

Biomarcatori e stratificazione del rischio nella GvHD: verso una medicina di precisione nel bambino e nell'adulto

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Disclosures of Pietro Merli

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
SOBI					X	X	
MEDAC						X	
Miltenyi					X		
Pierre Fabre					X		
Amgen					X		

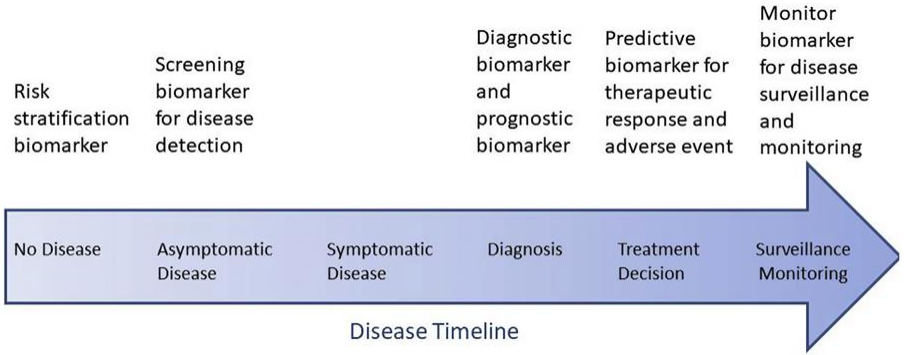
The problem

Assessment of GVHD severity at onset by Glucksberg criteria **does not** precisely predict response to treatment and non-relapse mortality (NRM).

GVHD Staging			
Stage	Skin	Liver (total bilirubin)	GI tract (diarrhea output/day)
0	No GVHD rash	<2 mg/dl	Adult: <500 ml/d *Child: <10 ml/kg/d
1	Maculopapular rash <25% body surface area	2-3 mg/dl	Adult: 500-999 ml/d Child: 10-19.9 ml/kg/d -or- persistent nausea, vomiting, or anorexia with a positive upper GI biopsy
2	Maculopapular rash 25-50% BSA	3.1-6 mg/d	Adult: 1000-1500ml/d Child: 20-30 ml/kg/d
3	Maculopapular rash >50% BSA	6.1-15 mg/dl	Adult: >1500ml/d Child: >30 ml/kg/d
4	Generalized erythroderma (>50% BSA) plus bullous formation or desquamation >5% BSA	>15 mg/dl	Severe abdominal pain with or without ileus, or grossly bloody diarrhea
*Use adult values for patients ≥ 50 kg			
Overall Clinical Grade: Grade 0: No GVHD of any organ Grade 1: Stage 1-2 skin and no liver OR GI tract involvement Grade2: Stage 3 skin and/or stage 1 liver and/or stage 1 GI tract Grade 3: Stage 0-3 skin with stage 2-3 liver and/or stage 2-3 GI tract Grade 4: Stage 4 skin, liver, and/or GI tract			

What are biomarkers?

- In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is **objectively measured** and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”
- World Health Organization (WHO), in coordination with the United Nations and the International Labor Organization, has defined a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”
- Medical signs have a long history of use in clinical practice—as old as medical practice itself—and biomarkers are merely the most objective, quantifiable medical signs modern laboratory science allows us to measure **reproducibly**.



Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Therapeutics. 2001; 69:89–95

Biomarkers in HSCT setting

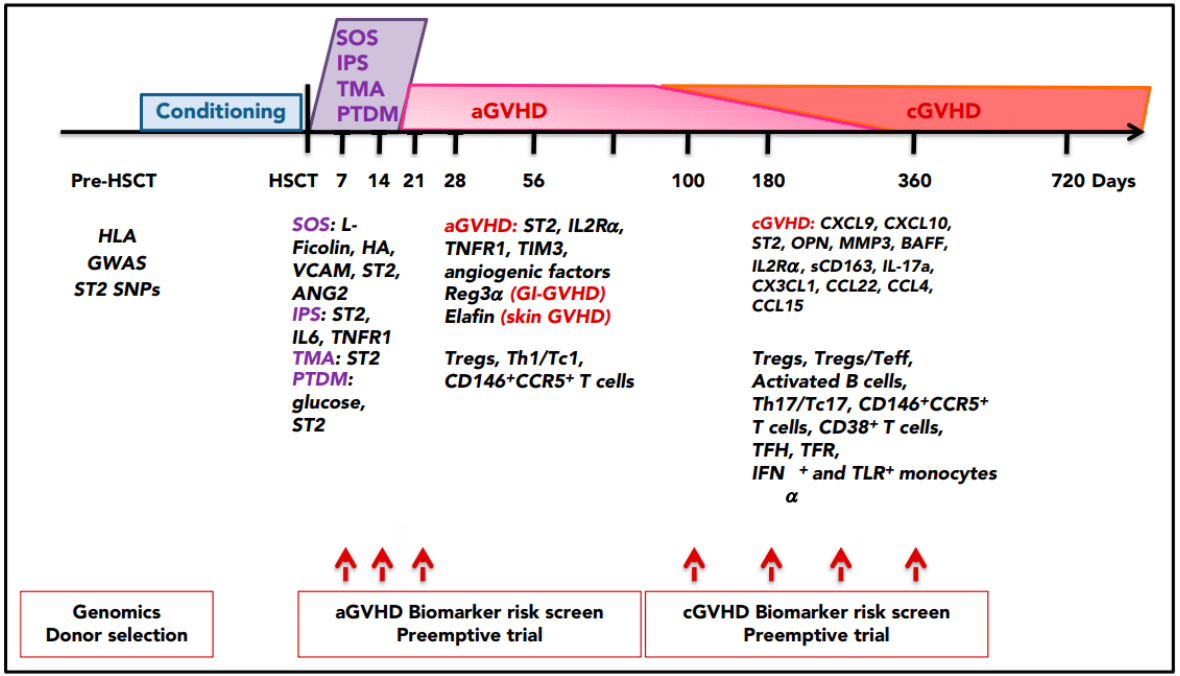


Figure 3. Most validated posttransplantation biomarkers by chronological outcomes. Chronological outcomes posttransplantation and biomarkers are all discussed in "Most validated and recent biomarkers." BAFF, B-cell–activating factor; HA, hyaluronic acid; MMP3, matrix metalloproteinase 3; OPN, osteopontin; Reg3α, regenerating islet-derived 3-α; sCD163, soluble CD163; Teff, T effector; TIM3, T-cell immunoglobulin mucin-3; TNFR1, tumor necrosis factor receptor-1.

Paczesny S., Blood 2018

Biomarkers in GvHD

TABLE 1 | Selected predictive and prognostic GVHD biomarker models other than the MAGIC algorithm probability.

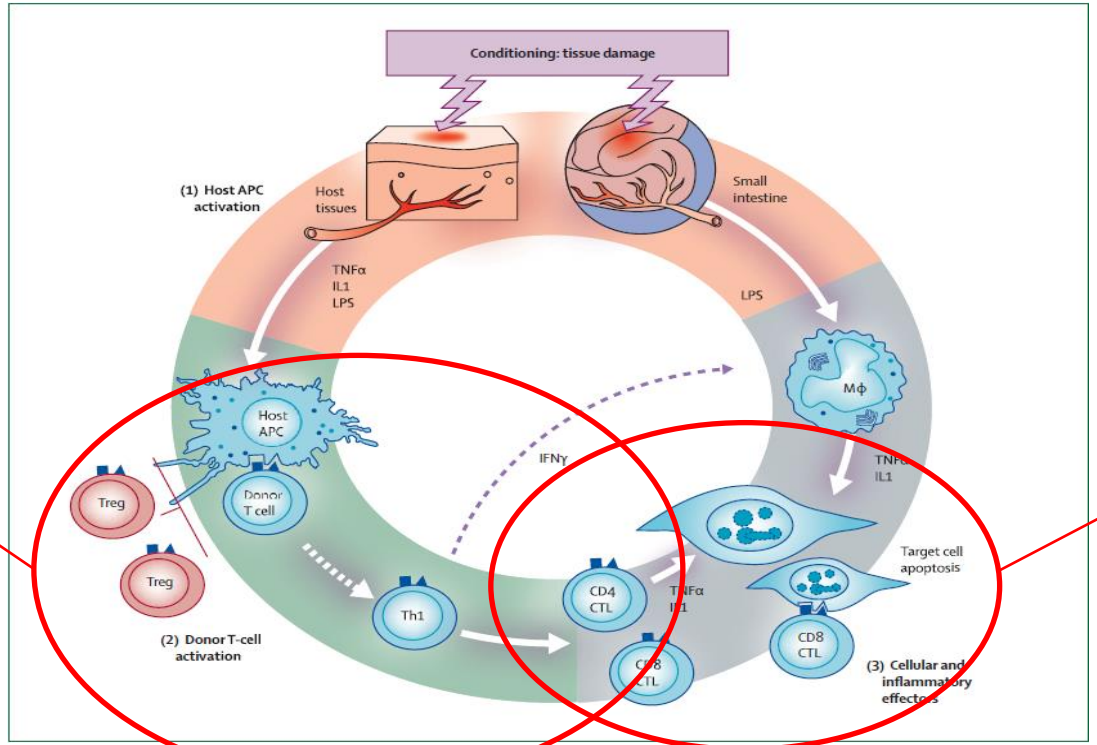
Study [reference]	Timepoint	Key findings
McDonald et al. [9]	GVHD onset	IL-6, ST2, TIM3, and TNFR1 individually predicted maximal GVHD severity and 1-year NRM; TIM3 alone provided the most predictive utility
Abu Zaid et al. [10]	Day 28 post-HCT	IL-6, ST2, TIM3, and REG3α individually predicted 2-year NRM; a model that incorporated all four biomarkers and clinical covariates (GVHD prophylaxis regimen, age, and CMV status) was the best predictor for NRM
Paczesny et al. [11]	GVHD onset	Composite panel of IL-2Rα, TNFR1, HGF, and IL-8 predicted 3-year NRM and OS
Levine et al. [12]	GVHD onset, Days 14 and Day 28 of treatment	At each timepoint, a composite panel of IL-2Rα, TNFR1, HGF, IL-8, and elafin predicted response to treatment and overall survival
Vander Lugt et al. [13]	Day 14 post-HCT and GVHD onset	At both timepoints, ST2 levels predicted 6-month NRM
Holtan et al. [17]	GVHD onset	Amphiregulin predicted 6-month NRM independently of clinical severity
Weissinger et al. [18]	Prior to and including GVHD onset	Urinary peptide pattern distinguished patients who developed GVHD from patients who did not develop GVHD
Weissinger et al. [21]	Serial monitoring from Day 7 to Day 35	Emergence of a urinary peptide pattern predicted GVHD grades II–IV

Abbreviations: CMV, cytomegalovirus; HGF, hepatocyte growth factor; IL-2Rα, interleukin-2 receptor α; IL-6, interleukin-6; IL-8, interleukin-8; REG3α, regenerating islet-derived protein 3-α; ST2, suppressor of tumorigenicity-2; TIM3, T-cell immunoglobulin and mucin domain 3; TNFR1, tumor necrosis factor receptor-1.

