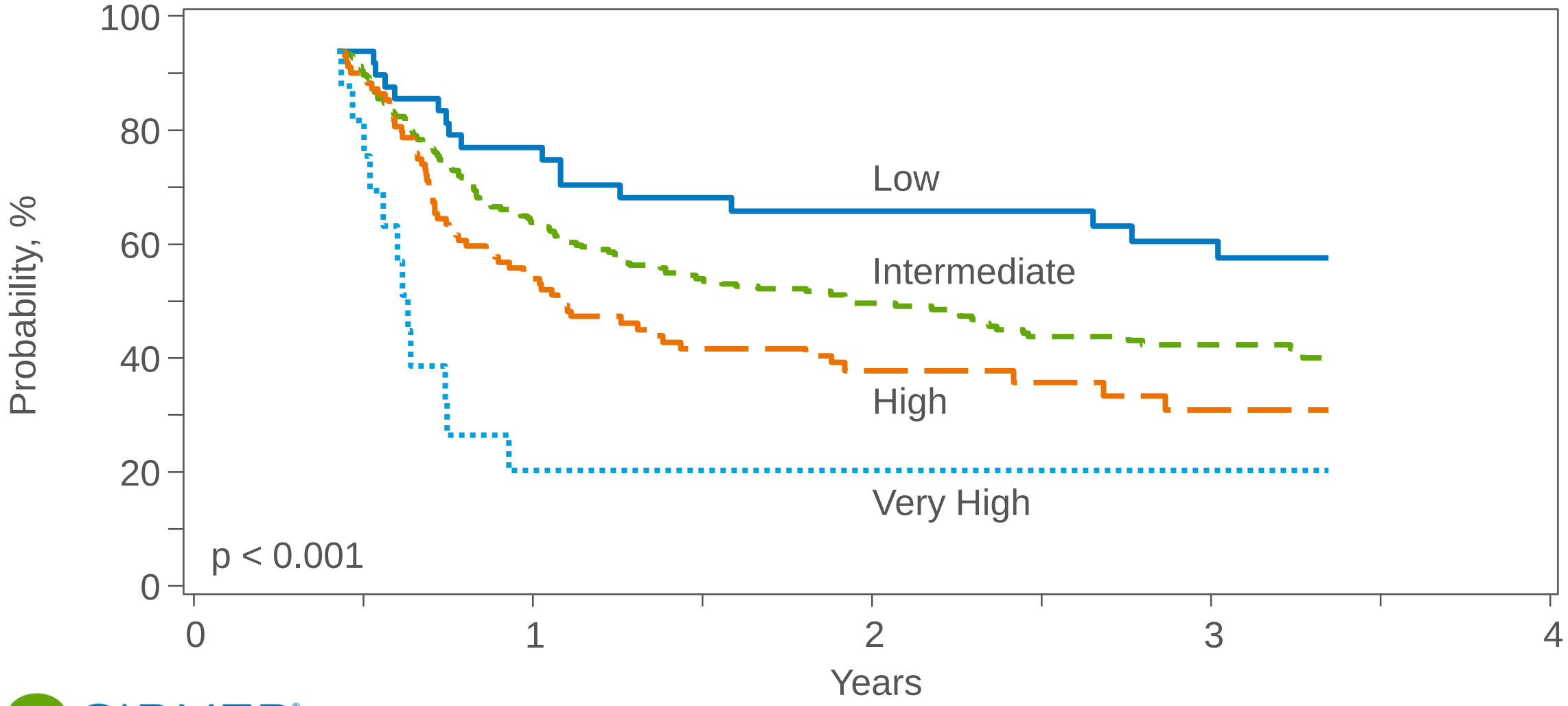
Overall Survival in HLA-matched Validation Cohort



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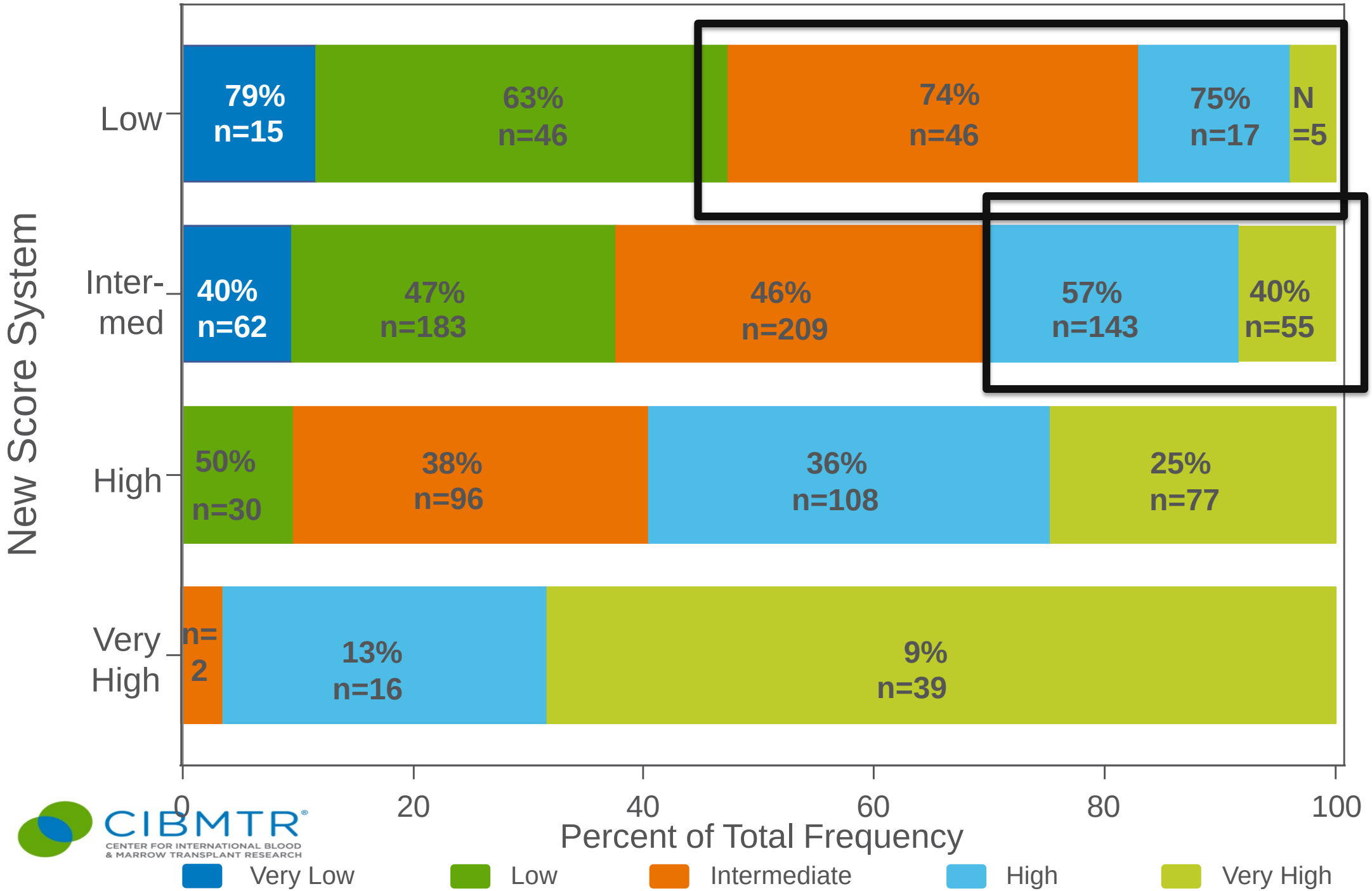
New Score System
Low
Inter-
med
High
Very
High
79%
n=15
63%
n=46
74%
n=46
75%
n=17
N
=5
40%
n=62
47%
n=183
46%
n=209
57%
n=143
40%
n=55
50%
n=30
38%
n=96
36%
n=108
25%
n=77
n=
2
13%
n=16
9%
n=39
0
20
40
60
80
100
Percent of Total Frequency
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Very Low
Low
Intermediate
High
Very High

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation

R.C. Lindsley, W. Saber, B.G. Mar, R. Redd, T. Wang, M.D. Haagenson, P.V. Grauman, Z.-H. Hu, S.R. Spellman, S.J. Lee, M.R. Verneris, K. Hsu, K. Fleischhauer, C. Cutler, J.H. Antin, D. Neuberg, and B.L. Ebert

ABSTRACT

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Approach

Cohort: 1514 MDS patients

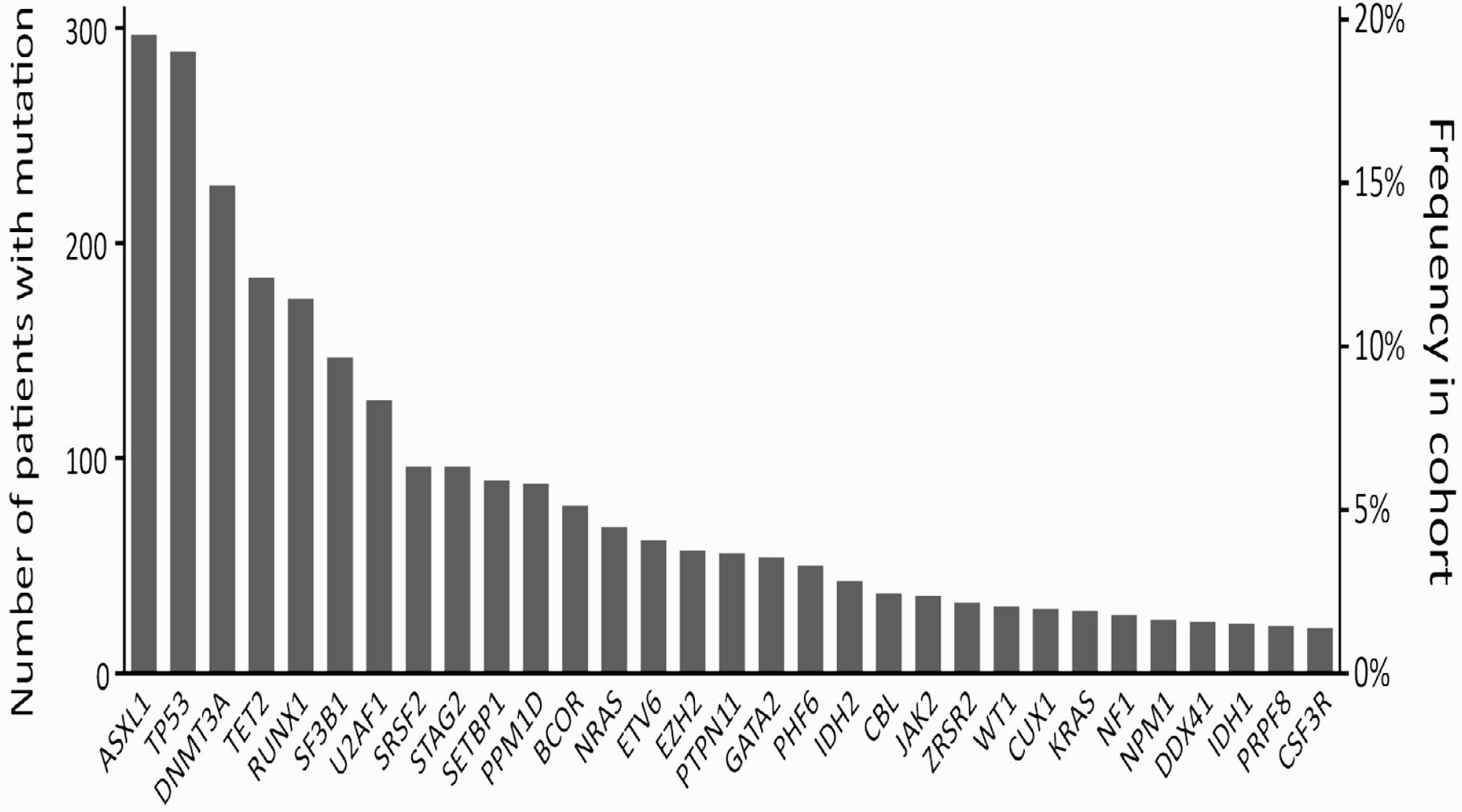
- Broadly representative: 130 transplant centers
- Uniform diagnosis: MDS
  - No CMML or MDS/MPN
  - Blasts <20%
- Year of transplant: 2005 – 2014

Analysis

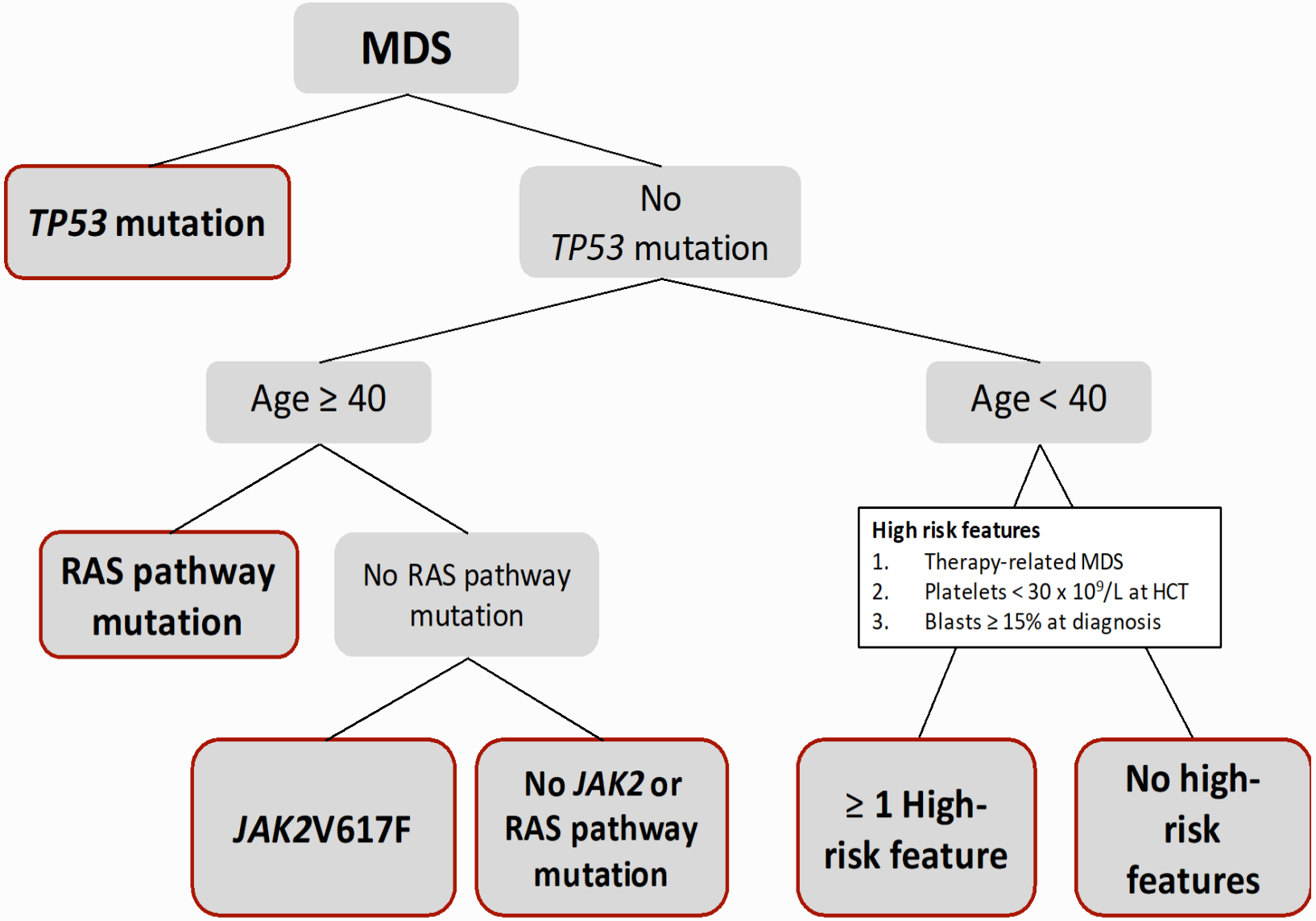
- Samples: pre-HCT whole blood (NMDP biorepository)
- Targeted sequencing: 129 candidate genes
  - Myeloid malignancies
  - Inherited or acquired bone marrow failure
- Clinical annotation: CIBMTR research database

