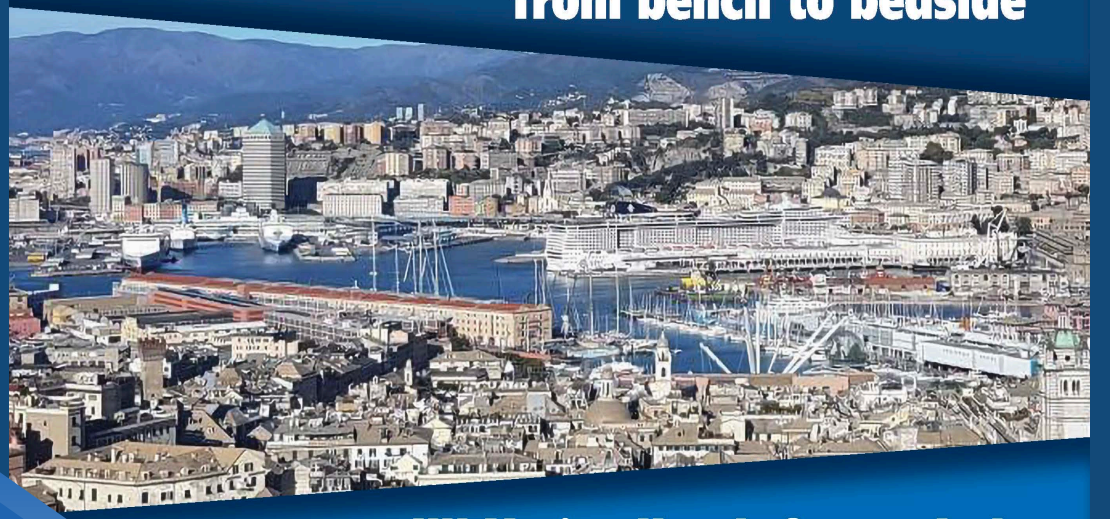
2023 Multiple Myeloma updates:
from bench to bedside