Levine JE., Am J Hematol 2025

GVHD pathophysiology and Biomarkers

TNFR1
TIM3
IL6



REG3α
ST2
AREG

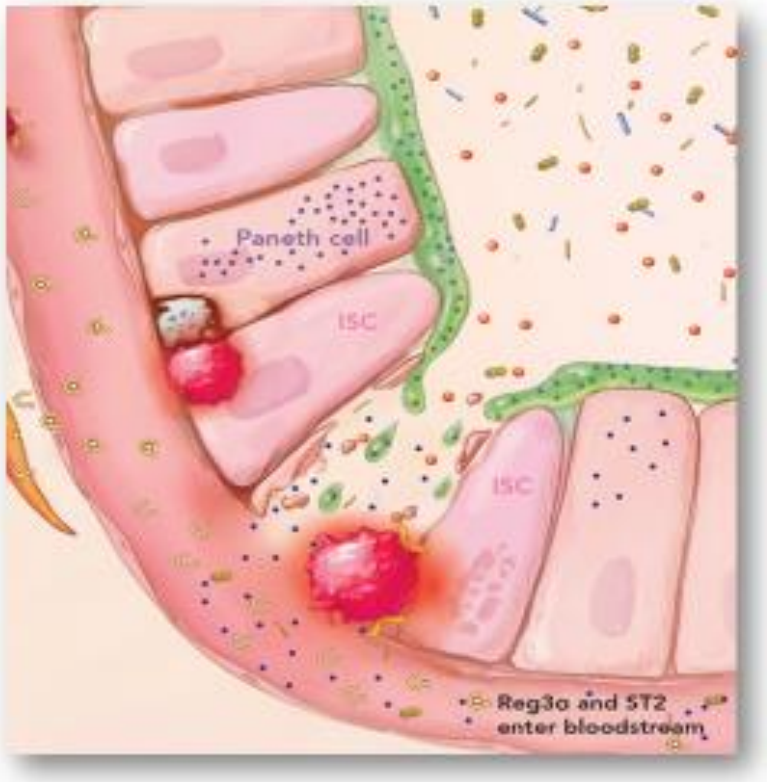
Modified from Ferrara JLM et al., Lancet 2009

MAGIC SERUM BIOMARKERS: A LIQUID BIOPSY

Reg3α: a cellular survival protein, released from dying Paneth cells in the crypts

ST2 (soluble IL-33 receptor), released from endothelium, mesenchyme and activated lymphocytes.

An equation combines the serum concentrations of ST2 and Reg3α in a single value: the MAGIC Algorithm Probability (MAP) that predicts NRM because it accurately estimates GI crypt damage



Ferrara et al, Blood Advances, 2018 Major-Monfried et al, Blood, 2018 Srinagesh et al, Blood Adv, 2019

Glossary

- Gluck… …mptoms
- Minn… …mptoms
- Manh… …mptoms
- MAG… …um
- MAP… …re
- Ann A…
- MAG… …d on signs/symptoms AND biomarkers

TABLE 2 | Validated MAGIC algorithm (ST2+REG3α) and thresholds for Ann Arbor scores.

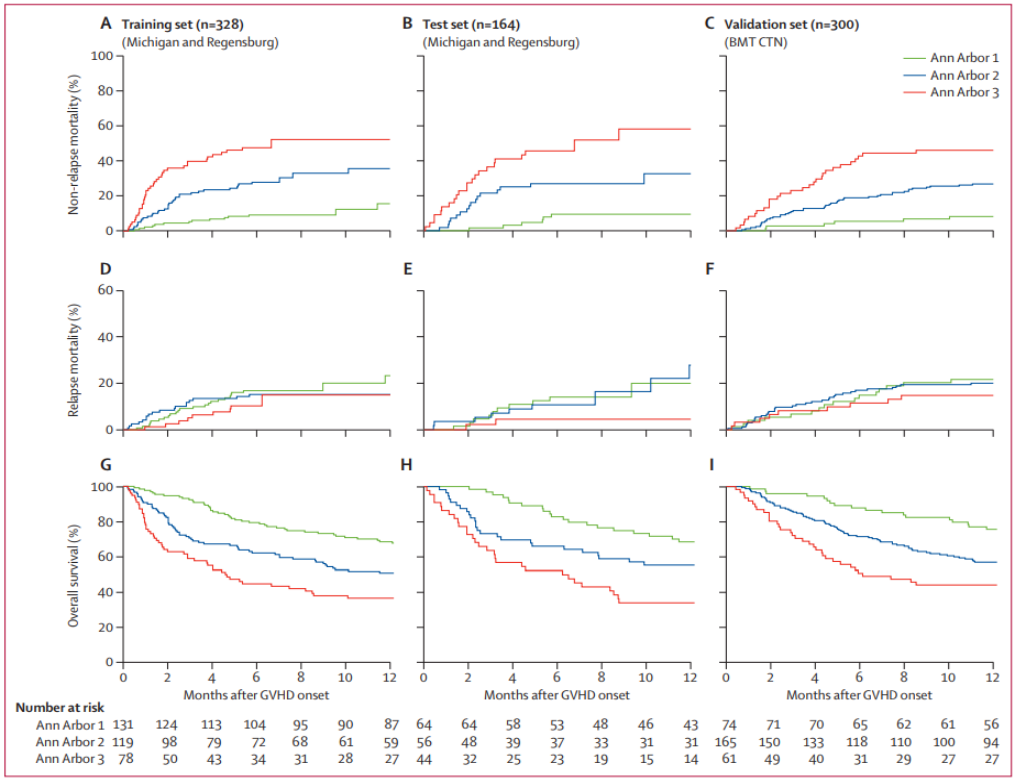
Ann Arbor score	MAP
AA1	0.001–0.140
AA2	0.141–0.290
AA3	0.291–0.999

Note: Algorithm:
$\log(-\log[1-\mathrm{MAP}]) = -11.263 + 1.844(\log_{10}\mathrm{ST2}) + 0.577(\log_{10}\mathrm{REG3}\alpha)$.



The MAGIC Consortium

GVHD Biomarker Algorithms
Can Predict Response to Treatment and Long-Term Outcomes



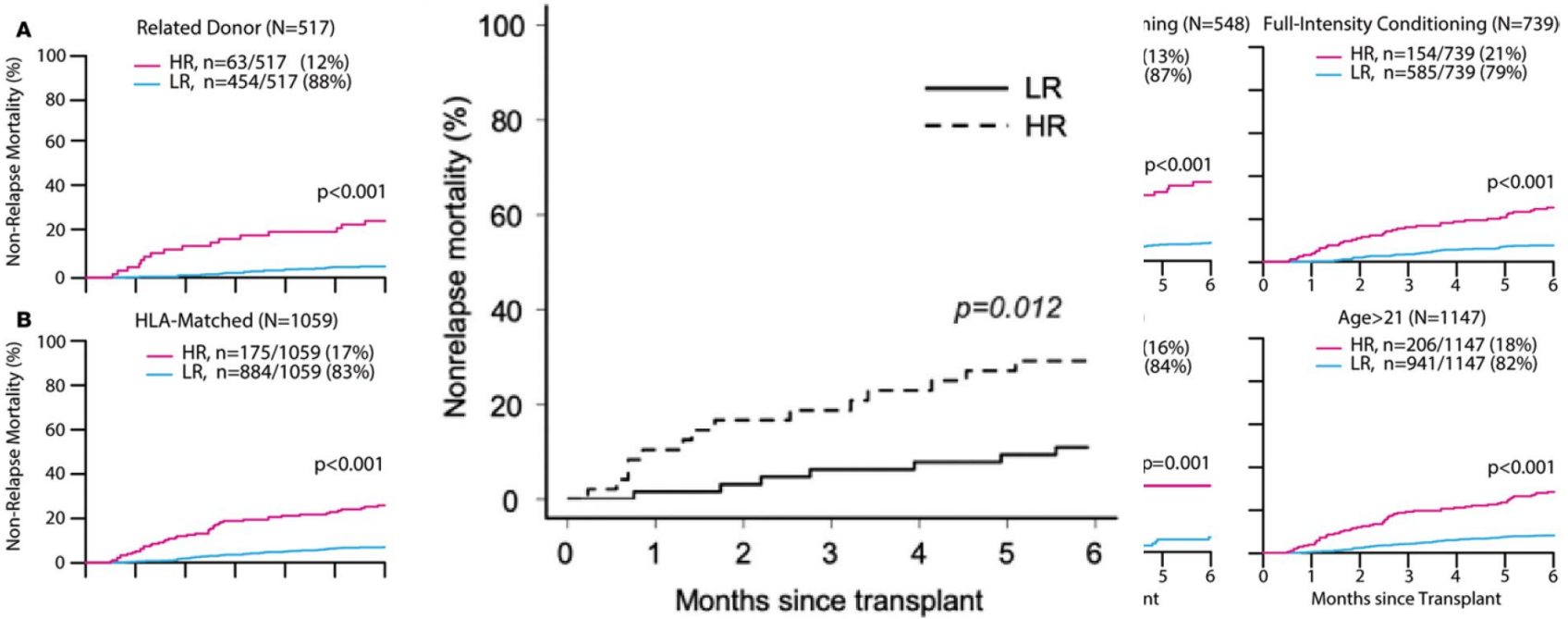
3 biomarkers:
- TFNR1
- ST2
- REG3α

log[-log(1-p)] = –9·169 + 0·598(log2TNFR1) –0·028 (log2REG3α) + 0·189 (log2ST2)