Overview of mutations

3497 mutations in 65 genes, ≥ 1 mutation in 79% of patients

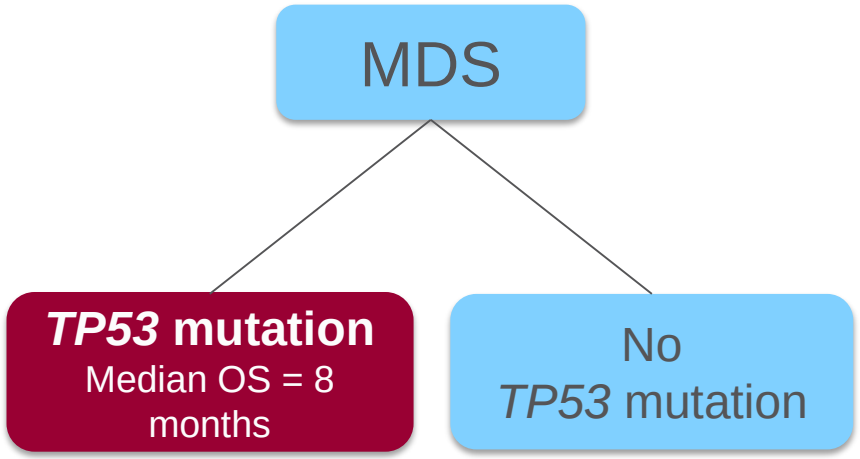


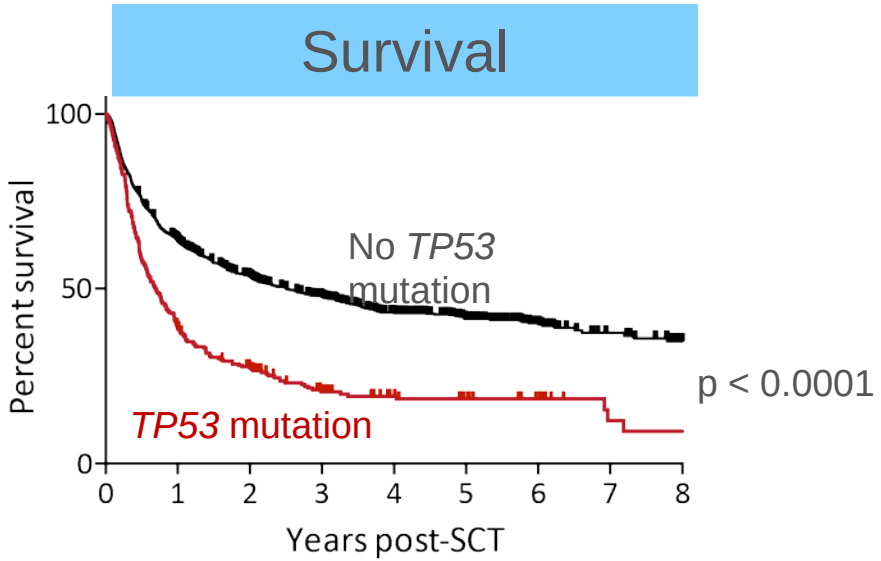
Multivariable Model for Overall Survival

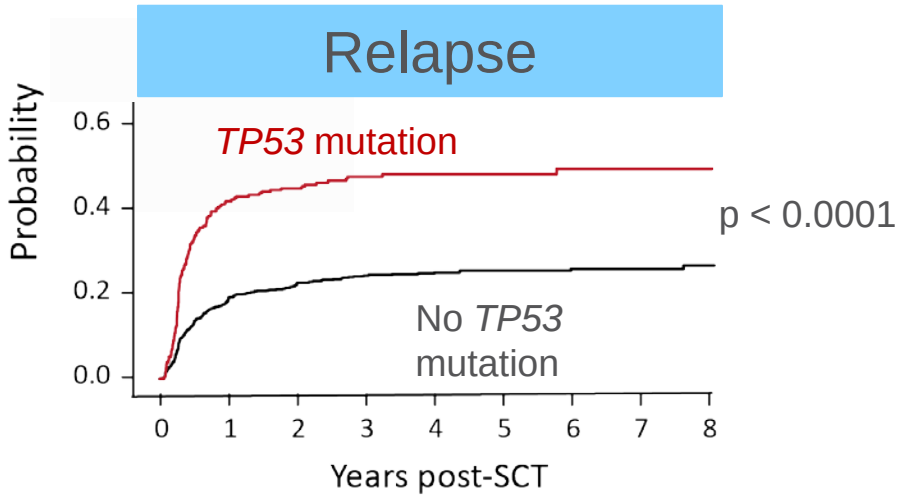


TP53 mutated MDS

Poor prognosis due to early relapse







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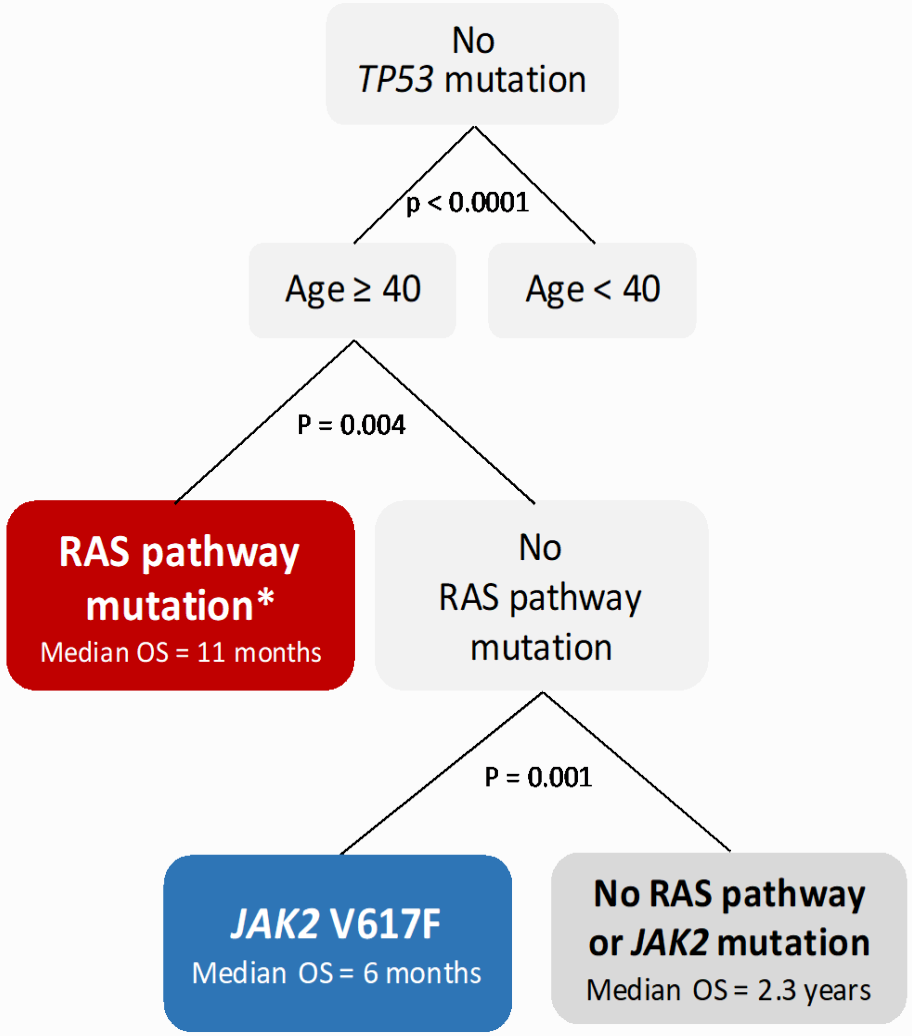
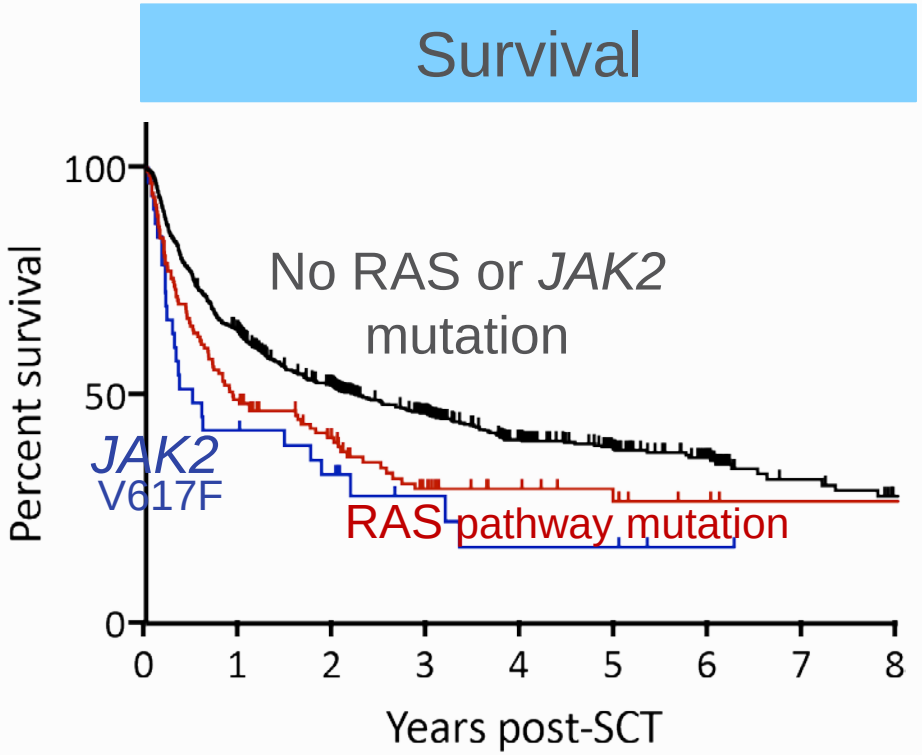
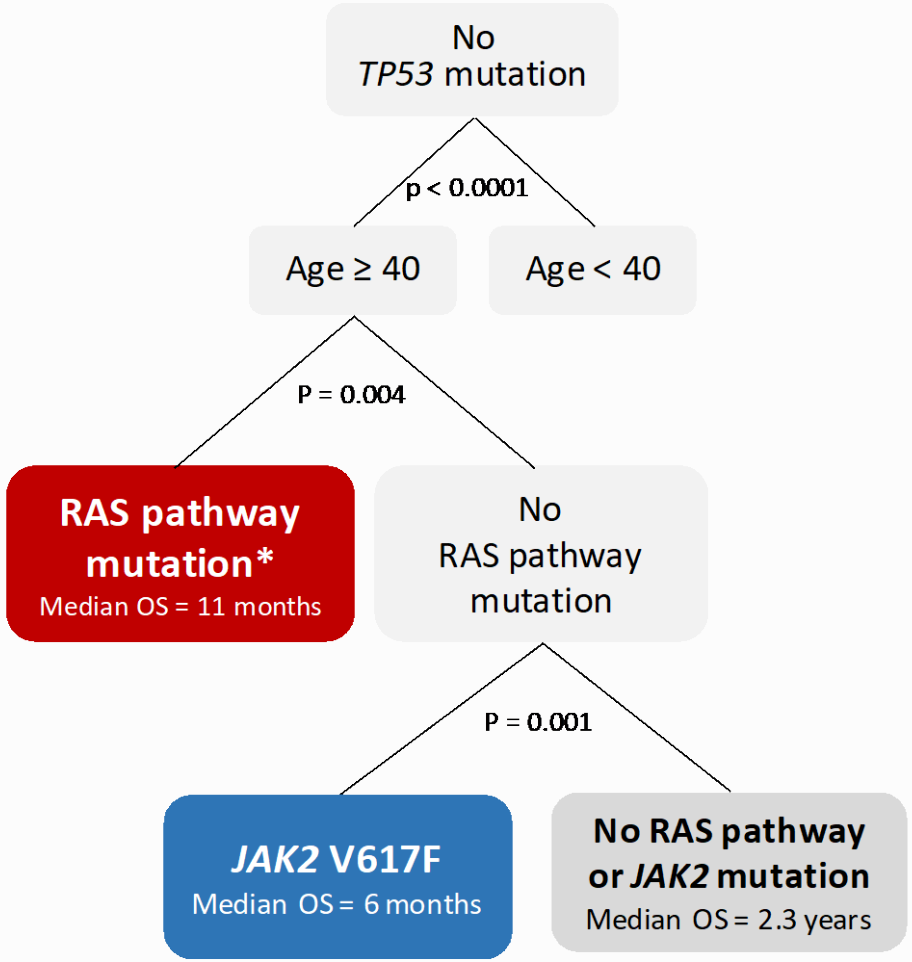
JAK2 and RAS pathway mutations in patients without *TP53* mutations



*RAS pathway: *NRAS, KRAS, CBL, PTPN11, NF1, RIT1, KIT, FLT3*

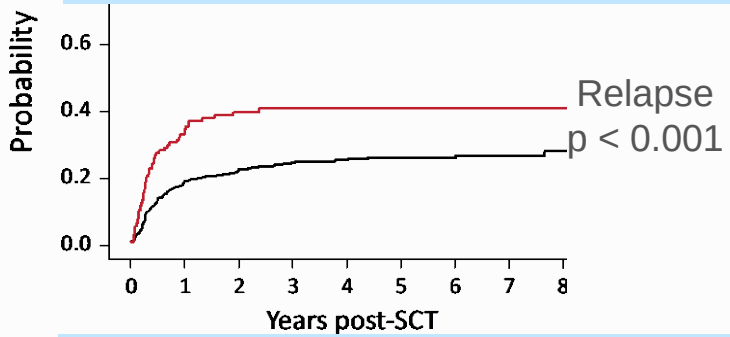


JAK2 and RAS pathway mutations in patients without *TP53* mutations

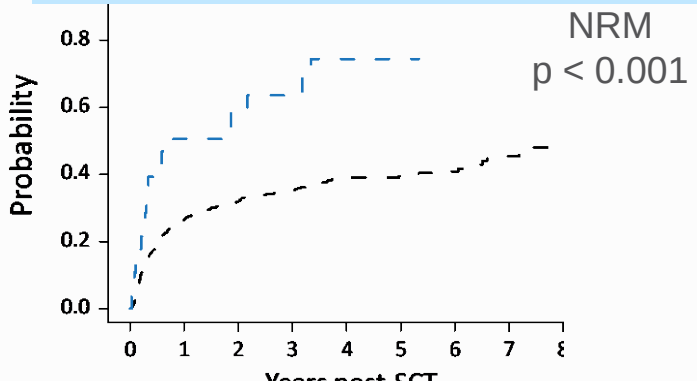


*RAS pathway: *NRAS, KRAS, CBL, PTPN11, NF1, RIT1, KIT, FLT3*

RAS pathway
Poor prognosis driven by relapse



***JAK2* V617F**
Poor prognosis driven by NRM

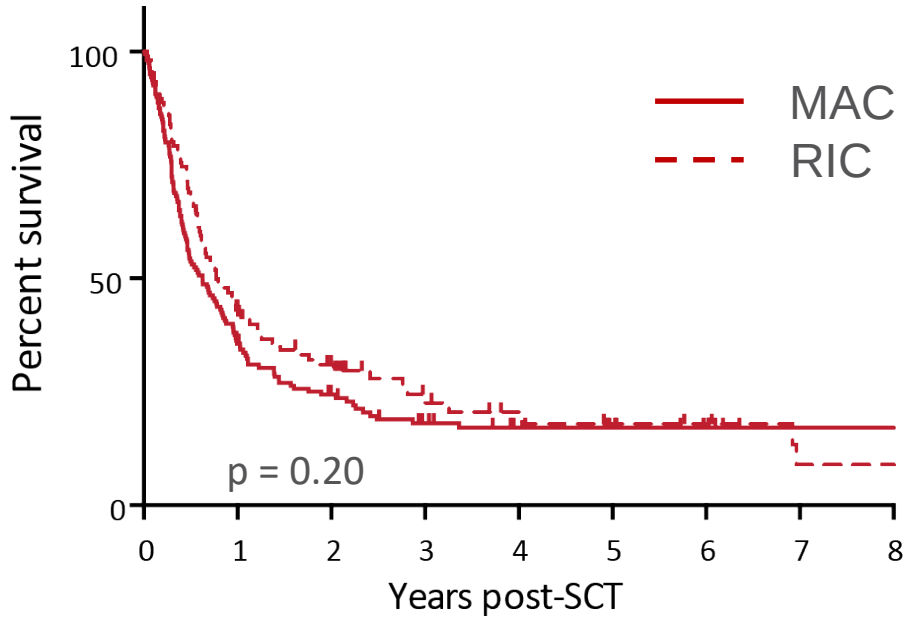


Does dose escalation (in TP53/RAS) of conditioning regimen intensity improve outcome?