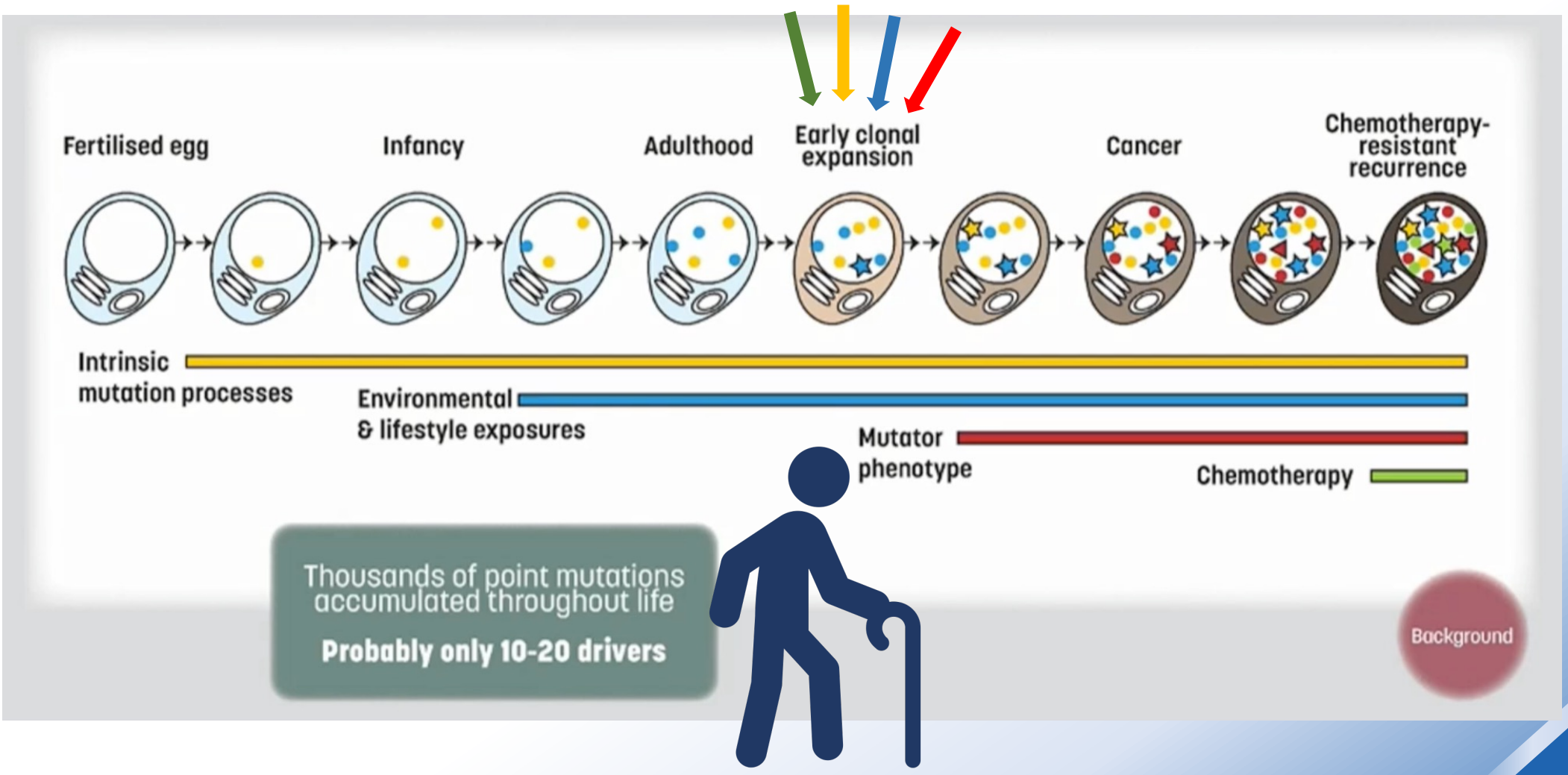


NH Marina Hotel, Genoa, Italy
20-21 November 2023

Carolina Terragna
IRCCS Azienda Ospedaliero-
Universitaria di Bologna

Clonal Hematopoiesis as a biomarker in Multiple Myeloma

DNA mutational rate throughout life



➔ the majority of mutations have no functional impact and do not impair functions however, few “driver” mutations might provide a selective advantage => ***clonal expansion*** possibly preceding cancer transformation

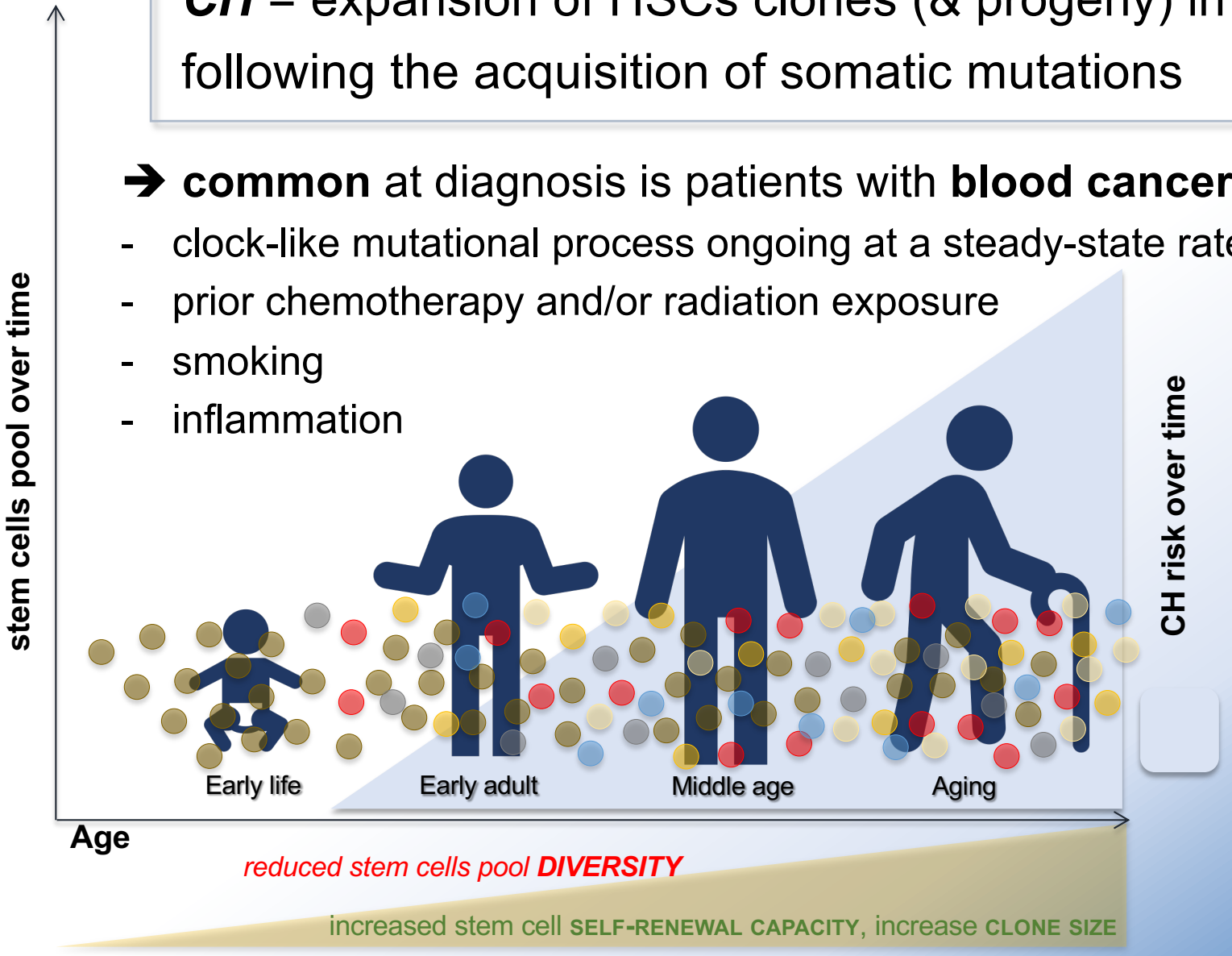
P. Campbell, IMS 2023

clonal haematopoiesis

CH = expansion of HSCs clones (& progeny) in the BM, following the acquisition of somatic mutations