Levine JE *et al*., Lancet Haematol 2015

GVHD Biomarker Algorithms
Can Predict the Occurrence of NRM and GVHD

2 biomarkers (ST2, REG3α); 7 days after HSCT



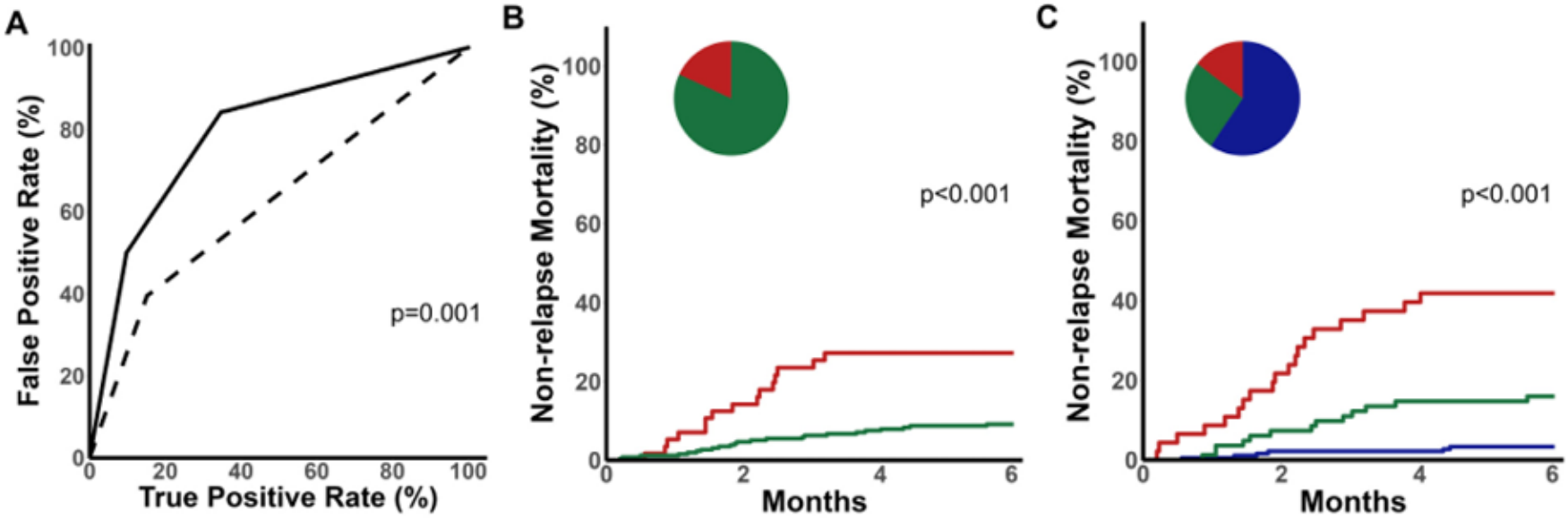
Hotta M *et al*., Transplant Cell Ther 2021

Hartwell MJ *et al*., JCI Insight 2017

Validation of biomarkers in the pediatric population

Characteristic	
Median age at BMT, years (range)	11 (0, 21)
PBSC	116 (37%)
Cord	45 (14%)
Conditioning regimen, n (%)	
Myeloablative	279 (89%)
Reduced intensity/nonmyeloablative	36 (11%)

GVHD prophylaxis, n (%)	



Qayed M et al., Transplant Cell Ther 2024

Combining clinical and biomarkers data

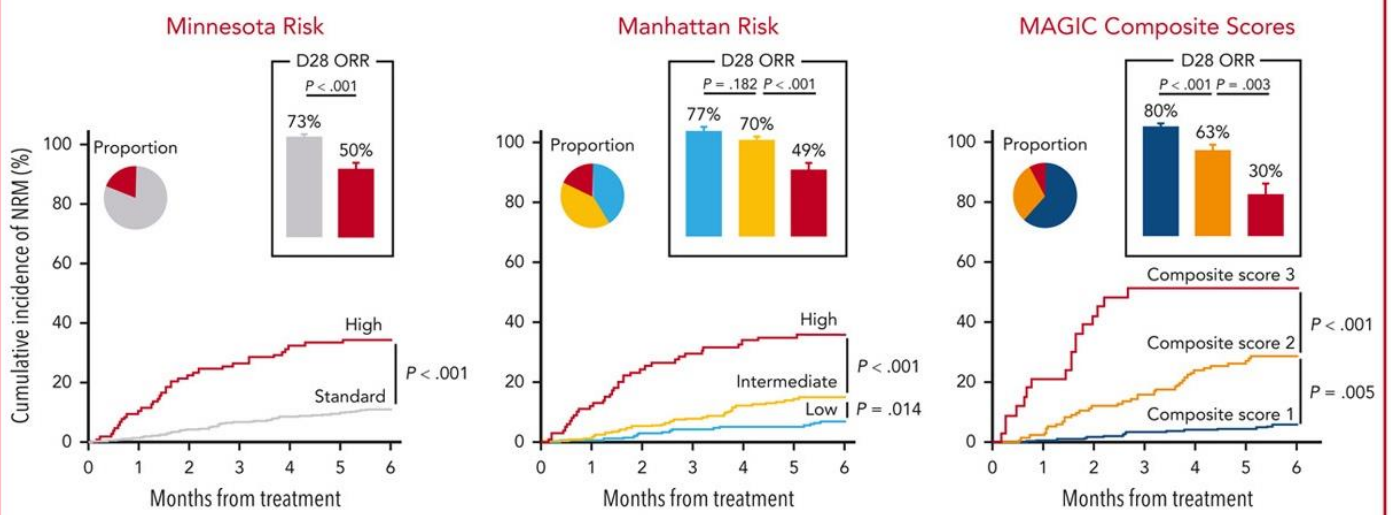
Context of Research

The Minnesota risk system for acute GVHD, modeled on clinical symptoms, lacks a low-risk stratum needed to minimize treatment

Aim of This Study

The objective was to develop new models that use clinical symptoms ± biomarkers to define 3 risk groups, including a low-risk stratum

Findings



Akahoshi Y et al., Blood 2024

Manhattan Risk and MAGIC Composite Score Calculator

Enter aGVHD staging for each target organ at the initiation of systemic treatment

Skin

- ○ Stage 0 - no GVHD rash
- ○ Stage 1 - <25% BSA
- ◉ Stage 2 - 25-50% BSA
- ○ Stage 3 - >50% BSA
- ○ Stage 4 - Generalized rash (>50% BSA) with bullous formation and/or desquamation >5%

Liver

- ○ Stage 0 - Total Bilirubin <2.0 mg/dl or non-GVHD hyperbilirubinemia
- ○ Stage 1 - Total Bilirubin 2.0-3.0 mg/dl
- ○ Stage 2 - Total Bilirubin 3.1-6.0 mg/dl
- ◉ Stage 3 - Total Bilirubin 6.1-15.0 mg/dl
- ○ Stage 4 - Total Bilirubin >15.0 mg/dl

Upper GI

- ◉ Stage 0 - no persistent N/V/A or N/V/A from non-GVHD causes
- ○ Stage 1 - persistent N/V/A

Lower GI Adult ⊙

- ○ Stage 0 - Diarrhea <500 ml/day or non-GVHD diarrhea
- ◉ Stage 1 - Diarrhea 500-999 ml/day
- ○ Stage 2 - Diarrhea 1000-1500 ml/day
- ○ Stage 3 - Diarrhea >1500 ml/day
- ○ Stage 4 - Severe abdominal pain with or without ileus or grossly bloody stool (volume independent)

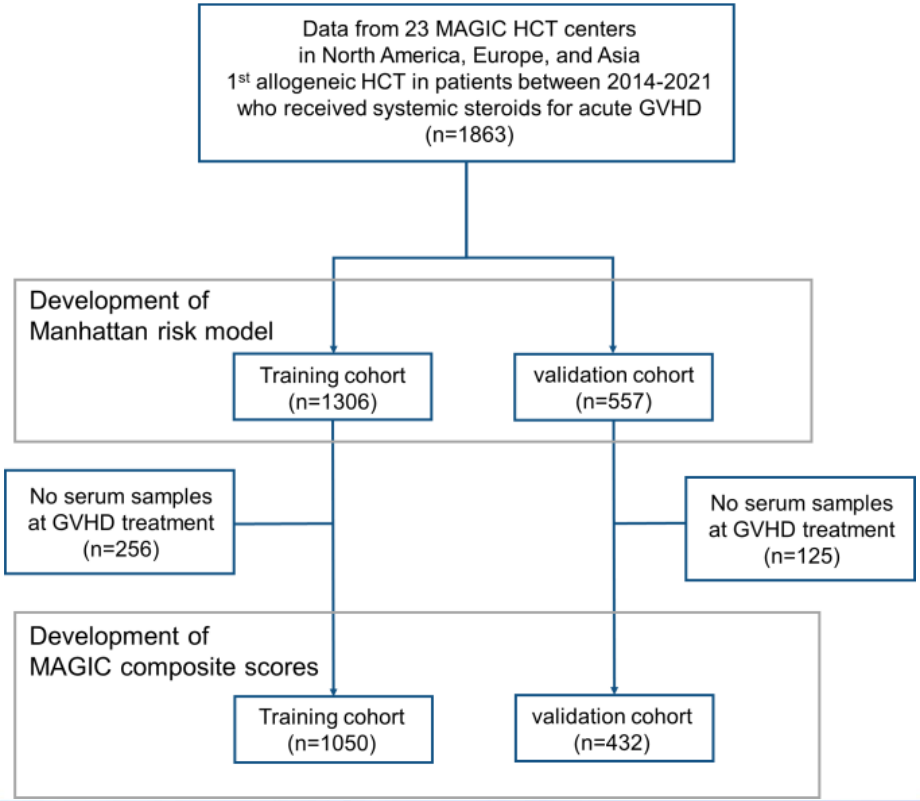
Legend/Help Text:

- N/V/A - Nausea, Vomiting, Anorexia
- Persistent N/V/A
  1. Nausea lasting >3 days **and/or**
  2. >2 vomiting episodes per day for at least 2 days **and/or**
  3. Anorexia with weight loss
- If diarrhea is reported in episodes: 1 episode = 200 mL

https://gvhdmagic.com

Akahoshi Y et al., Blood 2024

Manhattan (clinical) and Magic (combined) scores



Manhattan (clinical) and Magic (combined) scores

Table S3. GVHD phenotype in the training cohort

Skin	Liver	LGI	UGI	n	Skin	Liver	LGI	UGI	n
0	0	0	1	112	1	2	0	0	4
0	0	1	0	89	1	2	1	1	2
0	0	1	1	62	1	3	0	0	1
0	0	2	0	31	1	3	0	1	1
0	0	2	1	32	2	0	0	0	259
0	0	3	0	23	2	0	0	1	33
0	0	3	1	30	2	0	1	0	11
0	0	4	0	9	2	0	1	1	9
0	0	4	1	22	2	0	2	0	5
0	1	0	0	3	2	0	2	1	5
0	1	0	1	1	2	0	3	0	3
0	1	3	0	1	2	0	3	1	4
0	1	3	1	1	2	0	4	1	2
0	1	4	1	1	2	1	0	0	3
0	2	0	0	6	2	1	2	1	2
0	2	0	1	1	2	2	0	1	1
0	2	3	1	1	2	2	1	1	1
0	2	4	0	1	2	3	1	1	1
0	2	4	1	1	2	3	3	0	1
0	3	0	1	1	3	0	0	0	209
0	3	1	1	2	3	0	0	1	13
0	3	3	1	2	3	0	1	0	8
0	3	4	0	1	3	0	1	1	8
0	4	0	0	2	3	0	2	0	7
0	4	0	1	1	3	0	2	1	2
0	4	4	0	1	3	0	3	0	3
1	0	0	0	135	3	0	3	1	2
1	0	0	1	35	3	0	4	1	1
1	0	1	0	29	3	1	0	0	2
1	0	1	1	18	3	1	0	1	2
1	0	2	0	9	3	1	2	1	1
1	0	2	1	6	3	2	0	0	3
1	0	3	0	5	3	2	1	0	1
1	0	3	1	4	3	2	2	1	1
1	0	4	1	4	3	3	3	0	1
1	1	1	0	1	4	0	0	0	7
1	1	2	0	1	4	0	0	1	1
1	1	3	0	1	4	3	2	1	1

All other combinations had zero patients.

Manhattan (clinical) risk

Organ involvement	n (%)	6m NRM (%)	Day 28 ORR (%)	Manhattan risk
Isolated stage 1-2 Skin				Low

Organ involvement	N (%)	6m NRM (%)	Minnesota risk	Manhattan risk
Isolated stage 1 Skin	188 (10.1)	6.0	Standard	Low
Isolated stage 2 Skin	364 (19.5)	7.7	Standard	Low
Isolated UGI	174 (9.3)	6.9	Standard	Low
Stage 1 Skin + UGI	45 (2.4)	6.7	Standard	Low
Stage 1-3 Skin + Stage 1-4 Liver	19 (1.0)	27.4	Standard	High

Stage 1-3 Skin + Stage 2-3 LGI ± UGI	99 (17.8)	35.8	48.5	High
Stage 3-4 LGI ± UGI				High
Stage 4 Skin ± other organ involvement				High

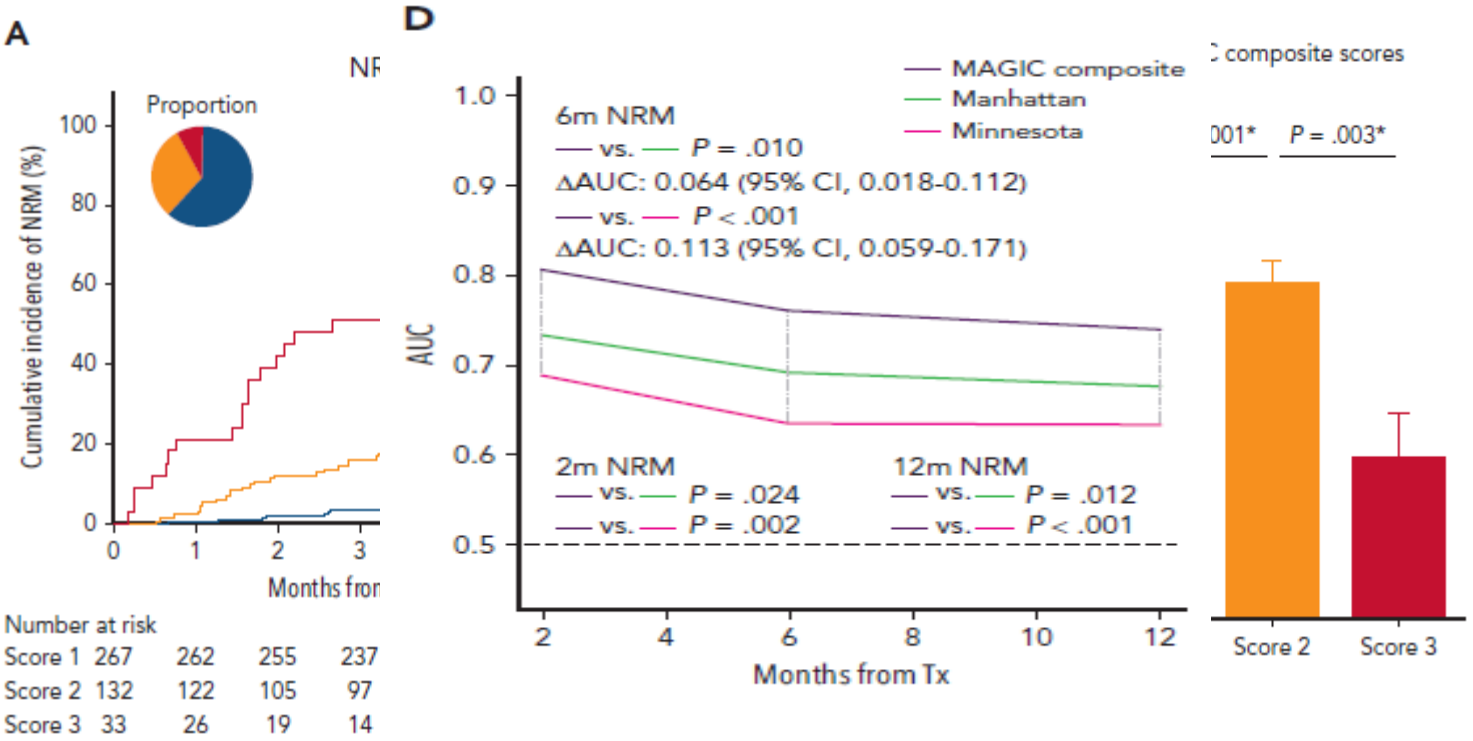
Magic (Composite) score

Table 3. Algorithm assignment to MAGIC composite scores of 9 categories determined by the Manhattan risk and AA scores in the training cohort

Manhattan risk	AA scores	n (%)	6-mo NRM (%)	CART	K-means
Low	AA1	296 (28.2)	3.1	MCS1	MCS1
Low	AA2	99 (9.4)	12.1	MCS1	MCS1
Low	AA3	36 (3.4)	27.8	MCS2	MCS2
Intermediate	AA1	247 (23.5)	8.7	MCS1	MCS1
Intermediate	AA2	125 (11.9)	18.4	MCS2	MCS2
Intermediate	AA3	74 (7.0)	29.7	MCS2	MCS2
High	AA1	50 (4.8)	20.4	MCS2	MCS2
High	AA2	52 (5.0)	29.1	MCS2	MCS2
High	AA3	71 (6.8)	56.3	MCS3	MCS3