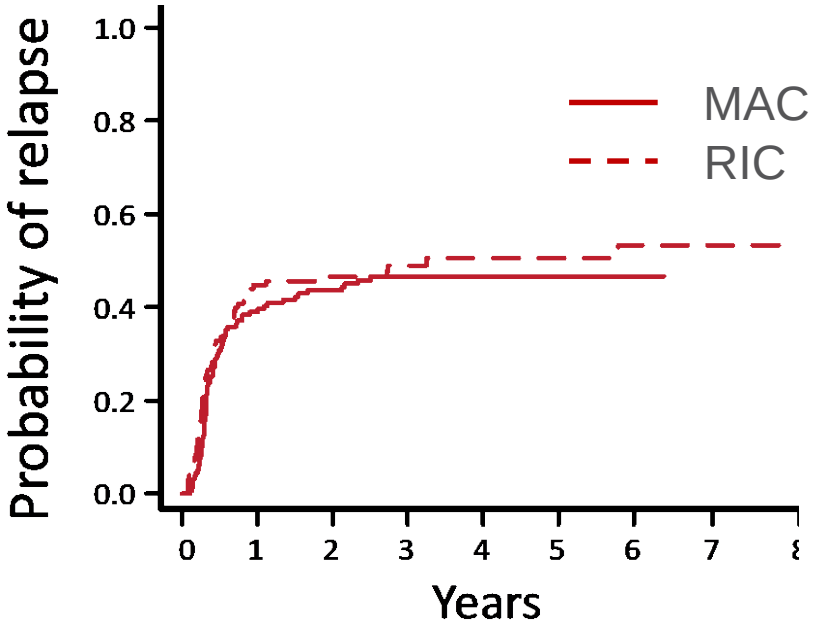
TP53 mutation

Myeloablative conditioning does not improve outcome

TP53 mutation
Overall Survival



TP53 mutation
Relapse



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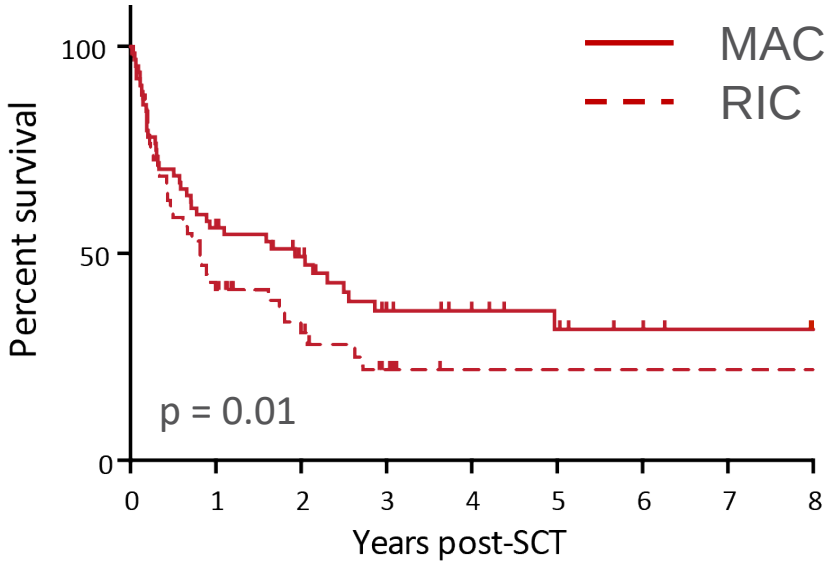
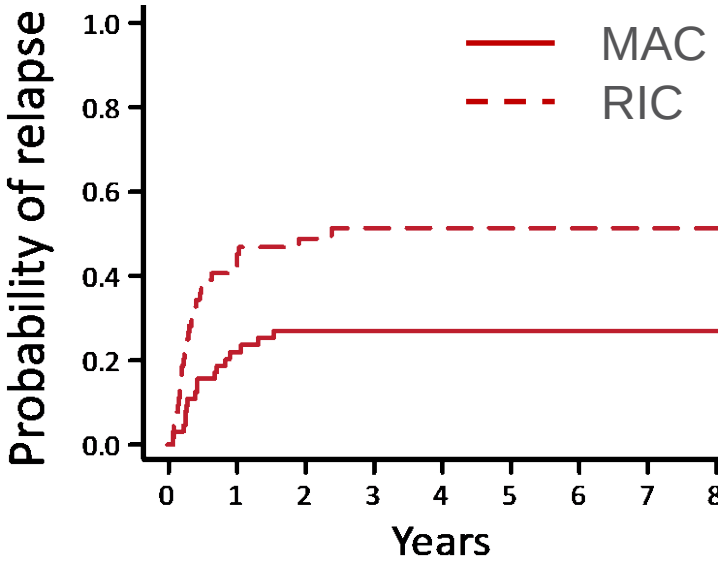
RAS pathway mutation

Myeloablative conditioning improves survival and reduces relapse

RAS pathway
Overall Survival



RAS pathway
Relapse



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Conclusions

TP53 mutations

- Poor prognosis, independent of age
  - Long-term survivors (20%)
  - No benefit to myeloablative conditioning

RAS pathway and *JAK2* mutations

- Poor prognosis in patients ≥ 40 without *TP53* mutations
  - RAS: high early relapse, improved OS and relapse with MAC
  - JAK2: high NRM, no decrease in NRM with RIC

A Personalized Prediction Model for Outcomes after Allogeneic Hematopoietic Stem Cell Transplant in Patients with Myelodysplastic Syndromes (MDS)

There are no conflicts of interest to disclose.

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Writing Committee

Aziz Nazha, Zhen-Huan Hu, Wang Tao, Betty Hamilton, Navneet Majhail, Coleman Lindsley, Ronald Sobecks, Uday Popat, Bart Scott, Wael Saber

On behalf of the CIBMTR® Chronic Leukemia Working Committee CIBMTR® is a research collaboration between National Marrow Donor Program®/Be The Match® and Medical College of Wisconsin.

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Background

Inclusion criteria:

- ✓ Pts diagnosed with MDS (WHO 2008) and registered at the CIBMTR database (2005-2014)
- ✓ Blasts < 20%

- Panel of **129** gene mutations
- Outcomes: **OS**, **Relapse**
- **RSF** algorithm was used to build the new model
- C-index used to evaluate the fit of the proposed model

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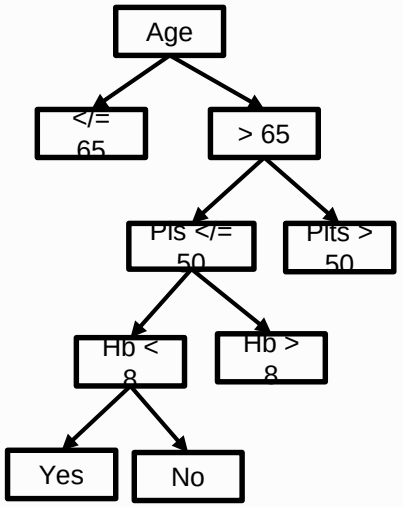
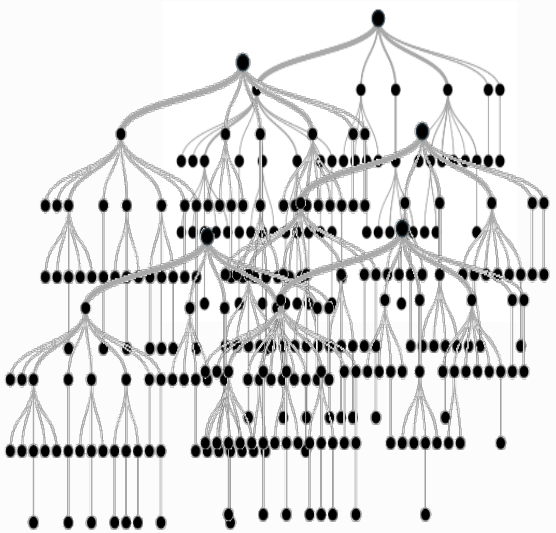
Methods: Machine Learning Model

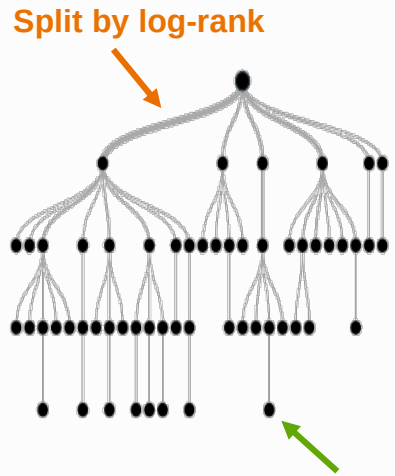
Decision-Tree



Random Forest



Random Survival Forest



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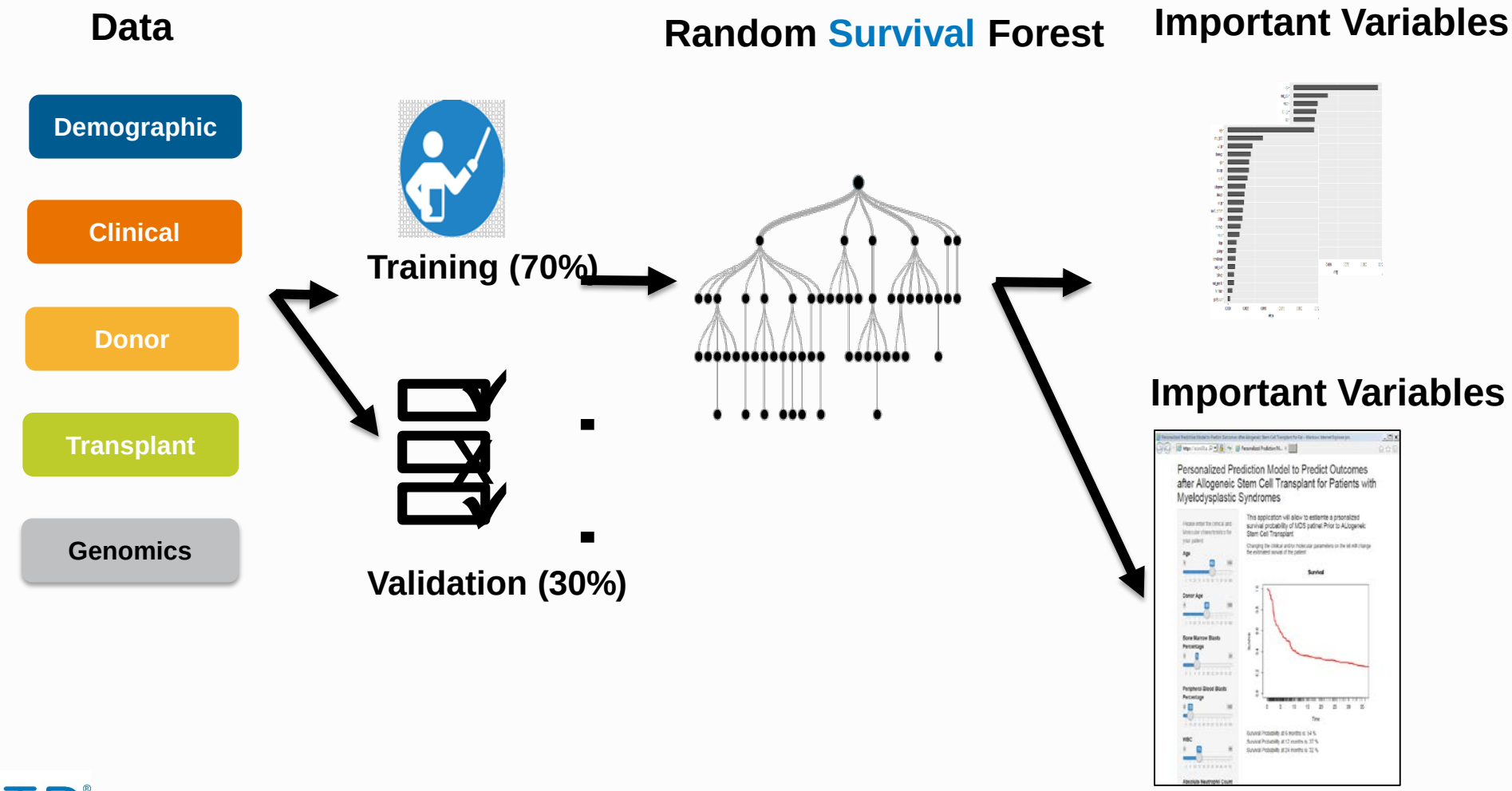
Results: New Model Building