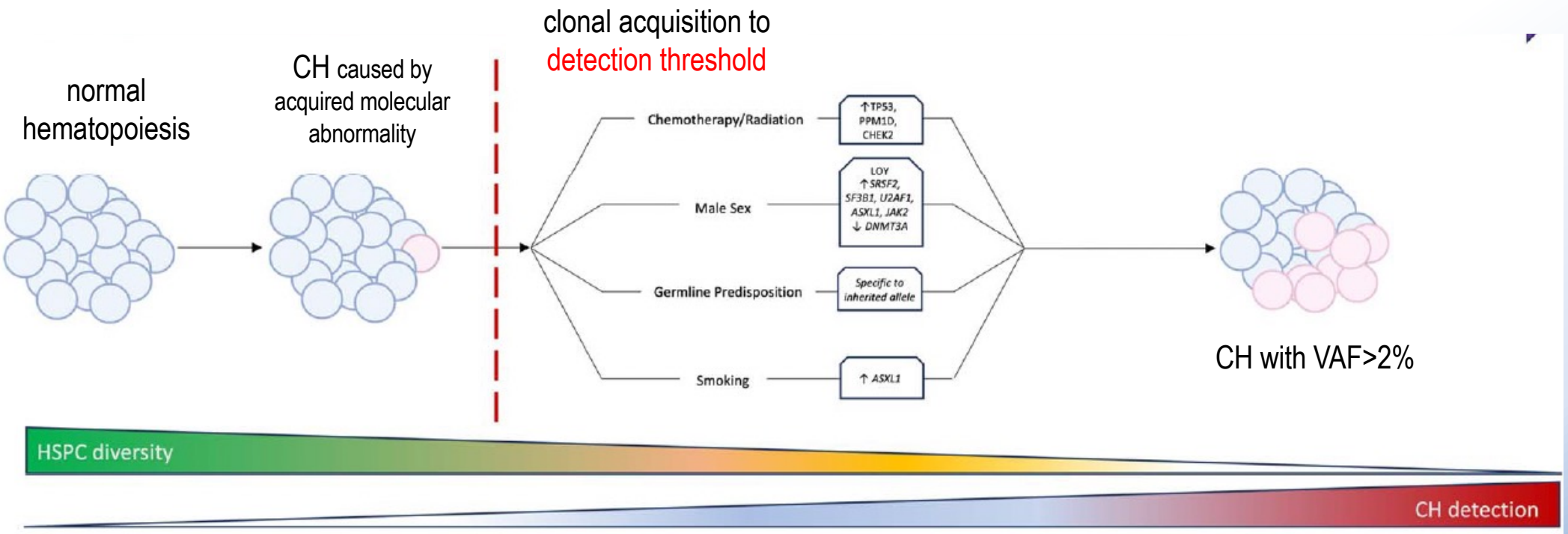
➔ **common** at diagnosis is patients with **blood cancers**, due to:

- clock-like mutational process ongoing at a steady-state rate throughout life
- prior chemotherapy and/or radiation exposure
- smoking
- inflammation



E.Borsi et al., manuscript in preparation

CHIP => clonal haematopoiesis of indeterminate potential



- CHIP can be detected in **10-20%** of individuals >70y
- 90% of CHIP cases carry mutation(s) in ***DNMT3A***, ***ASXL1*** and ***TET2*** (epigenetic modifiers); other frequently observed mutations in *JAK2*, *TP53*, *SF3B1* and *SRSF2*
- somatic CNAs in well-known myeloid malignancies drivers' loci can also occur in approximately 2% of individuals
- lymphoid CHIP less common (*NOTCH1*)

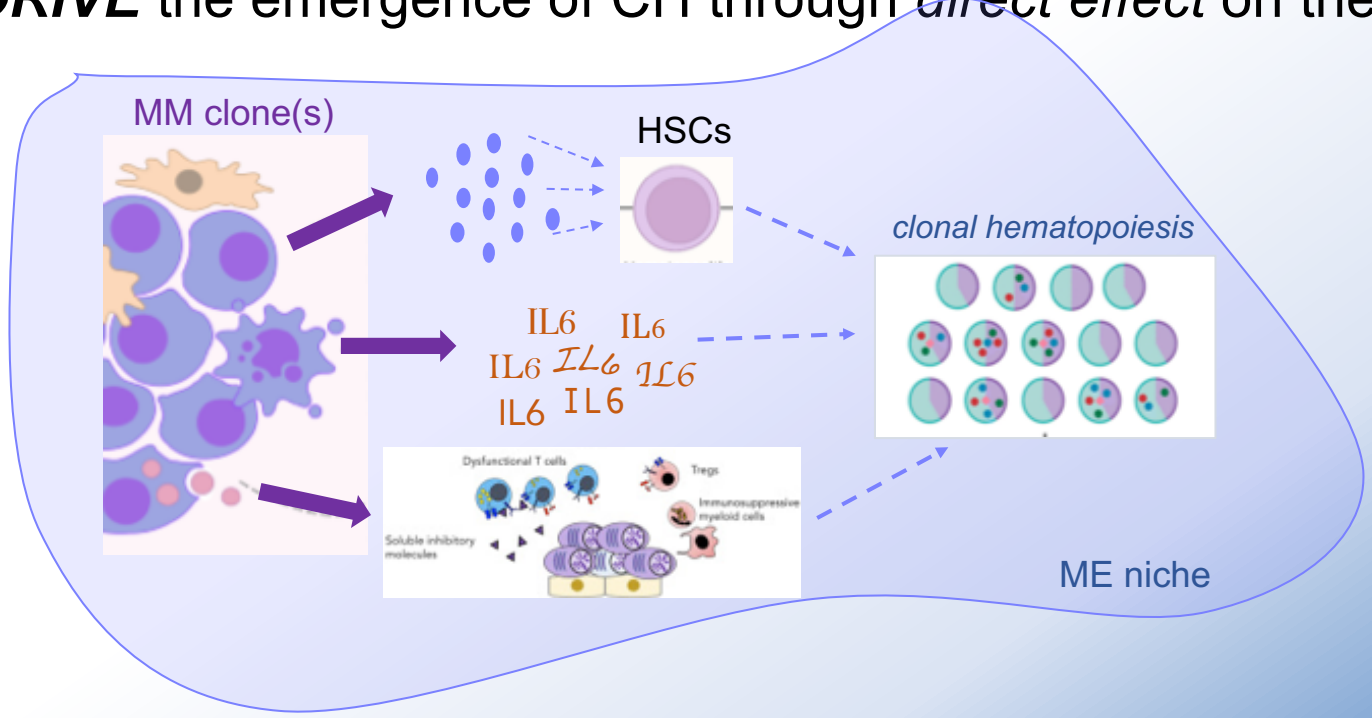
L.D.Weeks and B.L.Ebert, Blood (2023)