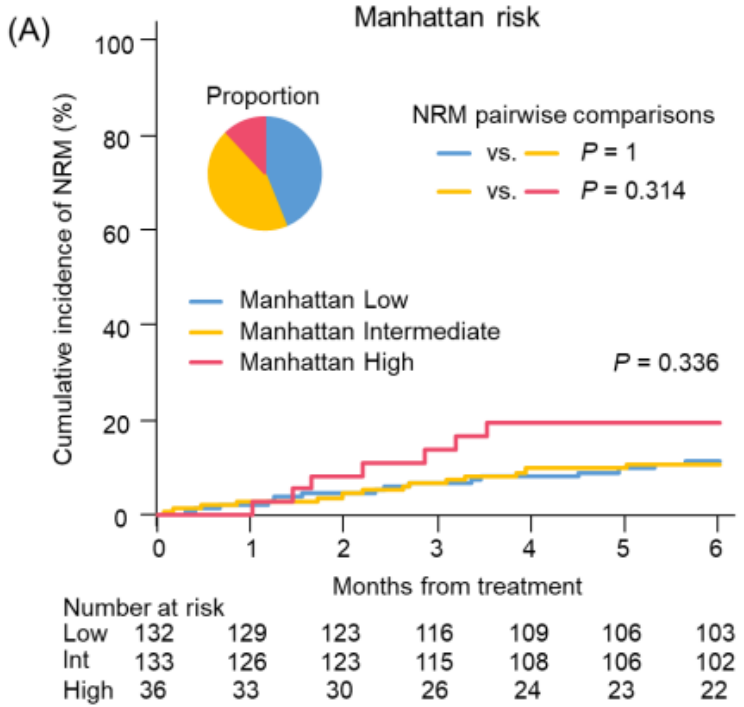
Akahoshi Y et al., Blood 2024

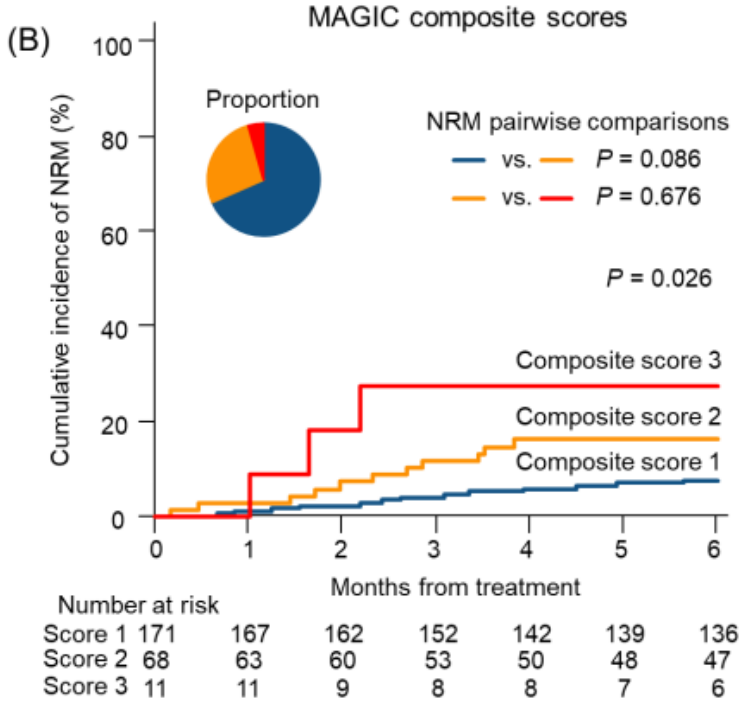
Magic (Composite) score



Akahoshi Y et al., Blood 2024

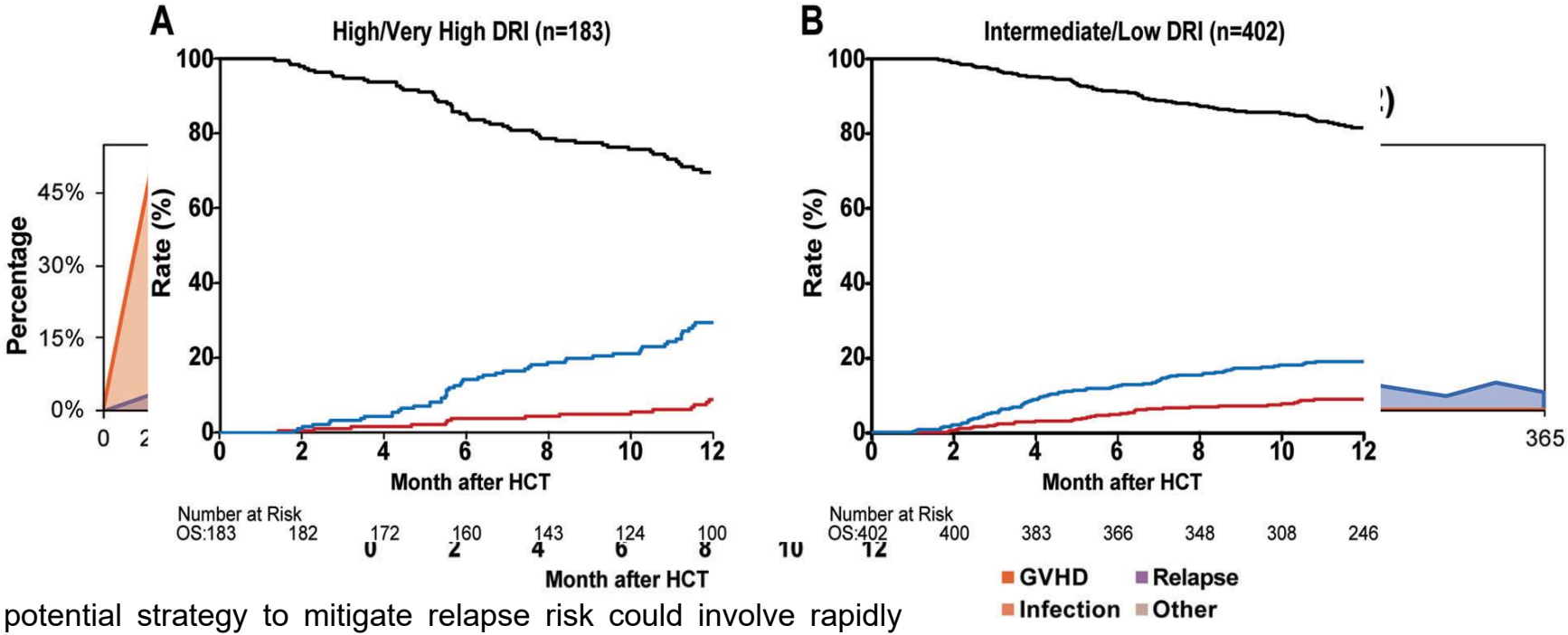
Magic score in PT/Cy





Akahoshi Y et al., Blood 2024

Disease risk and GVHD biomarkers can stratify patients for risk of relapse and NRM

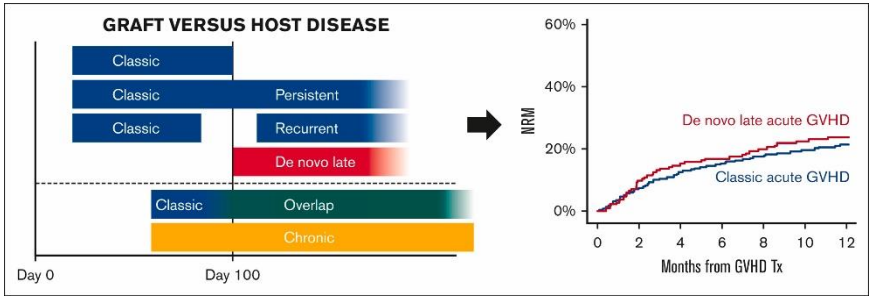


A potential strategy to mitigate relapse risk could involve rapidly tapering immunosuppression after day 28 in patients who are without GVHD and have a low MAP.

Aziz M *et al*., Leukemia 2020

MAP is useful in different settings

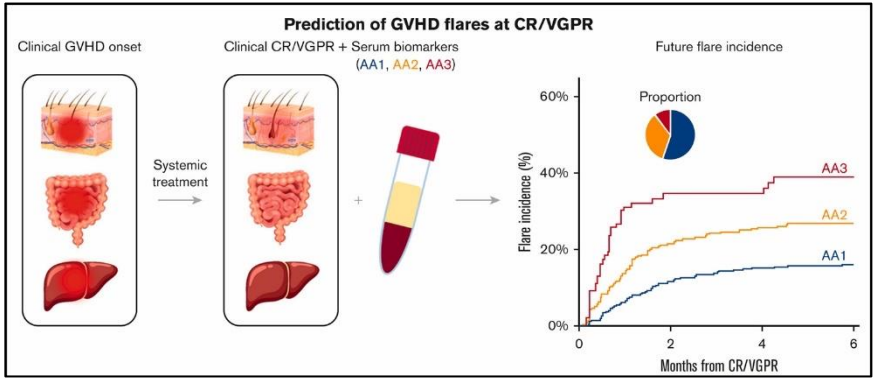
- De-novo late acute GVHD



Akahoshi Y et al., Blood Adv 2023

MAP is useful in different settings

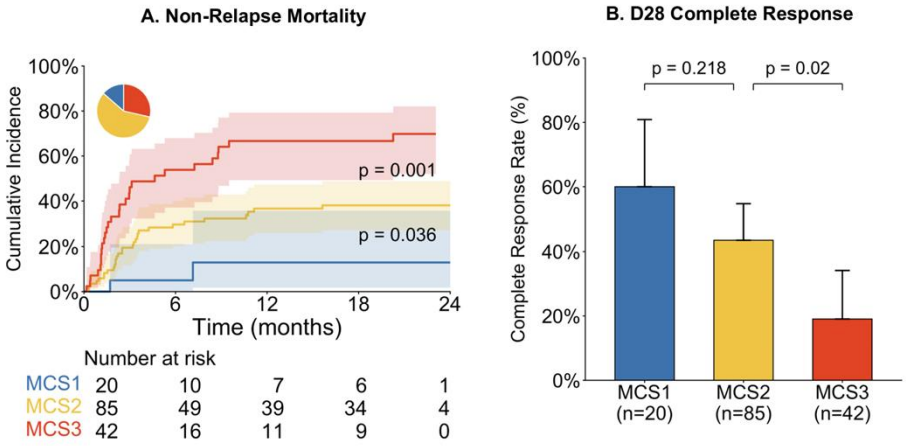
- De-novo late acute GVHD
- Flares of acute GVHD



Akahoshi Y et al., Blood Adv 2024

MAP is useful in different settings

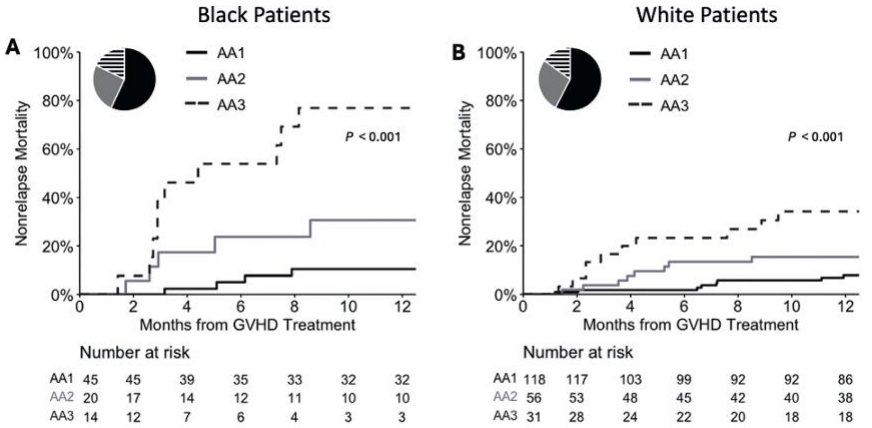
- De-novo late acute GVHD
- Flares of acute GVHD
- Second line therapies