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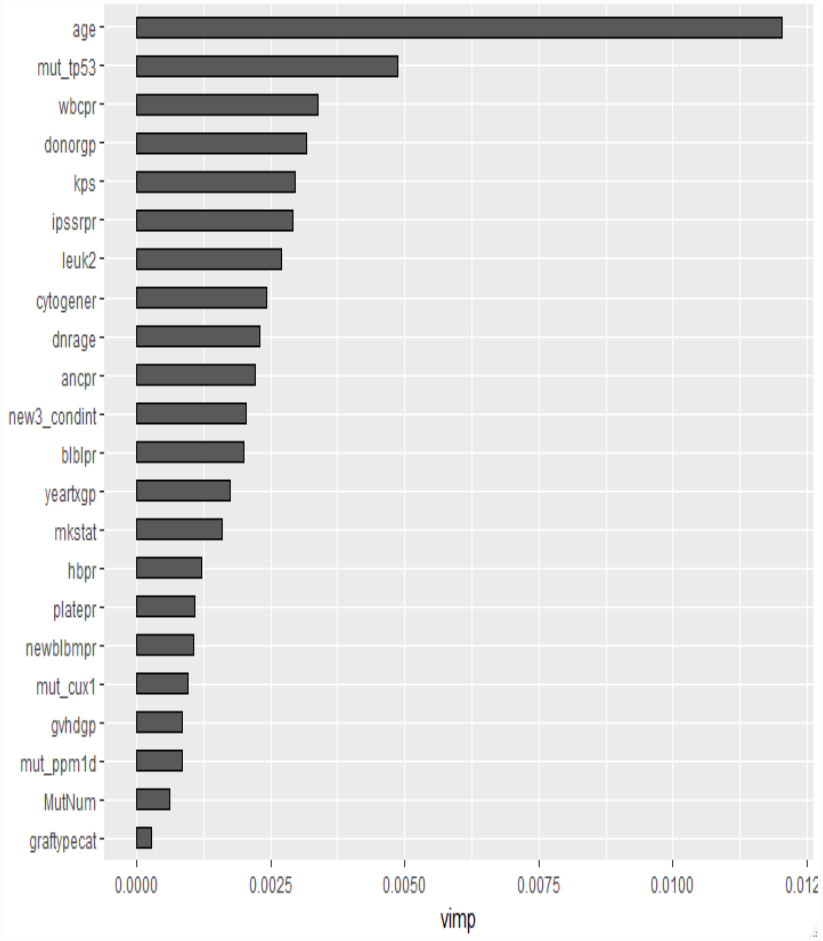
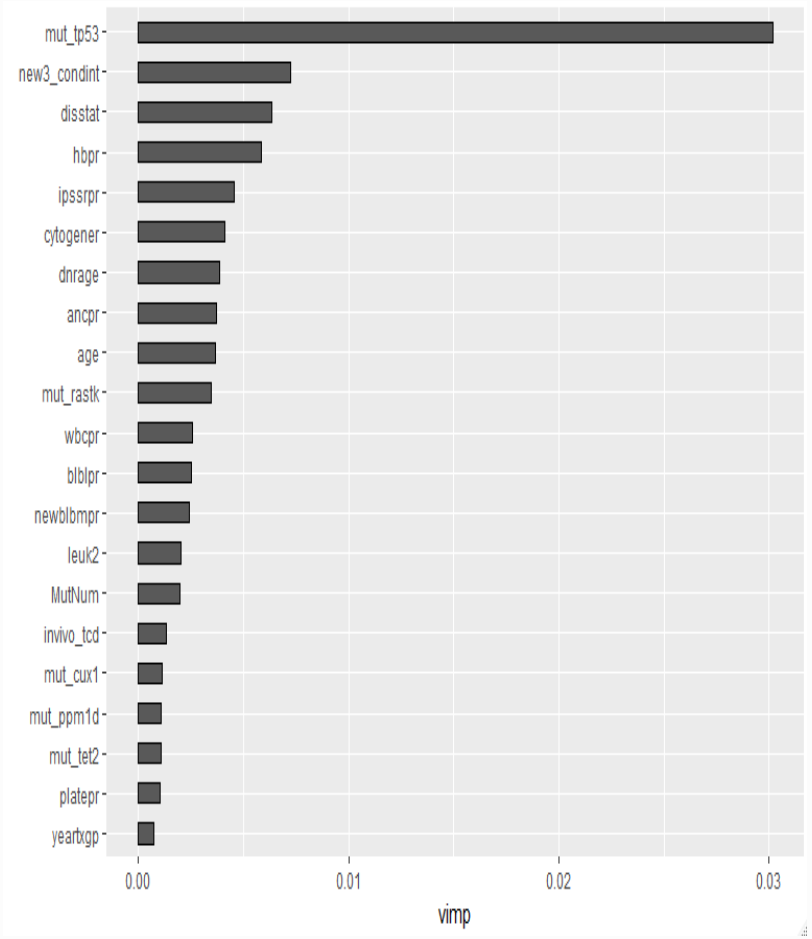
Results: Important Variables

OS



Relapse



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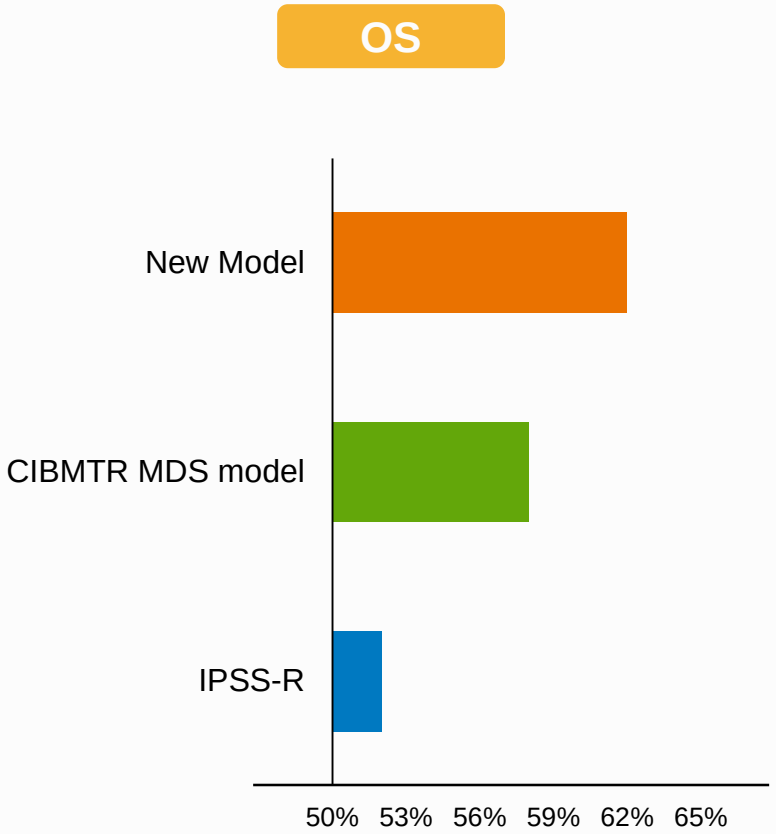
Results: C-index



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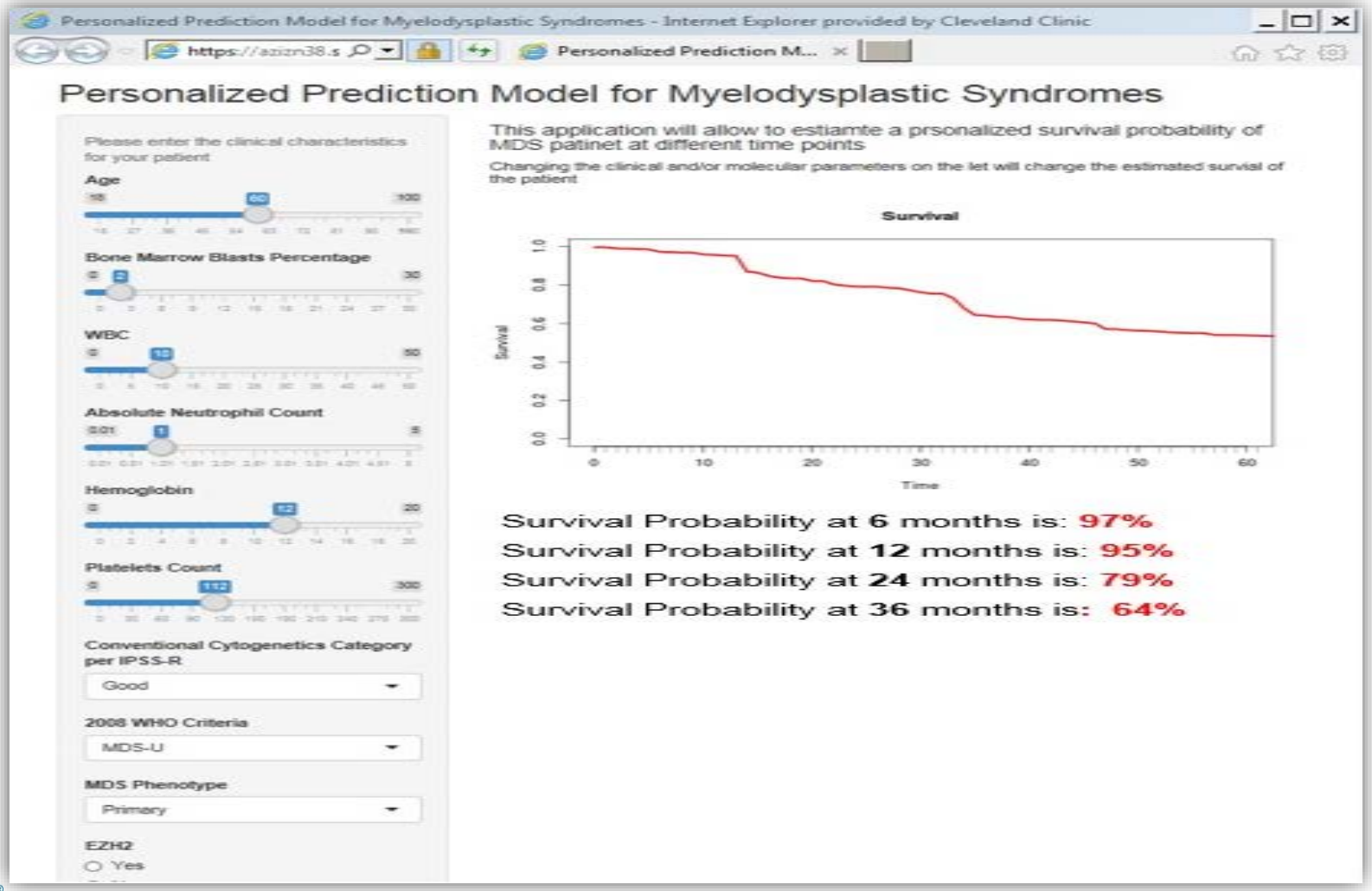
Results: Clinical Application



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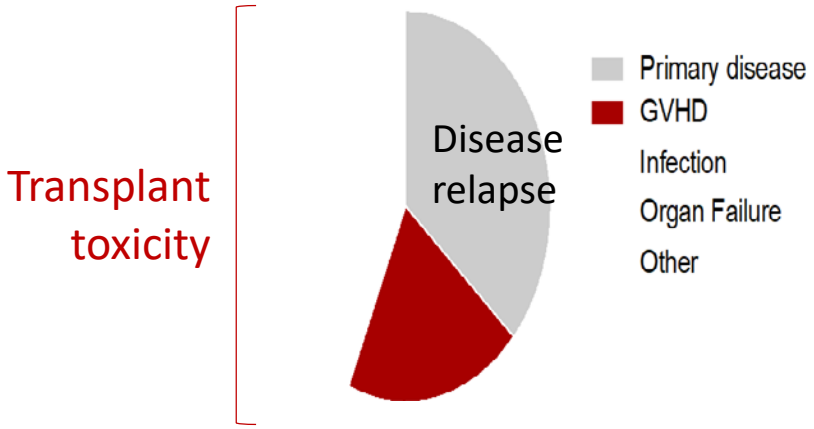
Myelodysplastic Syndrome

Allogeneic transplantation

Cause of death



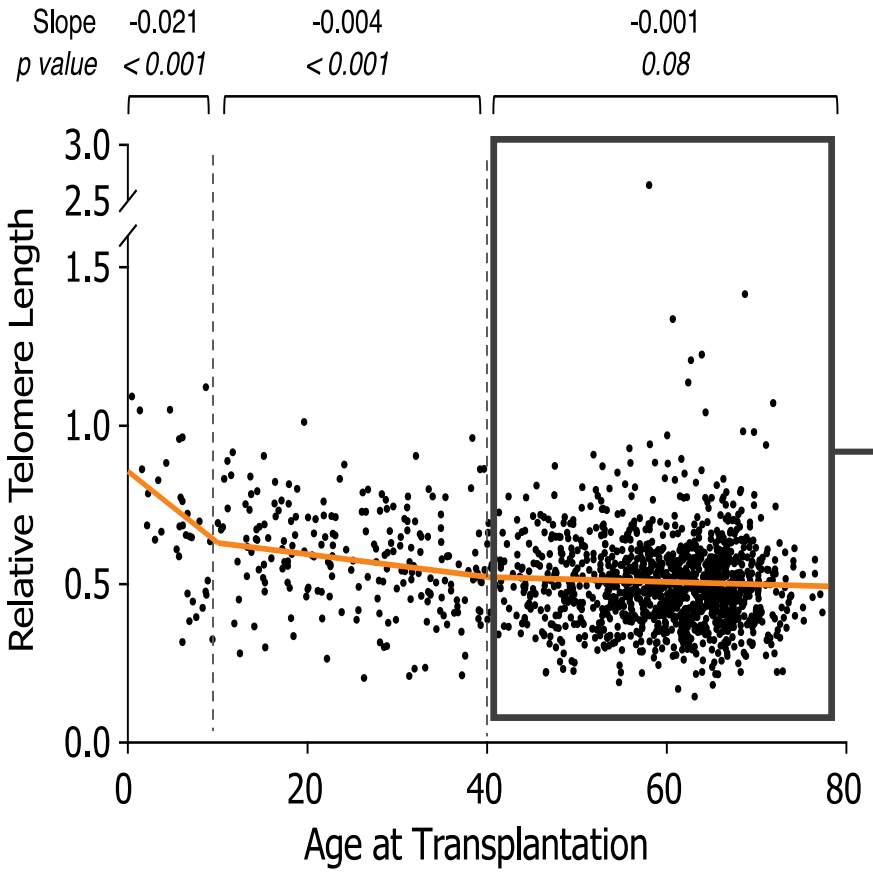
Conditioning intensity and outcome

	Relapse	Toxicity
Myeloablative	↓	↑
Reduced-intensity	↑	↓

Pasquini MC, Zhu X.

Telomere length in MDS

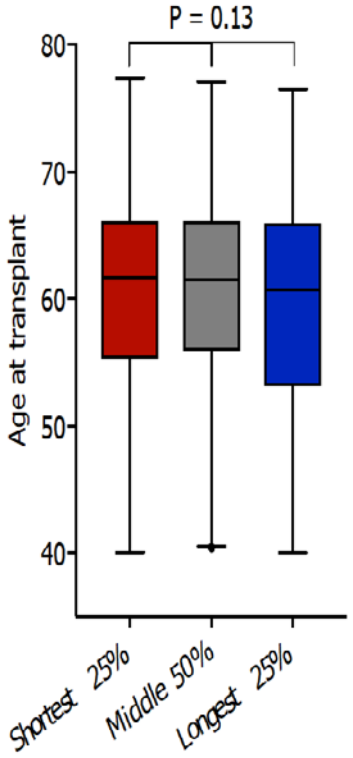
6 months to 77 years of age



Longest 25%
317 patients

Middle 50%
633 patients

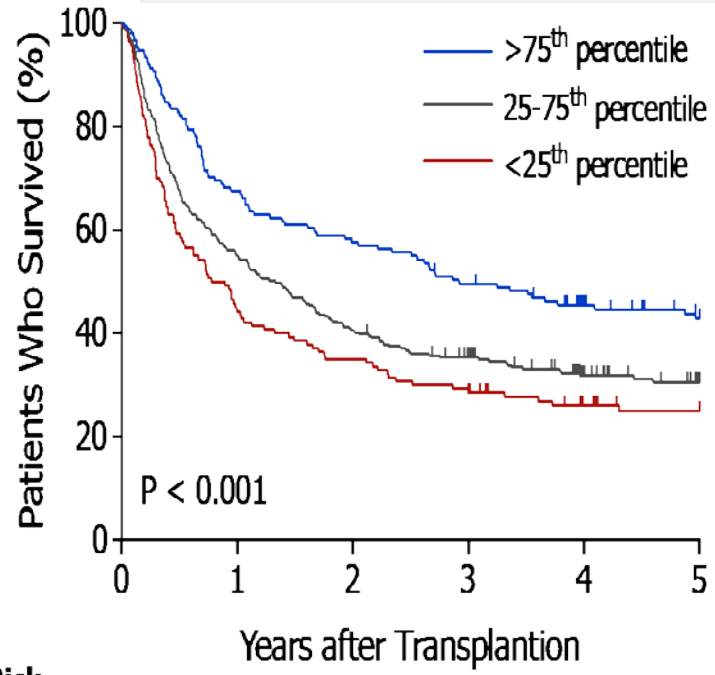
Shortest 25%
317 patients



Telomere length

Overall survival outcomes based on conditioning regimen

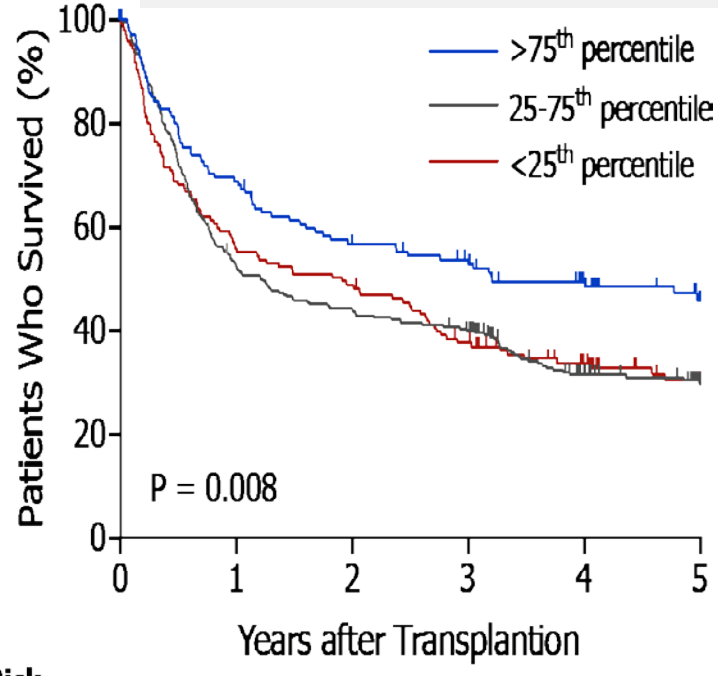
Myeloablative
(n=582)