CHIP is *common* in haematological diseases

➔ CHIP is *present* in patients with PC neoplasms (up to 30% of treated MM)

(MM incidence increases with aging: is CHIP a ***by-product*** *of age-related changes in HSCs??)*

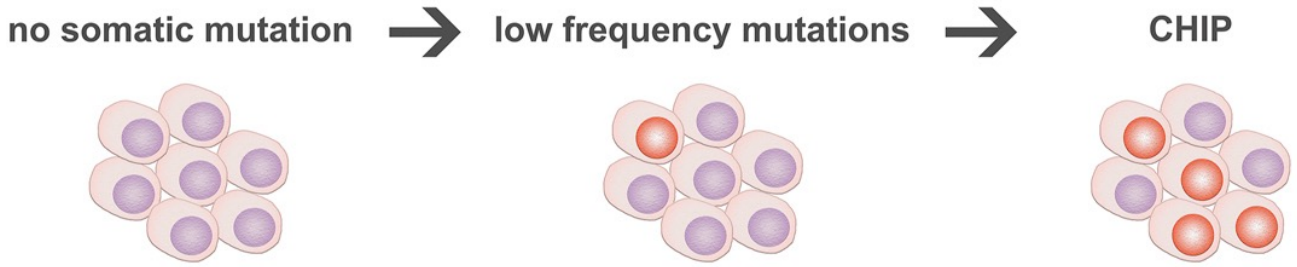
➔ MM might ***DRIVE*** the emergence of CH through *direct effect* on the BM niche