Sebastian T el a., pre-print

MAP is useful in different settings

- De-novo late acute GVHD
- Flares of acute GVHD
- Second line therapies
- Ethnic minorities



Rios CAO et al., Transplant Cell Ther 2024

MAP is useful in different settings

- De-novo late acute GVHD
- Flares of acute GVHD
- Second line therapies
- Ethnic minorities
- Early endpoints

CLINICAL TRIALS: PRIMARY ACUTE GVHD TREATMENT

Overall response rate (ORR) of clinical symptoms at four weeks after treatment:

- Validated surrogate for non-relapse mortality (NRM)
- Partial and complete responses do not have different long term outcomes
- Primary endpoint for clinical trials of acute GVHD treatment

ORR DISADVANTAGES

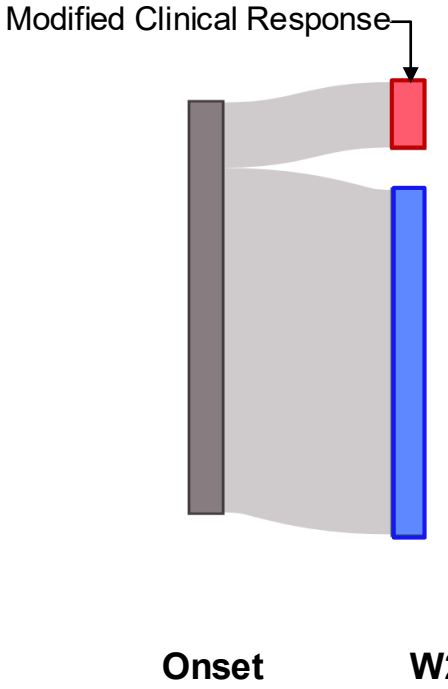
- Late responses (more than two weeks): problematic
- New therapies approved for steroid resistant disease
- Grade IV → Grade III = Grade II → Grade I, but different NRMs

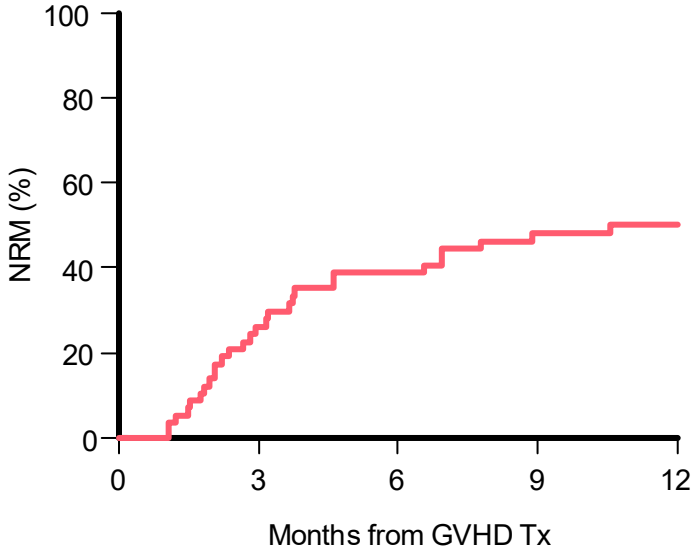
BETTER AND FASTER ENDPOINT NEEDED FOR TRIALS

Martin et al, BBMT, 2009

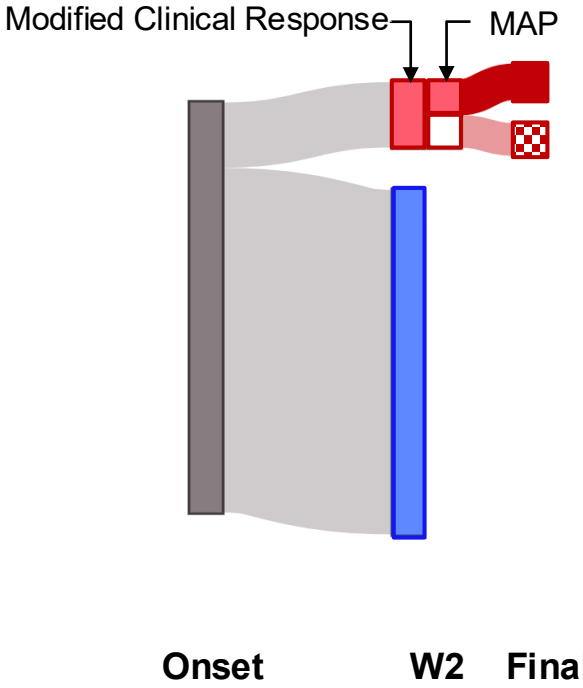
MacMillan et al, Blood, 2010

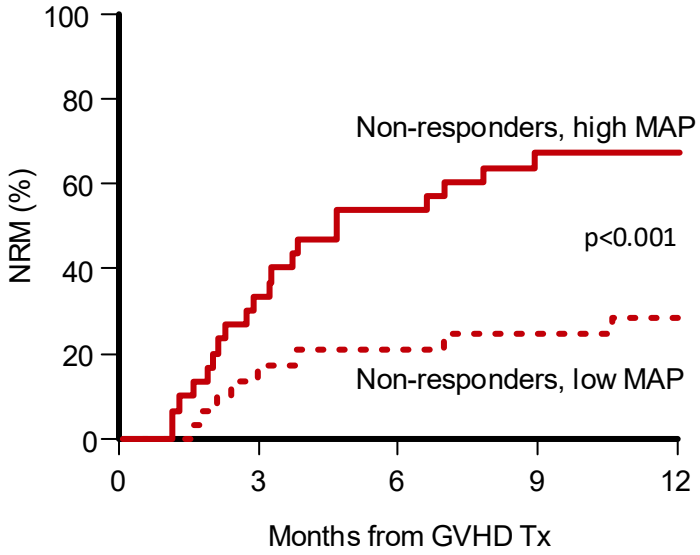
THE MAGIC RESPONSE: VALIDATION SET



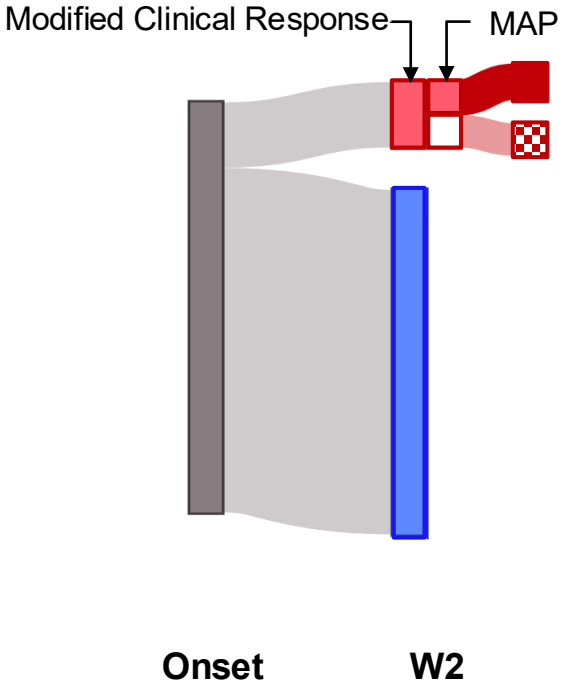


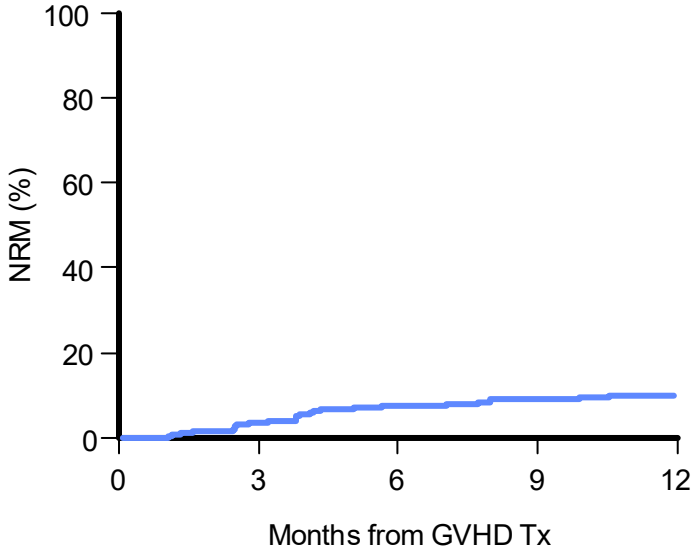
THE MAGIC RESPONSE IS A COMBINATION OF A MODIFIED CLINICAL RESPONSE AND THE MAP SCORE AT 2 WEEKS