No. at Risk

>75th percentile	151	102	87	74	58	46
25-75th percentile	291	160	118	97	69	49
<25th percentile	140	62	49	40	27	21

Reduced-intensity
(n=554)



No. at Risk

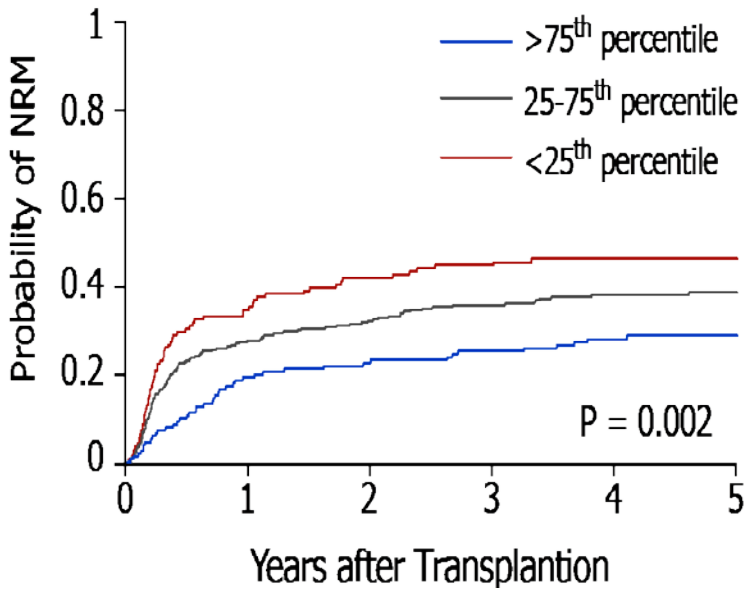
>75th percentile	136	93	74	66	49	31
25-75th percentile	274	143	120	107	65	44
<25th percentile	145	81	71	53	37	24

Shorter telomeres

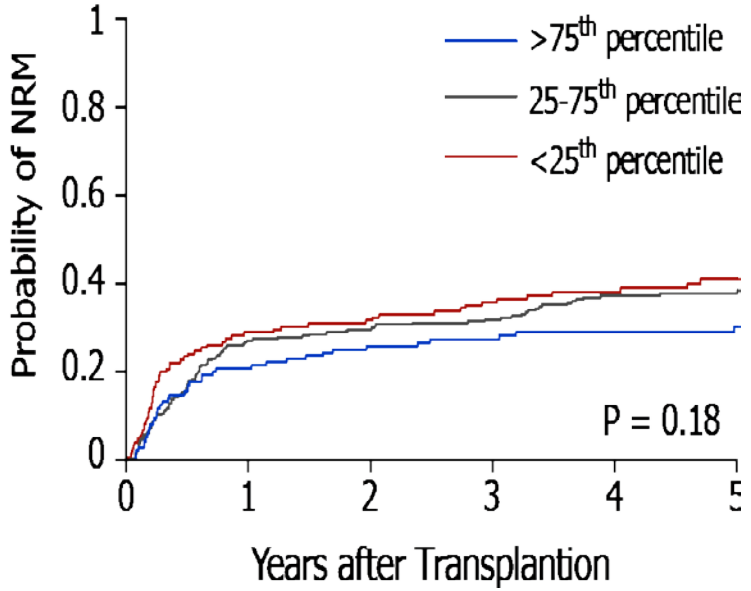
Increased NRM in patients receiving MAC

Non-relapse mortality

Myeloablative
(n=582)



Reduced-intensity
(n=554)

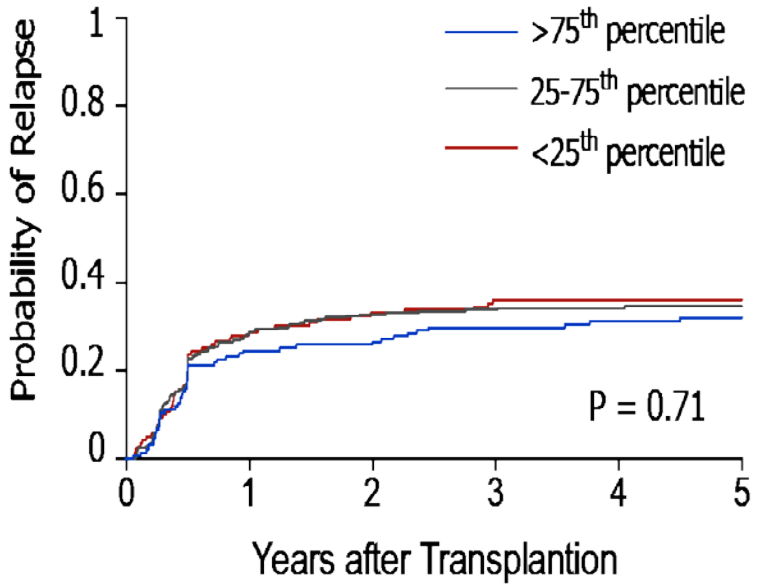


Shorter telomeres

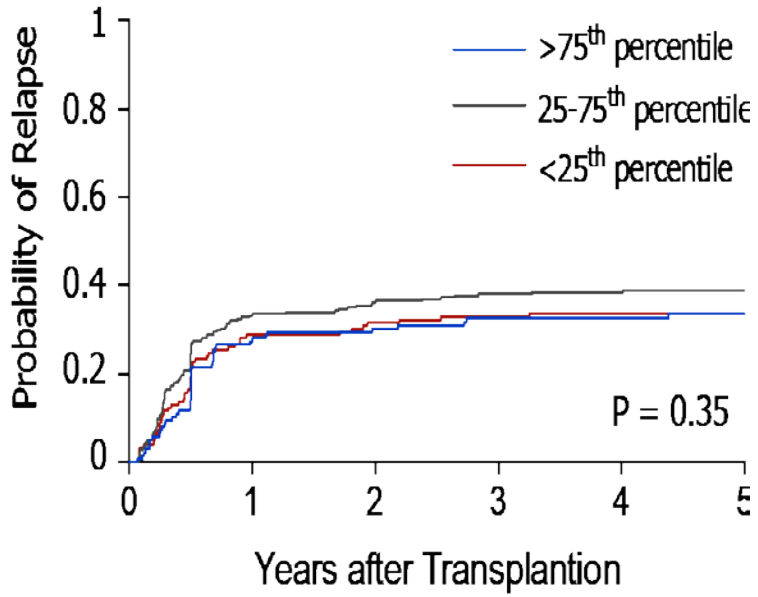
No impact on relapse risk

Relapse

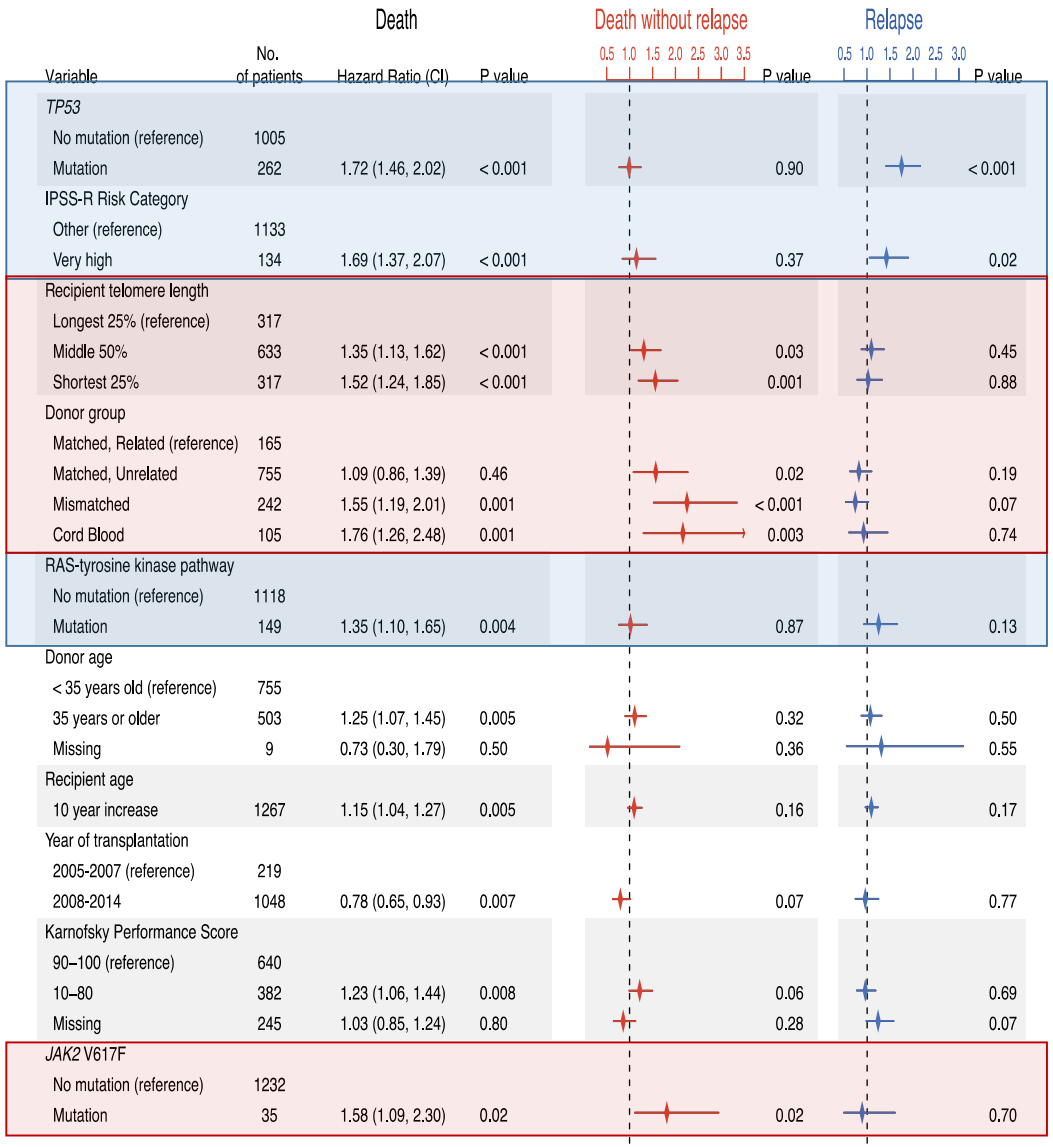
Myeloablative
(n=582)



Reduced-intensity
(n=554)



Multivariable models



Variable	No. of patients	Hazard Ratio (CI)	P value	P value	P value
TP53					
No mutation (reference)	1005				
Mutation	262	1.72 (1.46, 2.02)	< 0.001	0.90	< 0.001
IPSS-R Risk Category					
Other (reference)	1133				
Very high	134	1.69 (1.37, 2.07)	< 0.001	0.37	0.02
Recipient telomere length					
Longest 25% (reference)	317				
Middle 50%	633	1.35 (1.13, 1.62)	< 0.001	0.03	0.45
Shortest 25%	317	1.52 (1.24, 1.85)	< 0.001	0.001	0.88
Donor group					
Matched, Related (reference)	165				
Matched, Unrelated	755	1.09 (0.86, 1.39)	0.46	0.02	0.19
Mismatched	242	1.55 (1.19, 2.01)	0.001	< 0.001	0.07
Cord Blood	105	1.76 (1.26, 2.48)	0.001	0.003	0.74
RAS-tyrosine kinase pathway					
No mutation (reference)	1118				
Mutation	149	1.35 (1.10, 1.65)	0.004	0.87	0.13
Donor age					
< 35 years old (reference)	755				
35 years or older	503	1.25 (1.07, 1.45)	0.005	0.32	0.50
Missing	9	0.73 (0.30, 1.79)	0.50	0.36	0.55
Recipient age					
10 year increase	1267	1.15 (1.04, 1.27)	0.005	0.16	0.17
Year of transplantation					
2005-2007 (reference)	219				
2008-2014	1048	0.78 (0.65, 0.93)	0.007	0.07	0.77
Karnofsky Performance Score					
90–100 (reference)	640				
10–80	382	1.23 (1.06, 1.44)	0.008	0.06	0.69
Missing	245	1.03 (0.85, 1.24)	0.80	0.28	0.07
JAK2 V617F					
No mutation (reference)	1232				
Mutation	35	1.58 (1.09, 2.30)	0.02	0.02	0.70

Future Direction

“Precision Medicine Initiative to optimize HCT outcomes in MDS”

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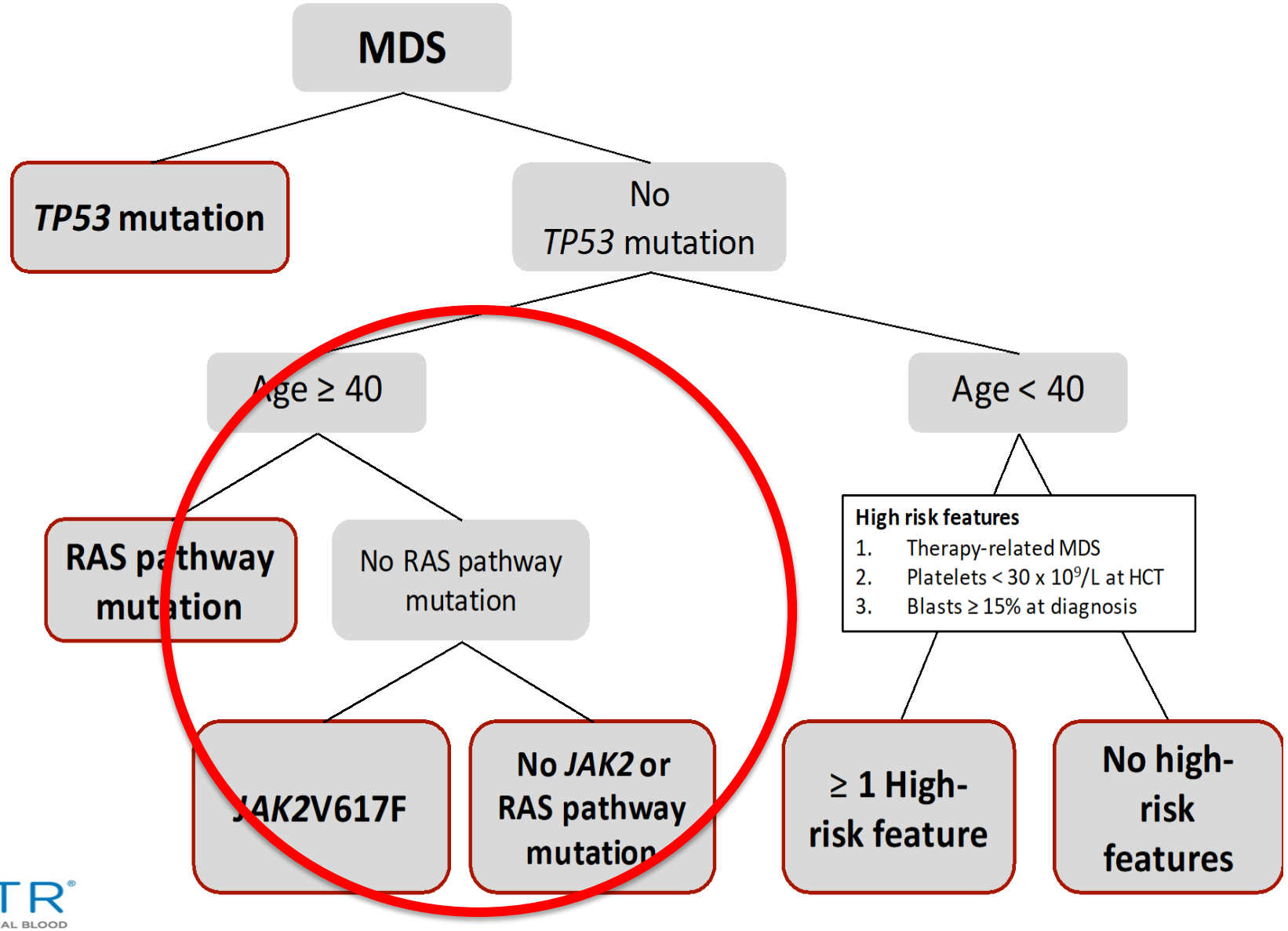
Multivariable Model for Overall Survival