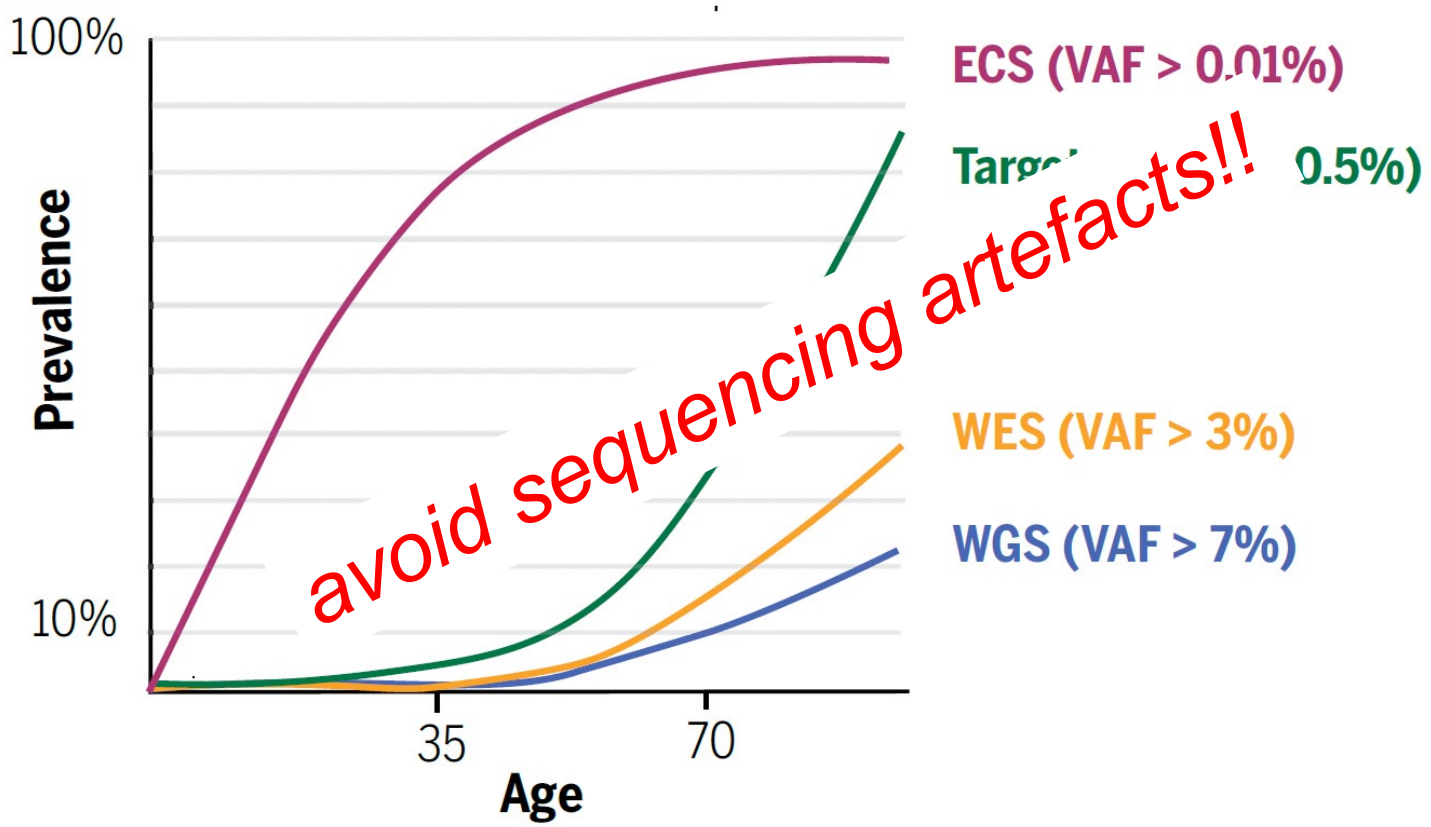


CHIP in MM is associated with a 11.5-fold risk of developing MDS/AML

***how** can CH be assessed*

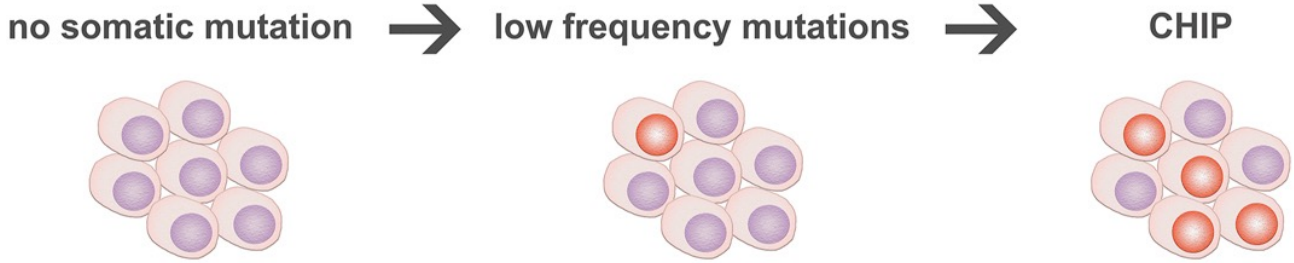
CH assessment by NGS

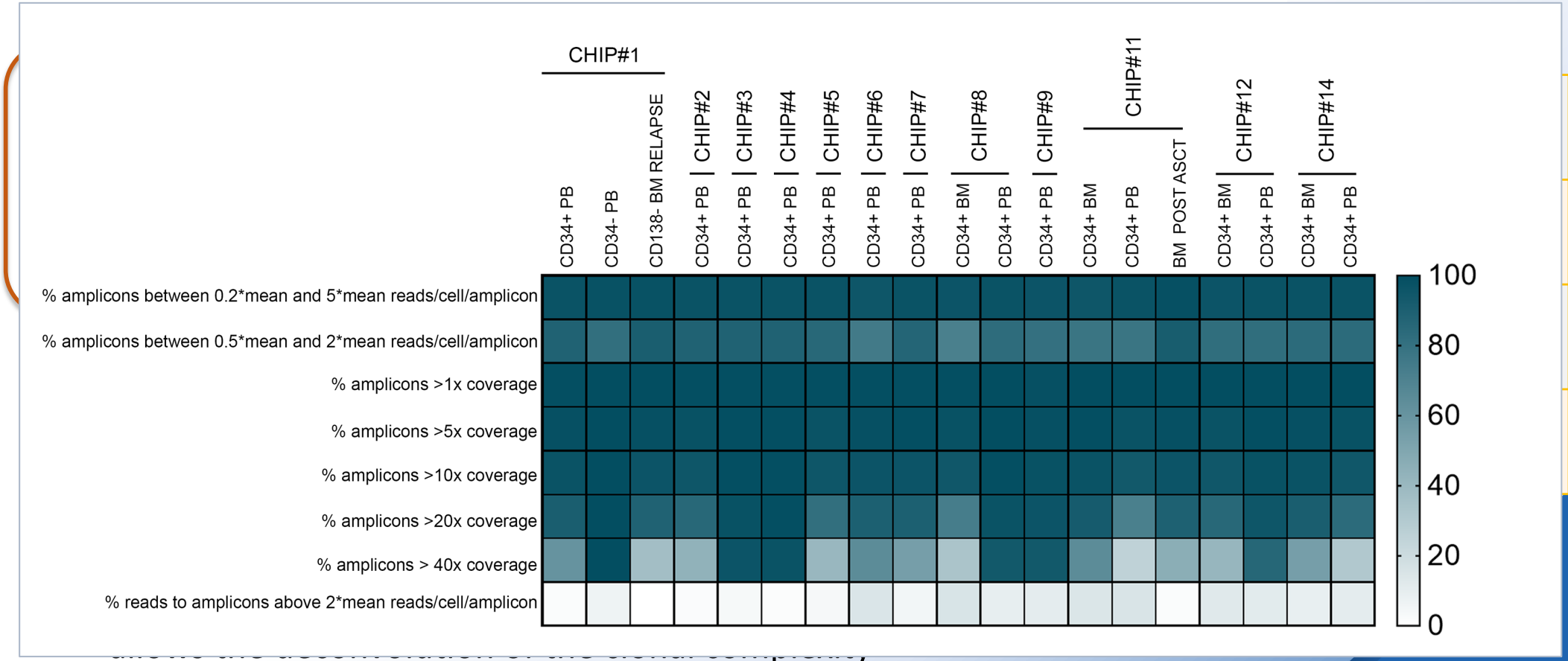




D.P.Steensma et al., Exp.Hematol. (2020)