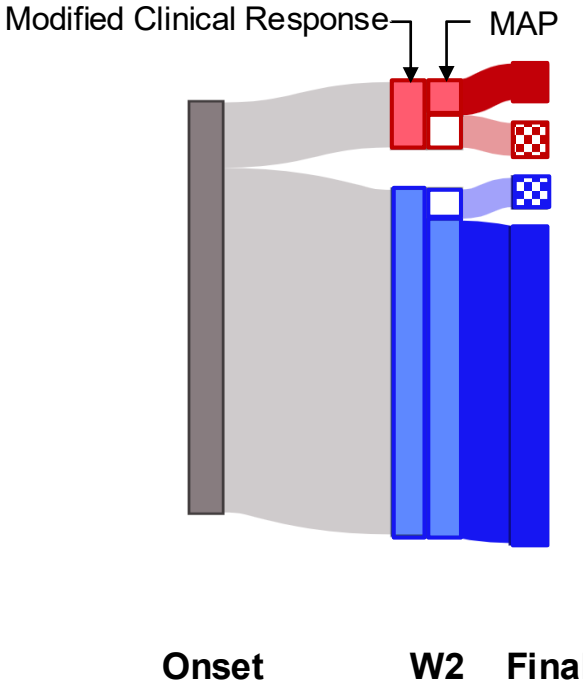


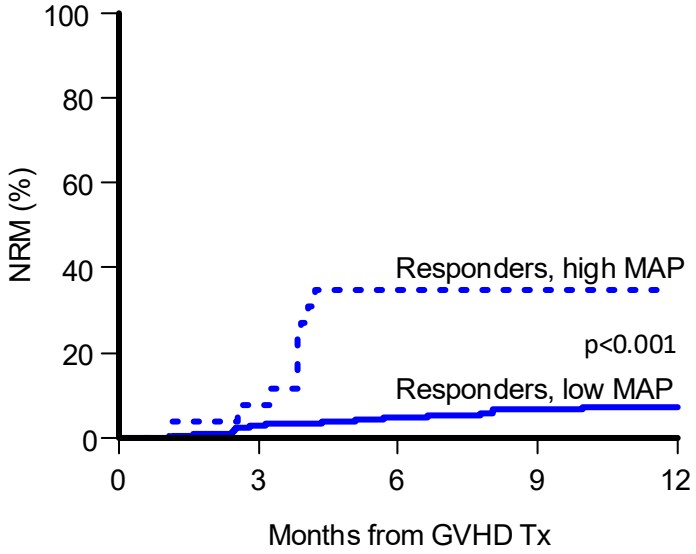
THE MAGIC RESPONSE IS A COMBINATION OF A MODIFIED CLINICAL RESPONSE AND THE MAP SCORE AT 2 WEEKS



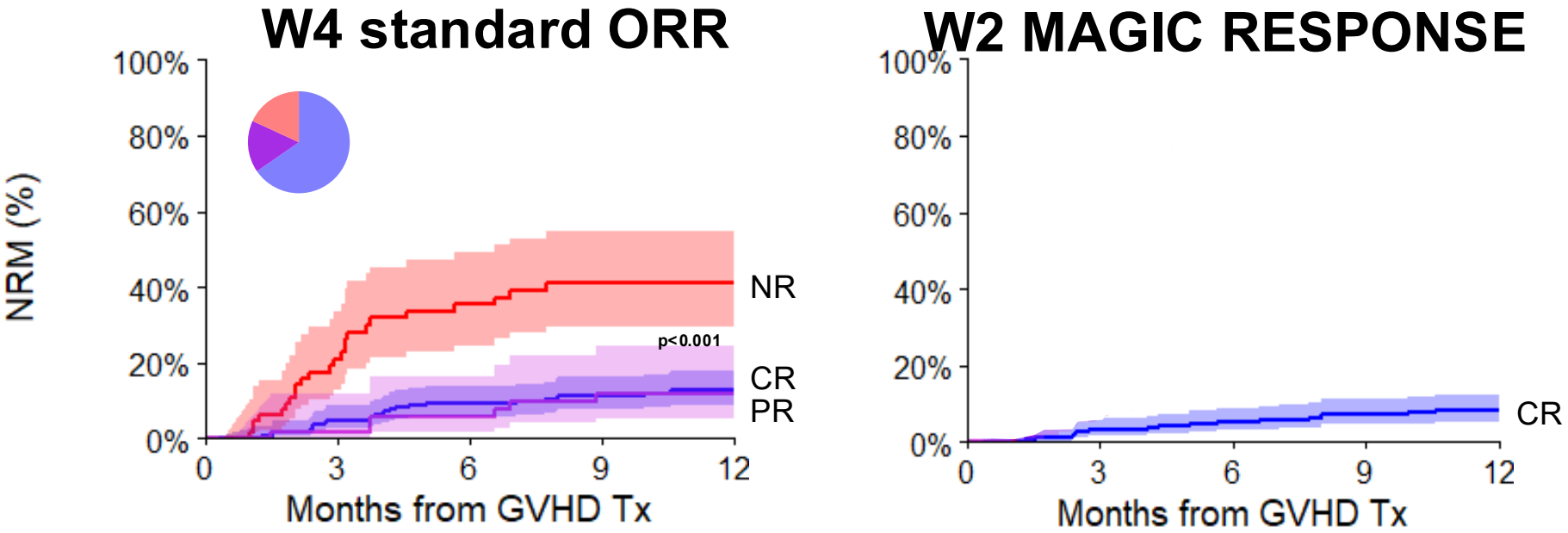


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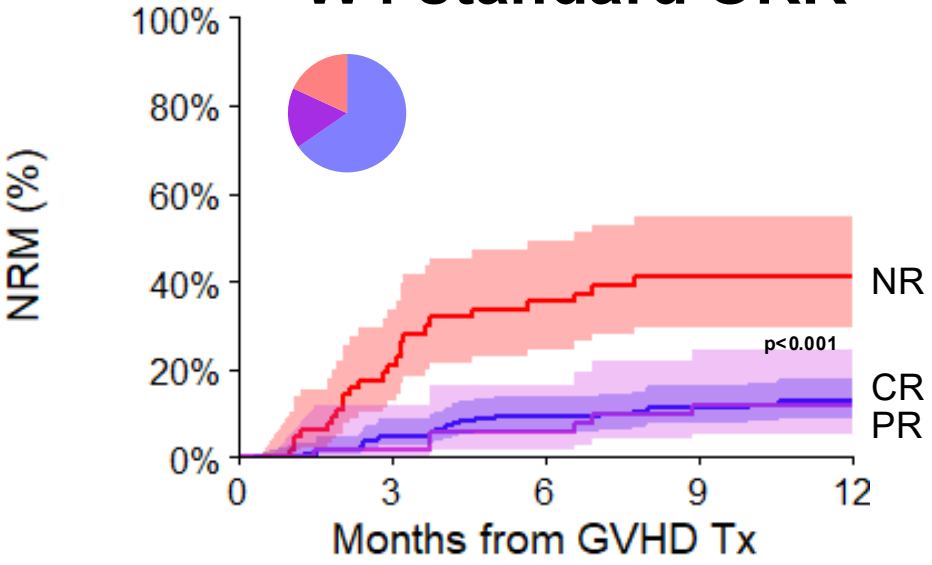