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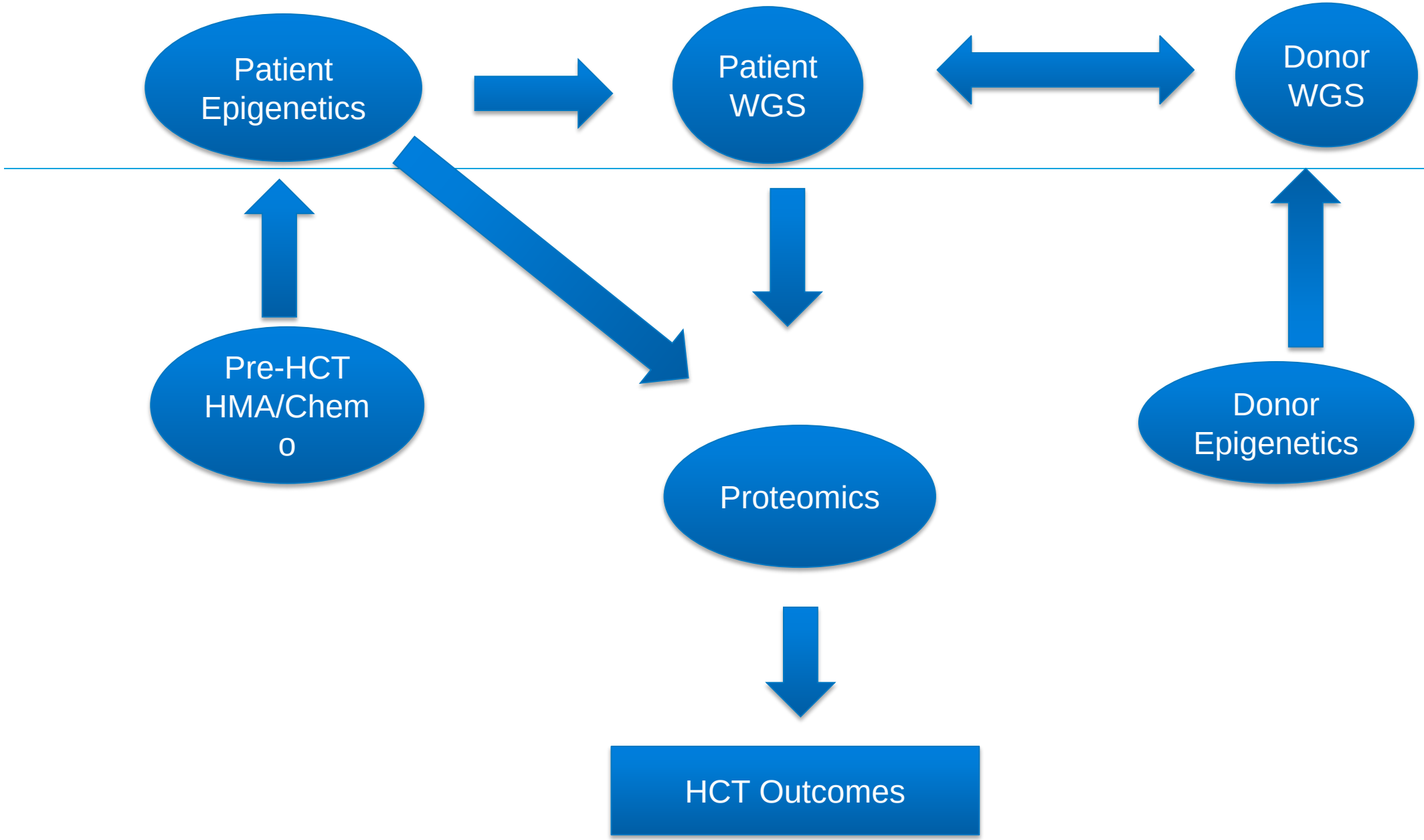

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Conclusions

-High quality centrally processed samples linked to clinically annotated database is an extremely valuable resource

-Big data, hypothesis free projects are expected to become the forefront and we need to position ourselves in order to lead

-A PMI TF is currently being assembled to provide recommendations to CIBMTR Advisory board so that the CIBMTR is well positioned

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Research Sample Life Cycle: Acute Graft-versus-Host Disease

Shernan Holtan, MD
University of Minnesota

November 9, 2018

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Outline

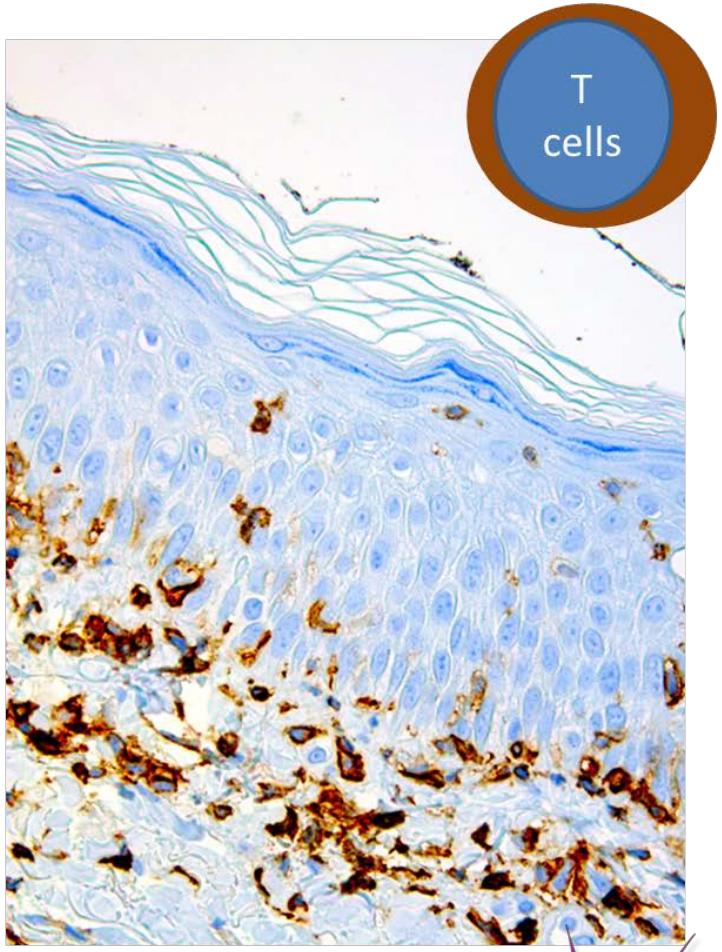
- Acute GVHD: models vs the human condition
- Patient story
- Using stored BMT CTN samples to test novel hypotheses
  - Epidermal growth factor (EGF)
  - Amphiregulin (AREG)
- New diagnostic and therapeutic options based on BMT CTN samples
  - Human chorionic gonadotropin/epidermal growth factor (off-label)
- Future directions

Acute GVHD Overview

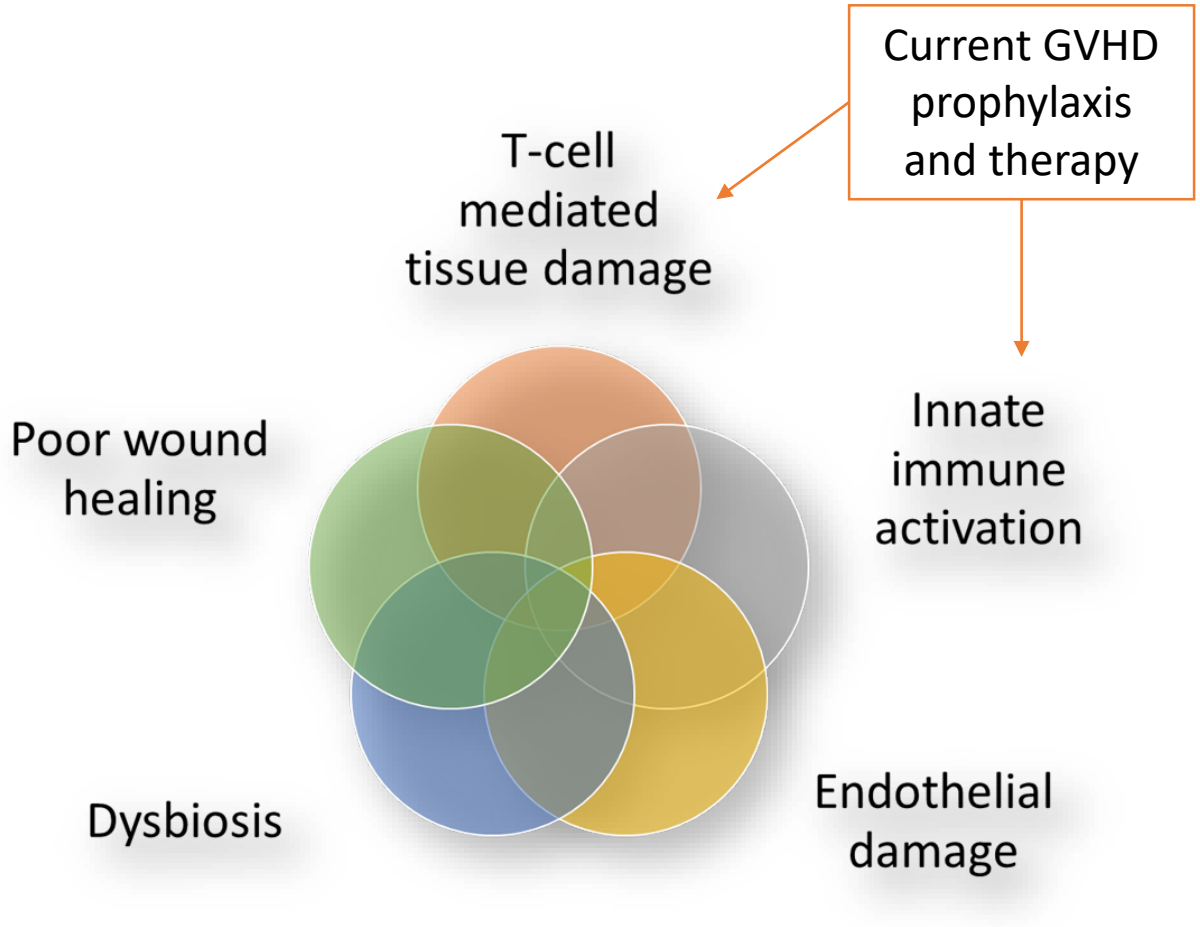
Acute GVHD
First 100 days (classic)
Rapid onset (days)
T-cell mediated

Target organs:
- Skin
- GI tract
- Liver

- Common complication of allogeneic hematopoietic cell transplantation
- Essentially all prophylaxis and treatment of GVHD targets the GRAFT
  - ATG
  - Campath
  - CD34 selection
  - CNI, sirolimus, etc…



GVHD models vs the clinical syndrome



He and Holtan (2018) Current Hematol Malig Reports

Meet “Steve”

- 60 year-old male
- Day +163 post-matched sibling BMT
- Low-grade fevers
- Subtle skin rash
- Feels nauseated
- Can’t eat
- Having 8-10 diarrhea episodes/day
- Diagnosed and treated for C diff

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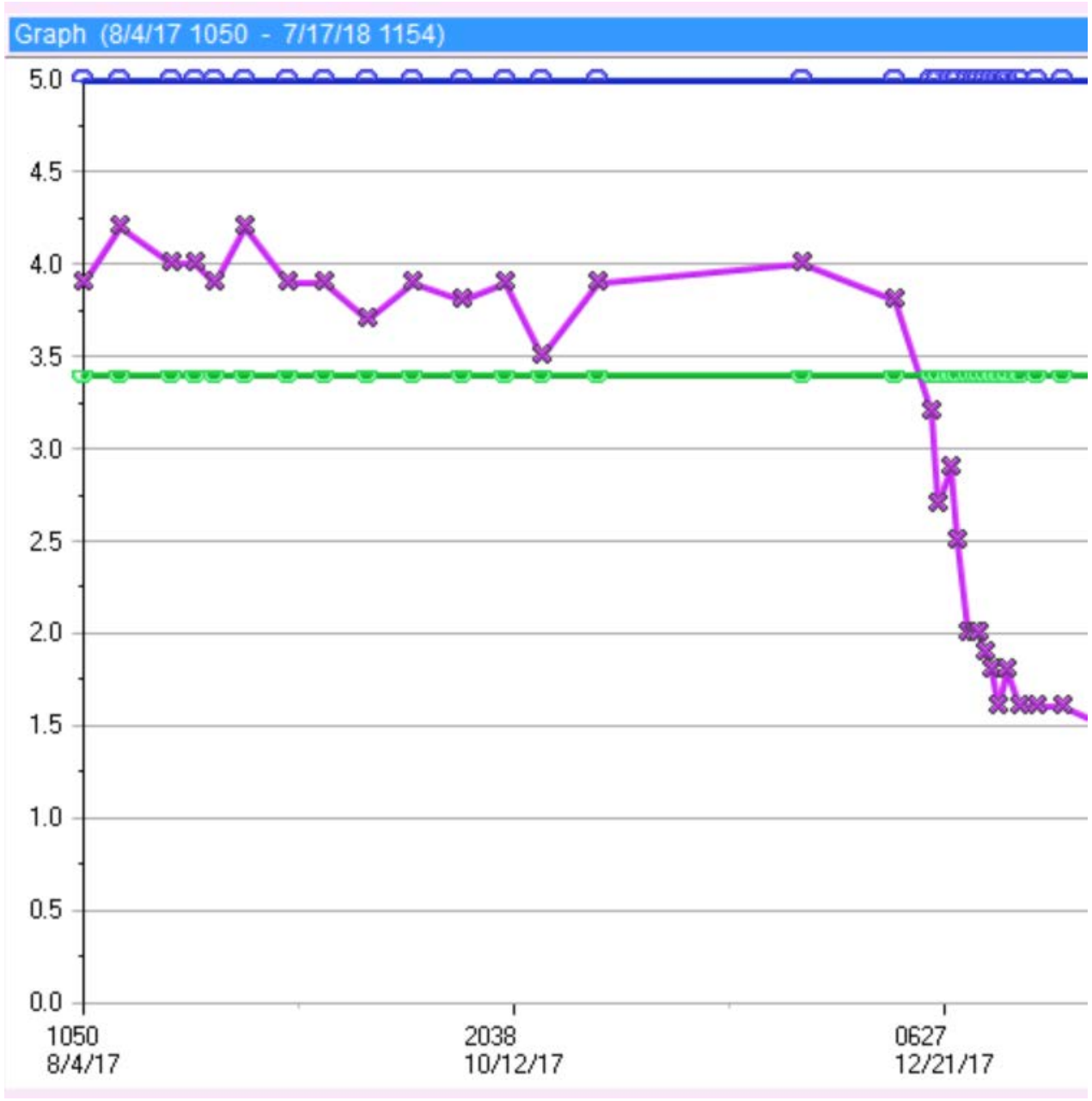
Grab your cape.

Let’s go!