CH assessment by *single-cell NGS*





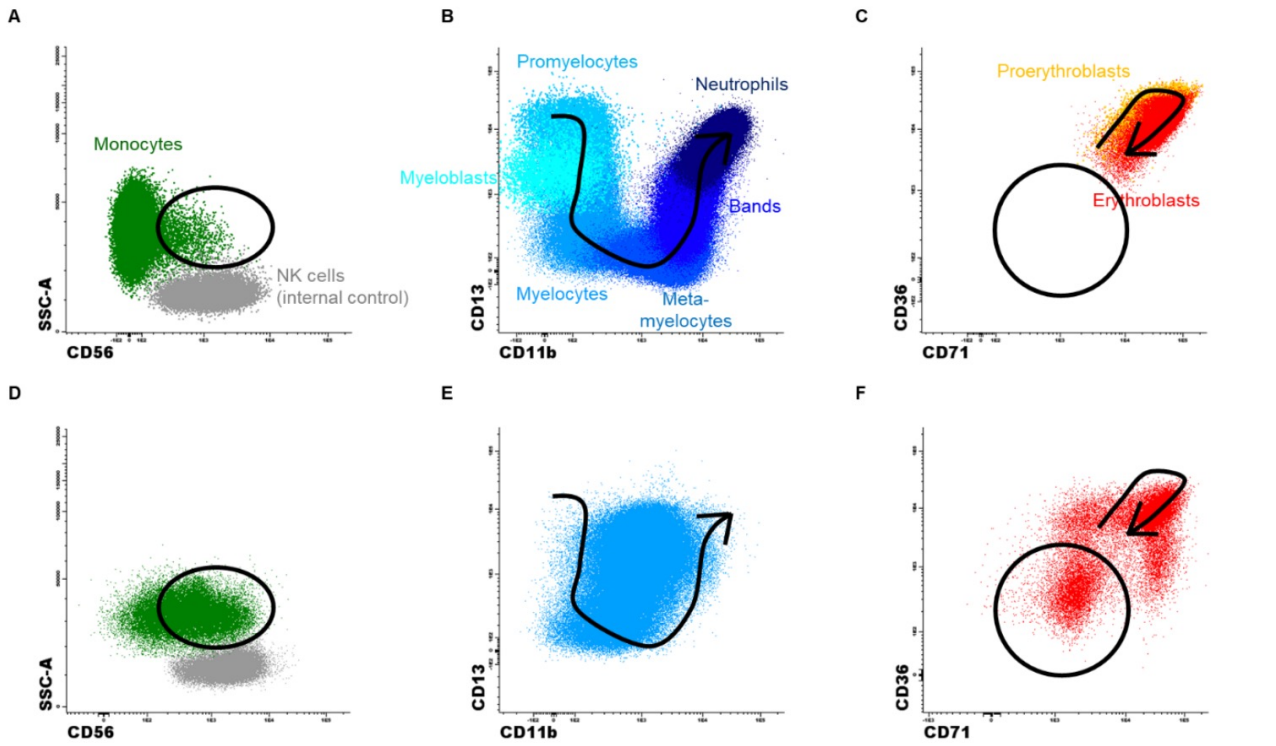
E.Borsi et al., manuscript in preparation

MDS-PA assessment by MC-FC

1. ***NGF Ab panel*** (CD138, CD27, CD38, CD56, CD45, CD19, CD117, CD81) => PC clonality & CD56+ monocytes

2. ***MDS-PA panel*** (HLADR, CD45. CD36, CD13, CD34, CD117, CD71) => neuthrophil and erythroid lineage altered maturation phenotypic pathways

patterns of expression
(monocytic, neutrophil and erythroid lineages)



NORMAL

MDS-like PA

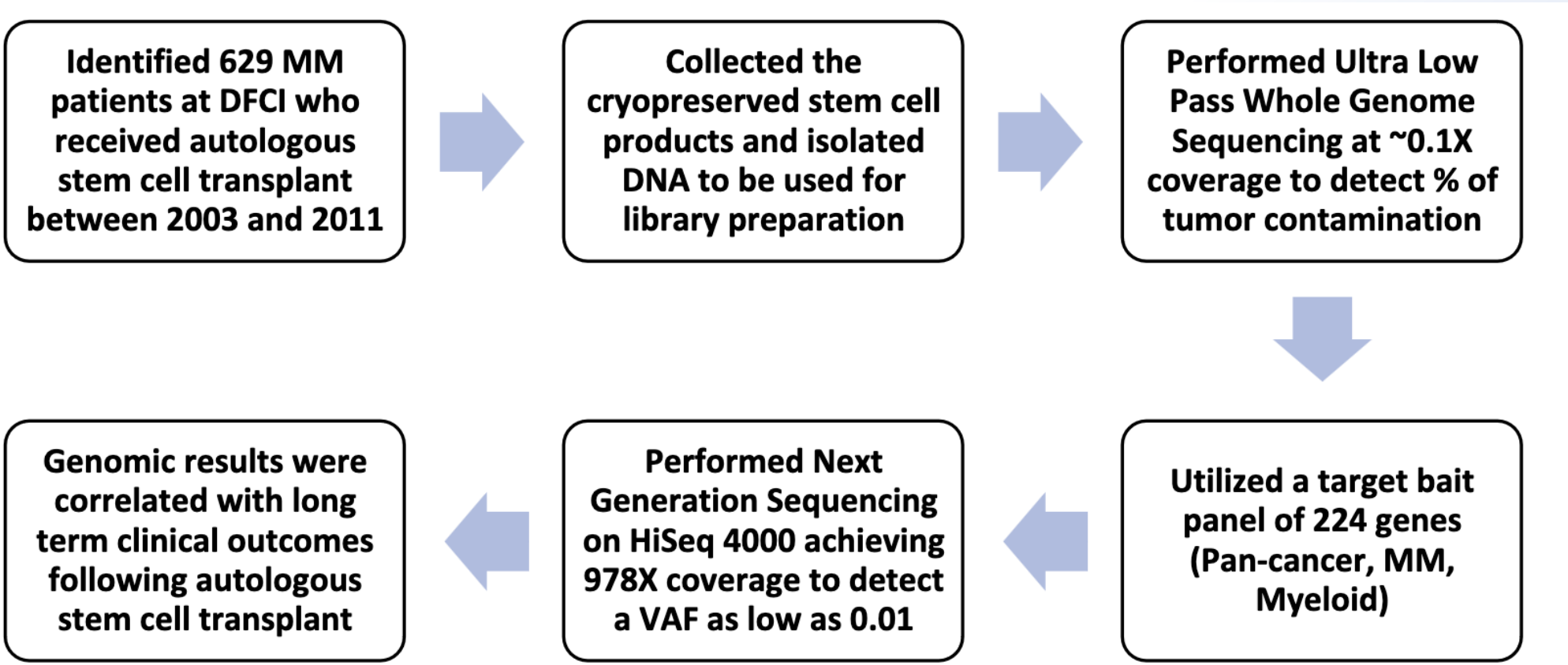
C.Maia et al., Blood (2020)