WEEK 4 RESPONSE VS WEEK 2 MAGIC RESPONSE: LONG TERM OUTCOMES

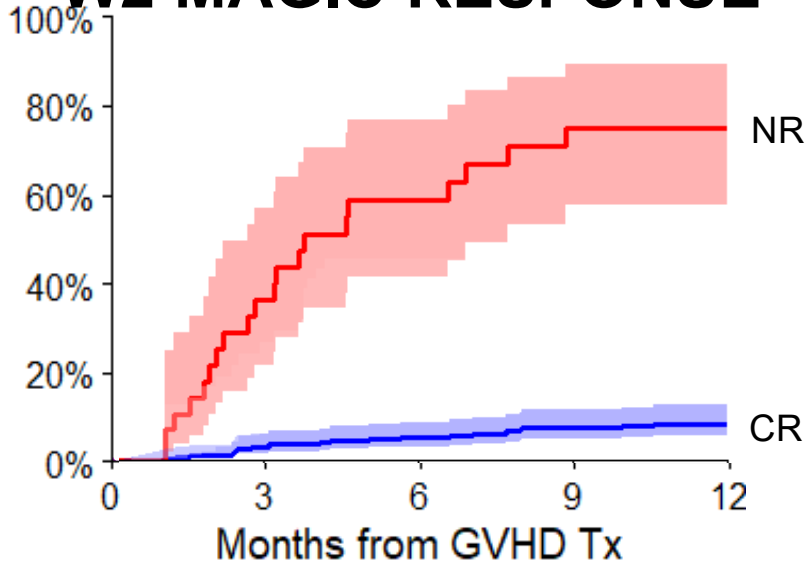


WEEK 4 RESPONSE VS WEEK 2 MAGIC RESPONSE: LONG TERM OUTCOMES

W4 standard ORR

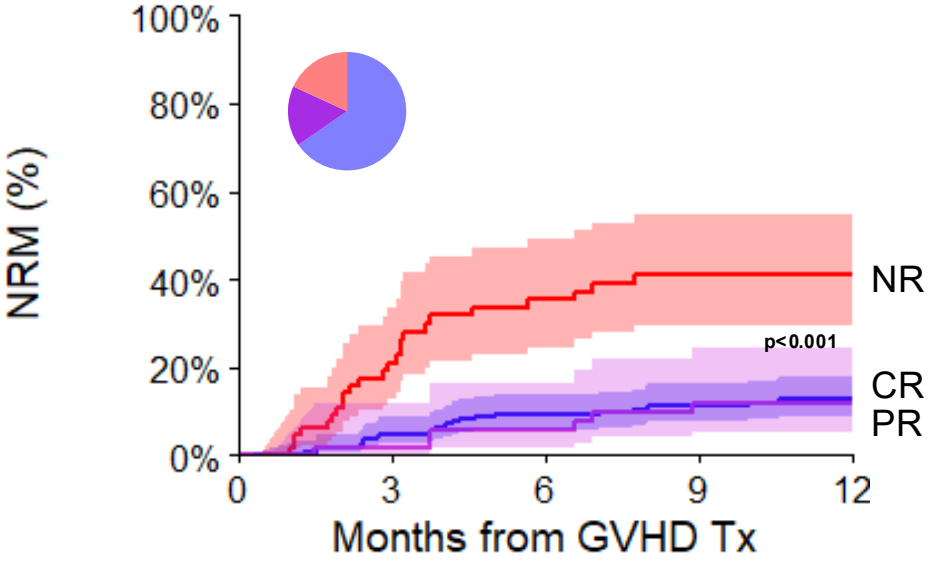


W2 MAGIC RESPONSE

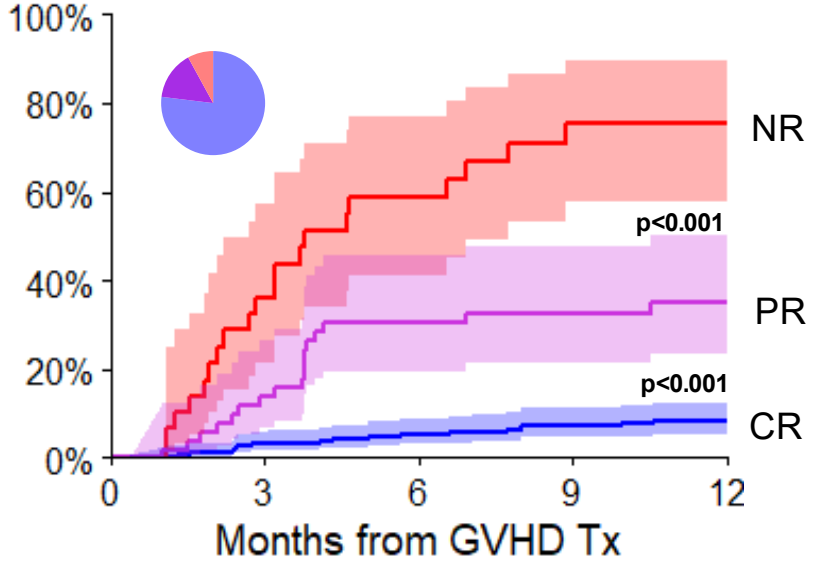


WEEK 4 RESPONSE VS WEEK 2 MAGIC RESPONSE: LONG TERM OUTCOMES

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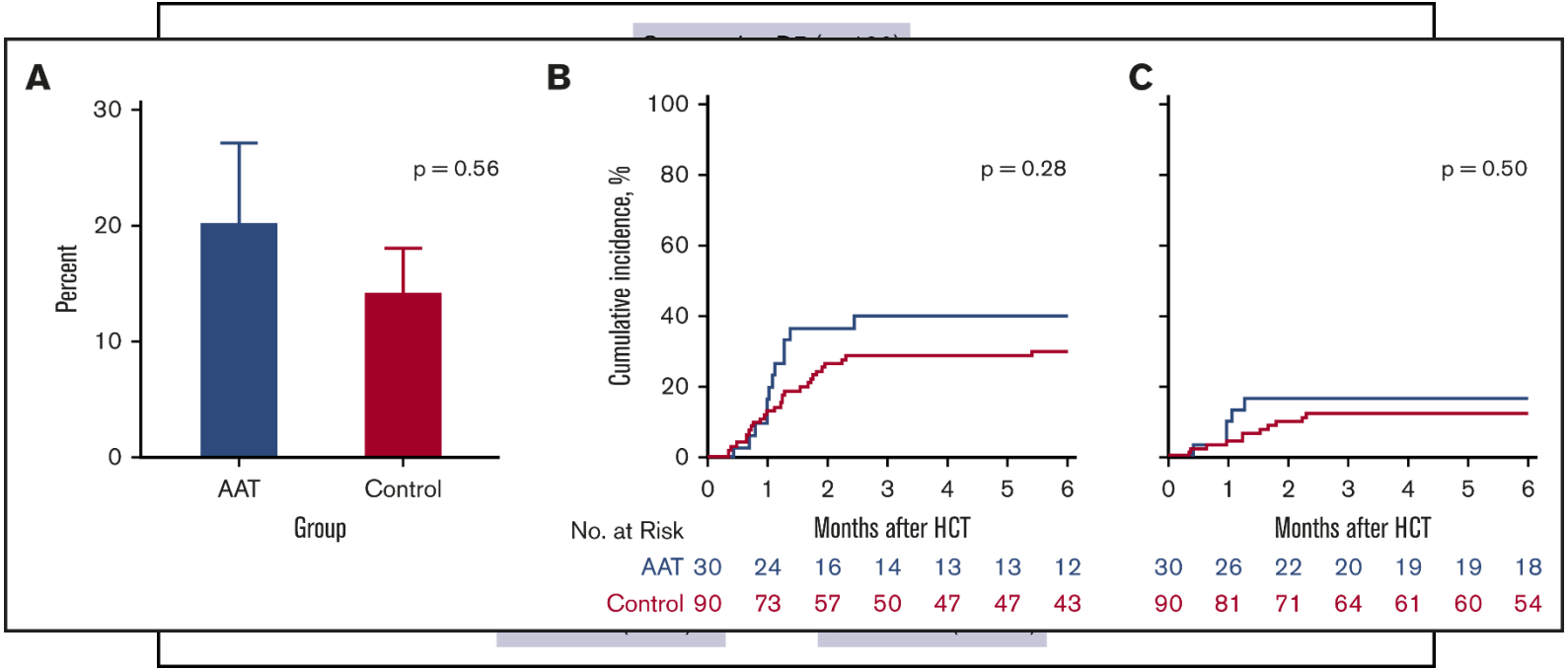
W2 MAGIC RESPONSE



Summary of evidences about MAP

Author (year)	Timepoint	Clinical implication
Hartwell (2017) Hotta (2021)	Day 7 post-HCT	High risk pts. might be candidates for pre-emptive therapy to prevent severe GVHD and death
Aziz (2020)	No GVHD at Day 28 post-HCT	Pts without GVHD and LR MAP on day 28 post-HCT might be candidates for early discontinuation of IS
Hartwell (2017) Robin (2022) Akahoshi (2024) Qayed (2024)	GVHD onset	Biomarker scores predict NRM at GVHD onset; may help guide treatment intensity
Major-Monfried (2018)	Day 7 post-treatment in patients without clinical response	MAP can identify a subset of pts. who will respond to treatment even if no clinical response after 7 days of steroid treatment; can consider watch and wait approach versus add second line therapy
Srinagesh (2019)	Day 28 post-treatment	MAP predicts long-term outcomes after 28 days of steroid treatment; can consider MAP as a response biomarker for clinical trials
Akahoshi (2024)	At time of first CR/VGPR	MAP predicts risk for GVHD flare and NRM after first CR/VGPR; can help guide steroid tapering in responding patients
Defilipp (2024)	Start of second line treatment	Greater benefit of rux for second line treatment if low MAP; poor outcomes with second line treatment with any agent if MAP is high

MAP to guide clinical trials - 1

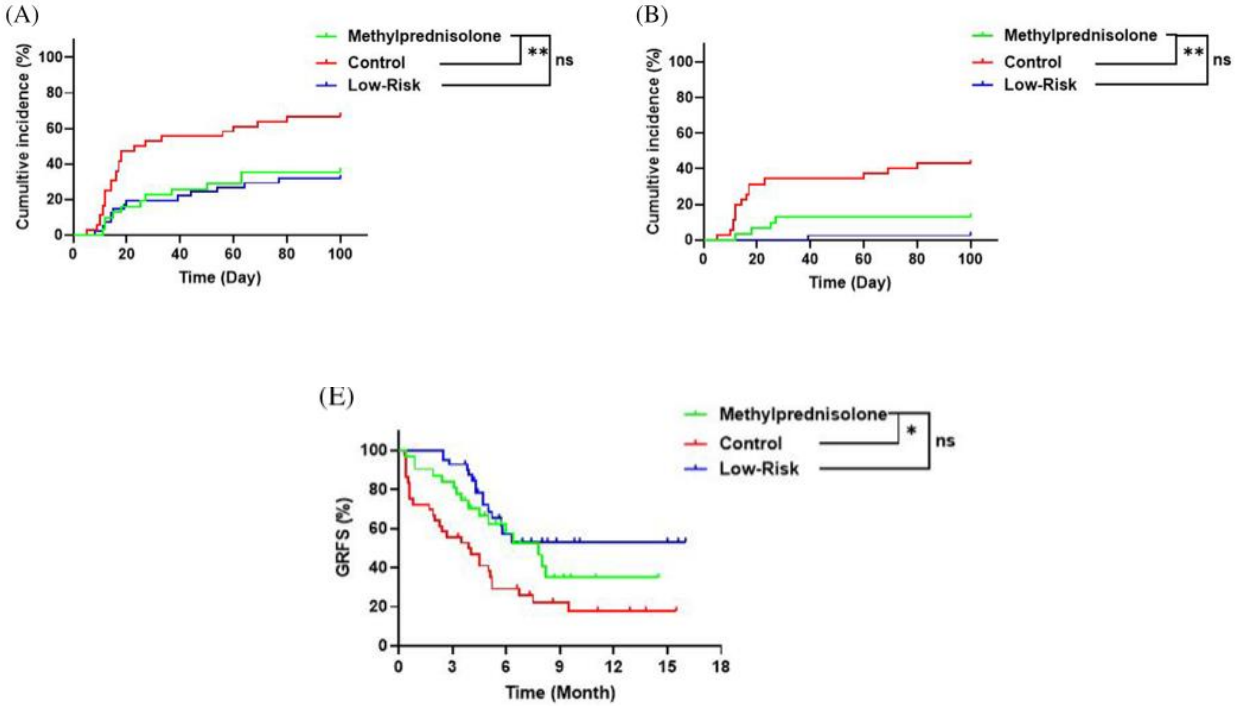


high MAP (defined as > 0.16) on either day 7 or 14

Gergoudis SC et al., Blood Adv 2020

MAP to guide clinical trials - 2

- 108 screened patients weekly for 4 weeks
- lower MAP threshold (> 0.14) to define the higher risk group
- Enrolled patients were randomized to receive either 2 mg/kg/day methylprednisolone, tapering in the absence of GVHD, or observation



Zeng Q et al., Am J Hematol 2023

Conclusions

- Strong evidences about usefulness of biomarkers in acute GvHD
- Just 2 biomarkers (ST2 and REG3α, sign of gastrointestinal damage) are able to predict NRM
- Useful in different setting
- Not only for treatment intensification (high risk patients), but also for IS tapering (low risk patients with malignant disorders)
- The value in clinical trials has to be proved
- Still not in clinical practice
- Will be studied also in the forthcoming FORUM2 trial