- Serum albumin drops down to 1.4
- Does not get better with PO vancomycin
- Started on TPN and methylprednisolone for probable GVHD, undergoes endoscopy

Grab your cape.

SPECIMEN(S):
A: Duodenal biopsy
B: Stomach biopsy

FINAL DIAGNOSIS:
A. DUODENAL BIOPSY:
- Mild active duodenitis
- Apoptotic bodies identified, consistent with mild graft versus host disease (GVHD)

B. STOMACH BIOPSY:
- Mild chronic gastritis
- Apoptotic bodies identified, consistent with mild graft versus host disease (GVHD)
- No H. pylori like organisms identified on routine staining
- Negative for intestinal metaplasia or dysplasia

COMMENT:
CMV IHC is in progress and the results will be submitted in an addendum report

I have personally reviewed all specimens and/or slides, including the listed special stains, and used them
with my medical judgement to determine or confirm the final diagnosis.

Electronically signed out by:

Mahmoud Khalifa, M.D., PhD, UMPhysicians

Endoscopy with biopsy shows mild acute GVHD.

Does he really have “mild” acute GVHD?

How can we tell?

Grab your cape.

Addition of biomarkers to clinical risk

- Ann Arbor (AA) biomarkers ST2 (inflammation and damage) and REG3a (GI damage) tested at GVHD onset:
  - AA1 and AA2 = less severe disease, but candidates for BMT CTN 1501
  - AA3 = more severe disease, excluded from BMT CTN 1501
- University of Minnesota approach in development:
  - Imbalance of circulating tissue repair factors
  - Prognostic information comes from:
    - Severity of damage
    - Likelihood that host can recover from the damage (regenerative capacity)

Using samples from BMT CTN 0302/0802 to test novel hypotheses about recovery from GVHD

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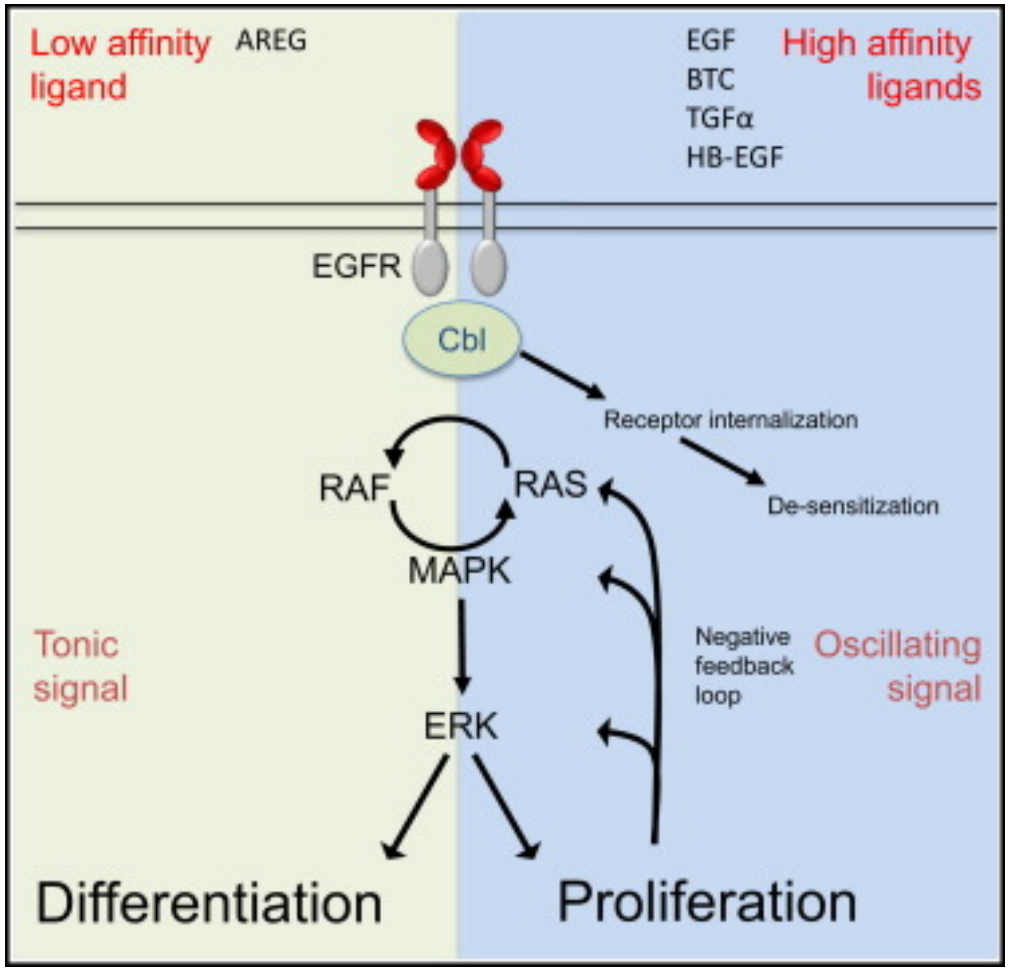
Grab your cape.

Let's go!

Biomarkers of tissue repair in acute GVHD

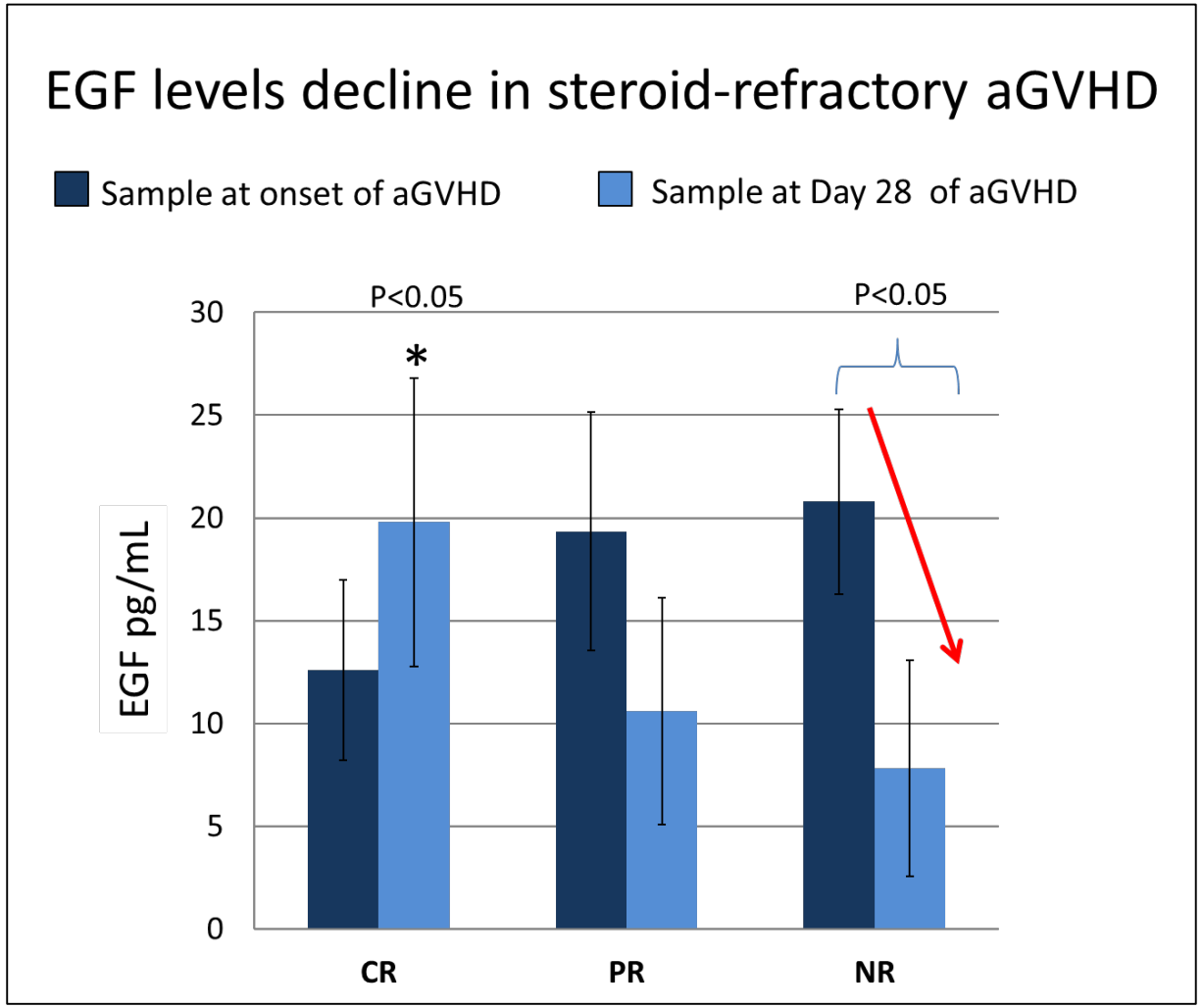
- **Epidermal growth factor (EGF)**
  - Strong growth stimulator
  - Normally ~25-75 pg/ml in plasma
- **Amphiregulin (AREG)**
  - 10x weaker ligand than EGF
  - Normal high expression in GI tract
  - Should not be in circulation



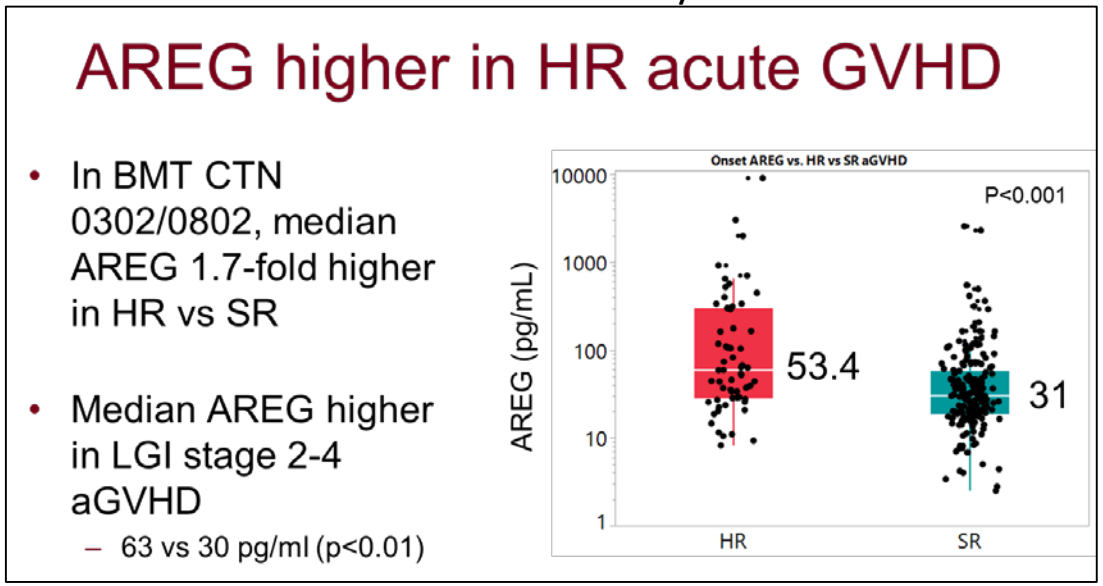
Zaiss et al (2015) Immunity

Plasma EGF is low, AREG is high in severe acute GVHD

BMT CTN 0802 (multicenter acute GVHD clinical trial)



BMT CTN 0302/0802

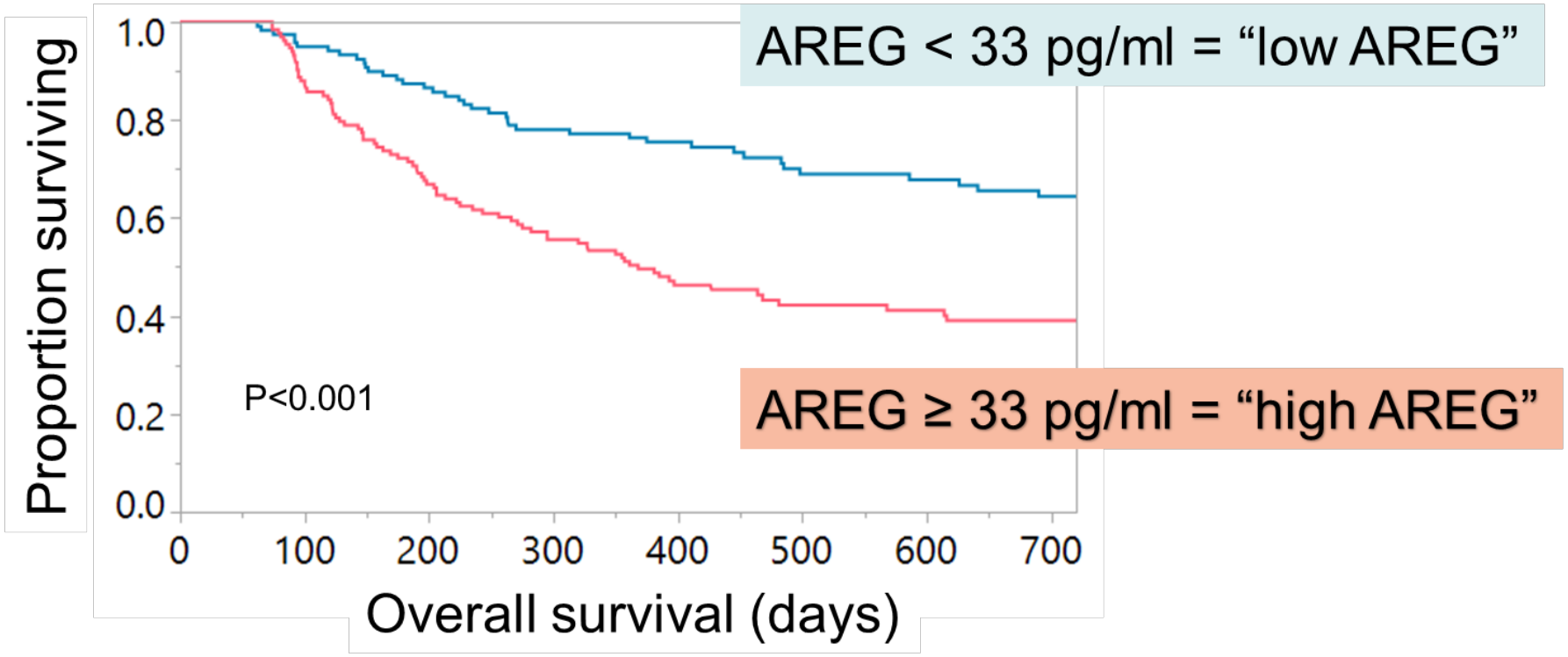


- Elevated AREG/EGF ratio at GVHD diagnosis is associated with a **9.4-fold increased** risk of death
- Our patient's baseline tissue repair biomarkers:
  - AREG = 182.3 pg/ml
  - EGF = 5.3 pg/ml

Holtan et al (2015) BBMT
Holtan et al (2018) Blood Advances
Holtan et al (2016) Blood