*CH **prevalence** and **clinical** impact in MM*

CHIP & MM

RATIONALE => prevalence of CHIP is higher in patients exposed to cytotoxic chemotherapy or radiation & is associated with worse clinical outcomes

AIM: to explore the prevalence od CHIP in MM patients at the time of ASCT



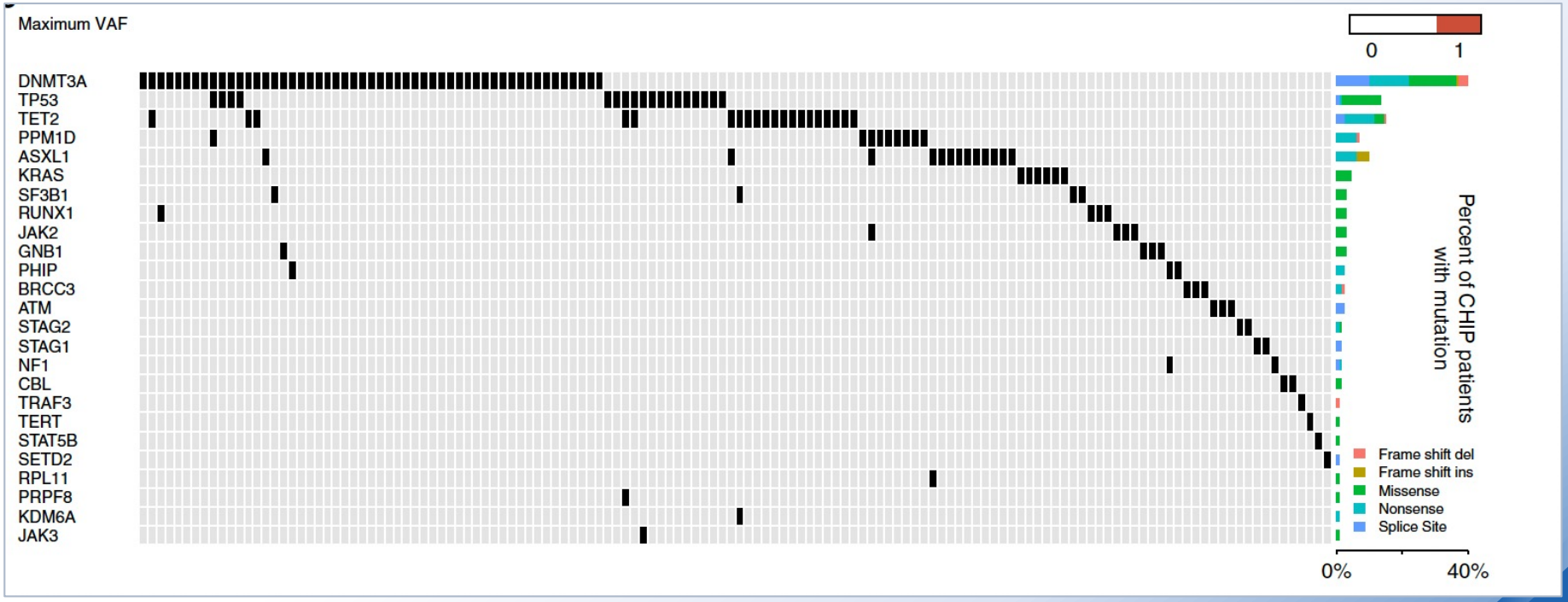
T.H..Mouhieddin et al., Nat.Comm (2020)

CHIP mutational *spectrum* in MM

- 88/629 MM patients (**14%**) with mutation with a VAF $\geq 0.02\%$
- 136/629 MM patients (**22%**) with mutation with a VAF $\geq 0.01\%$

  24/629 MM patients (**4%**) had VAF $\geq 0.1\%$

  ➔ median VAF = 0.027% (very low plasma cells contamination)
- CHIP prior to ASCT *was not associated* with an increased risk of TMN