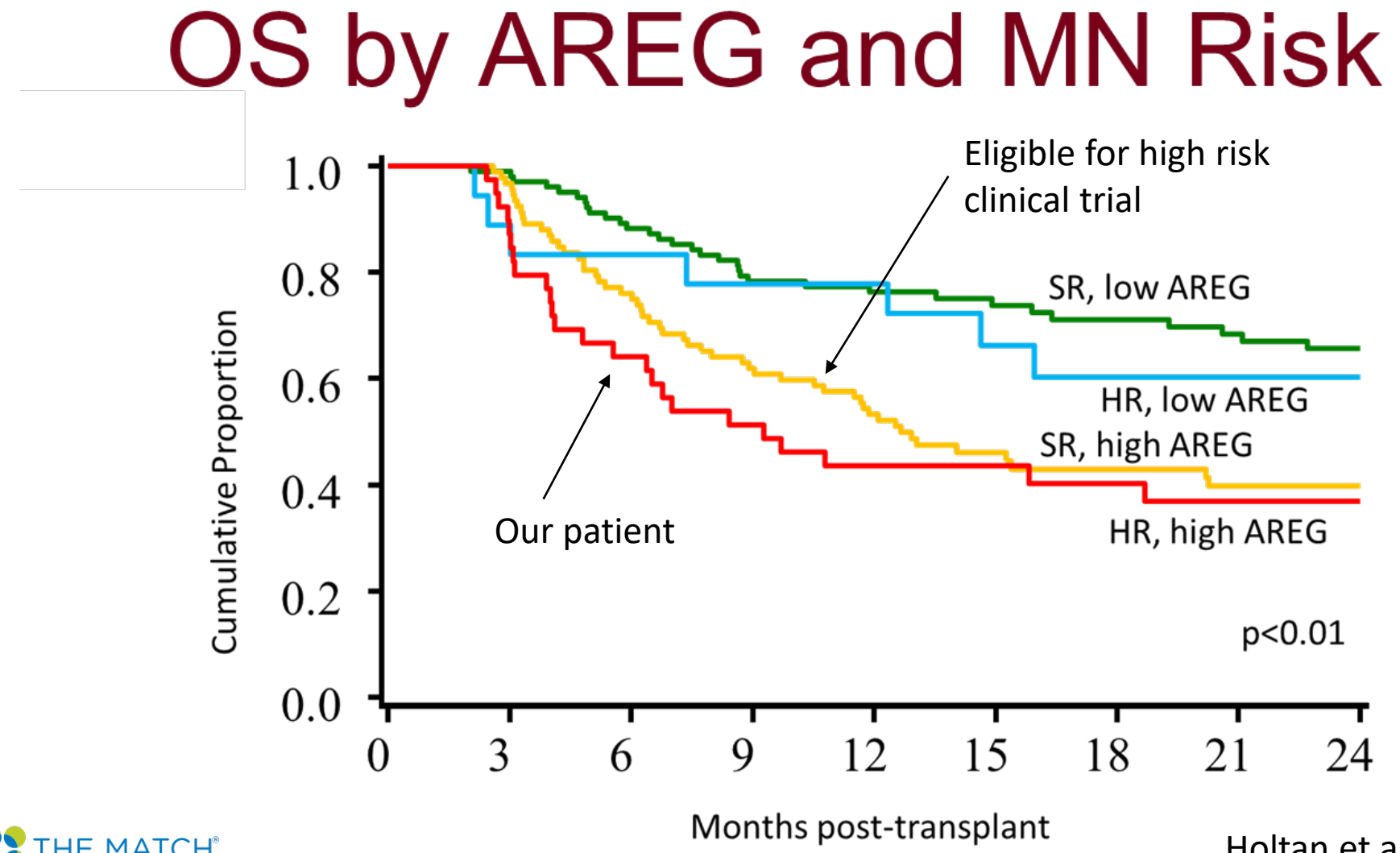
AREG can be tested in serum or plasma (BMT CTN 0302/0802)

AREG cutoff by 2-fold cross-validation



Holtan et al (2018) Blood Advances

AREG-modified Minnesota Acute GVHD Risk Score



Holtan et al (2018) Blood Advances

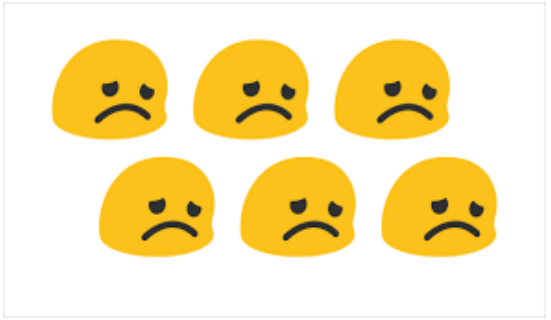
Proportion of plasma AREG to EGF

EGF AREG

Healthy tissue homeostasis

EGF AREG

Unresolved tissue damage



How can we use this for therapy?

Regenerative therapy based upon our tissue repair biomarkers?

- High AREG reflects unresolved damage, little value in inhibiting it
- Increase available EGF, but how?
  - Parenteral recombinant EGF not available

Circulating EGF increases by 20-fold in normal pregnancy

	Pregnant Median (N=16)	Control Median (N=11)	Fold difference	P
	Higher in pregnancy			
GROa	1018.6	341.6	3.0	<0.001
PDGF-AA	10740.6	563.8	19.1	<0.001
TGFa	11.7	1.8	6.5	0.002
EGF	**489.4**	**24.8**	**19.7**	**0.003**
PDGF-AB/BB	10760.7	3410.4	3.2	0.005

Holtan, Chen, Kaimal et al. (2015) *J of Immunol Research*

EGF is concentrated in urinary-derived hCG preparations

EGF median 50,833 pg/ml

Holtan and Panoskaltsis-Mortari (2015) *BMT*
Holtan (unpublished)

MT2014-12

Phase I/II Study of Human Chorionic Gonadotropin and Epidermal Growth Factor Supplementation (Pregnyl®) to Support Tolerance and Repair As Adjunct Therapy in High-Risk or Refractory Acute Graft-Versus-Host Disease

ARM 1
(High Risk)

Standard of care
Steroids

+

Pregnyl supplementation
QOD x 1 week

ARM 2
(Steroid Dependent/ Refractory)

Standard of care
Steroids (increased if dependent)
+

Second line agent (if refractory)

+

Pregnyl supplementation
QOD x 2 weeks

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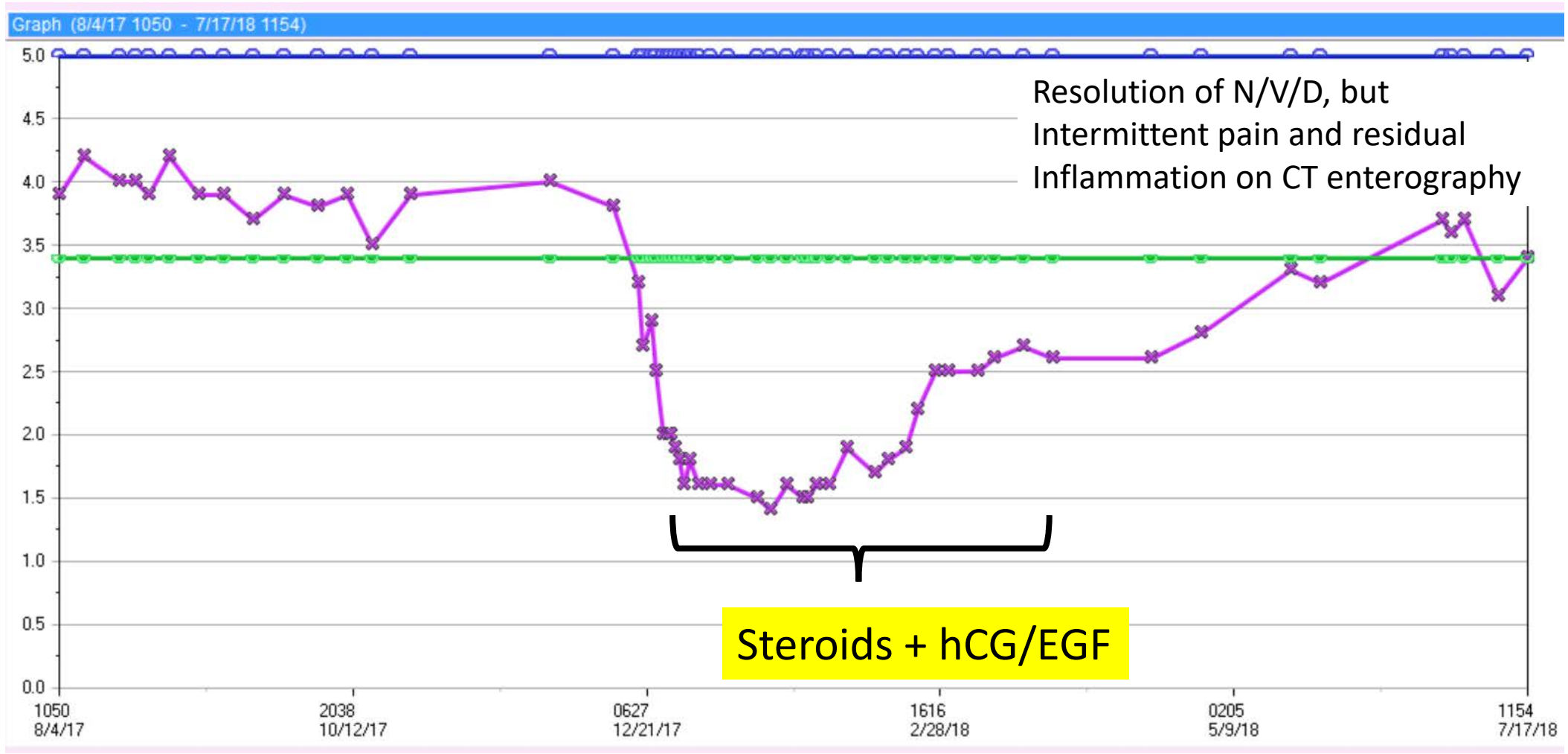
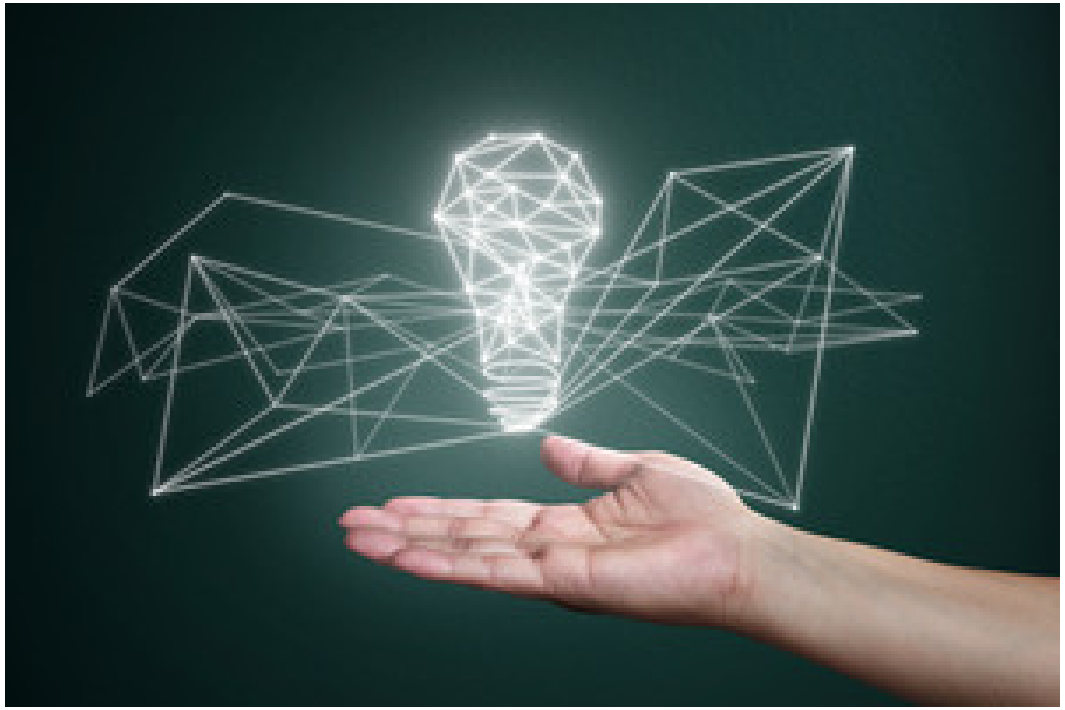
Our patient took >6 months to normalize serum albumin, AREG 21.8 pg/ml and EGF 5.9 pg/ml at end of treatment

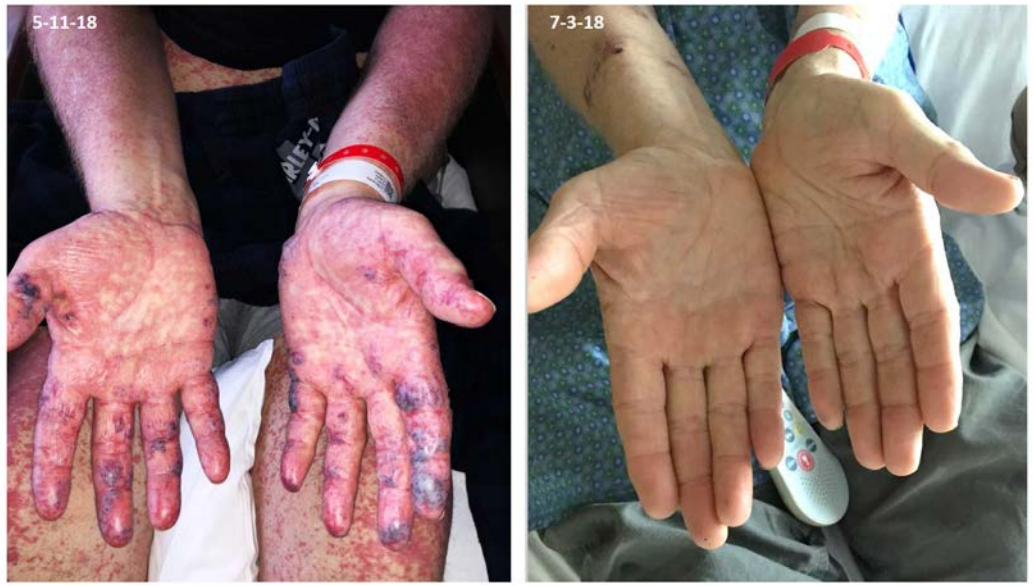


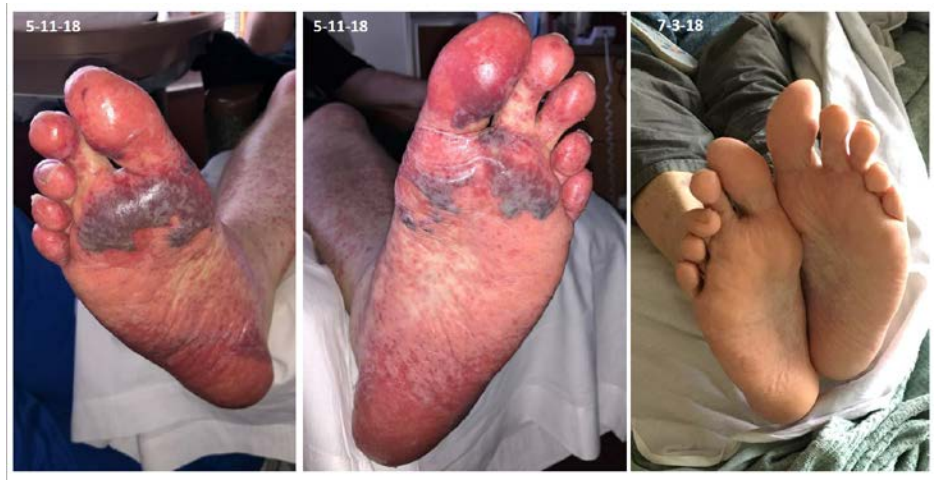
Future Directions



Concept of tissue repair in GVHD and inflammatory diseases gaining traction

- Mucosal healing more difficult to ascertain than skin
  - Inflammatory bowel disease: reassessed no sooner than 6 weeks
  - Might be monitored by blood biomarkers: MONITr (IBD), AREG (GVHD, ?IBD)
- Current goal = clinical complete response
- Future goal 1 = clinical complete response + complete tissue repair?
  - Resolve subclinical damage to prevent late effects
- Figure goal 2 = prediction and prevention of tissue damage in the first place?





Summary

- Tissue repair factors are altered at GVHD onset
  - Validated with 0302/0802 samples
  - EGF is low at acute GVHD onset, very low in steroid refractory GVHD
  - AREG is high, 33+ pg/ml indicates high risk
- Prospective trial for high-risk/refractory acute GVHD developed based upon results
- Future samples may help us know when we can taper/stop immunosuppression

Thank you!

sgholtan@umn.edu

@sghmd

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Questions?

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Thank you!

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