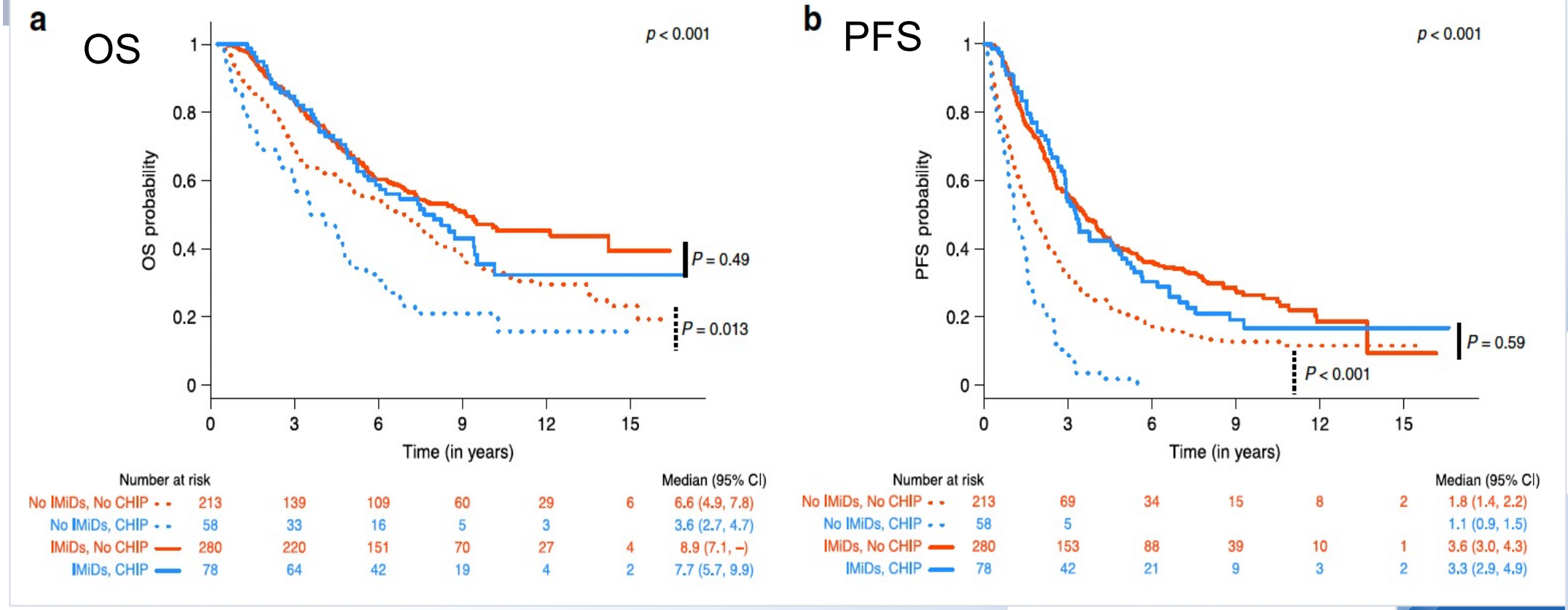
=> *CHIP is common in MM patients*

T.H..Mouhieddin et al., Nat.Comm (2020)

CHIP association with outcome

OS

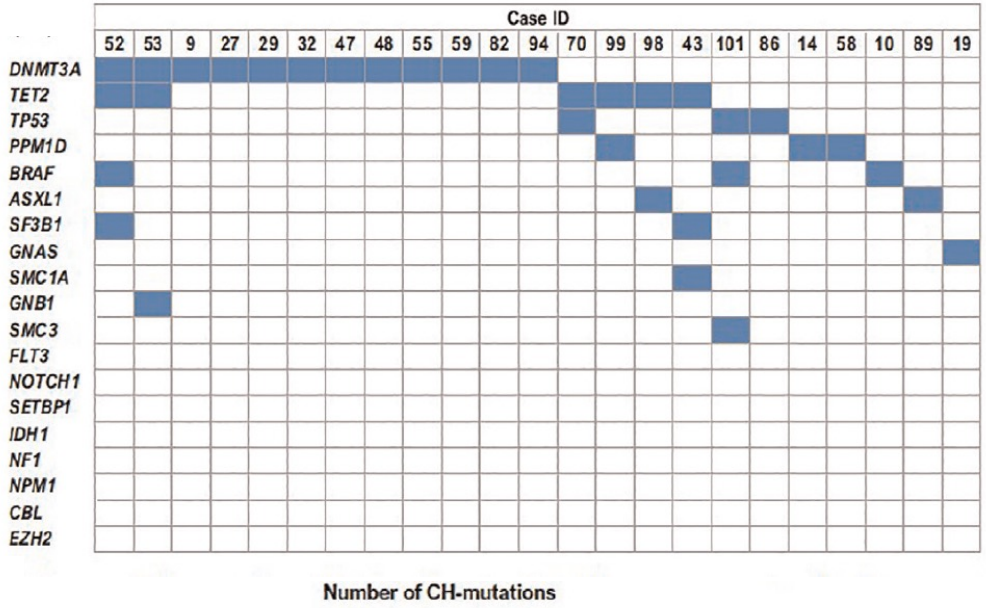
Term	N (%)	HR (LCI, UCI)	p–value
CHIP			
No	493 (78)	Reference	
Yes	136 (22)	1.34 (1.05, 1.70)	0.020



=> adverse impact on outcome was completely abrogated by LEN

T.H..Mouhieddin et al., Nat.Comm (2020)

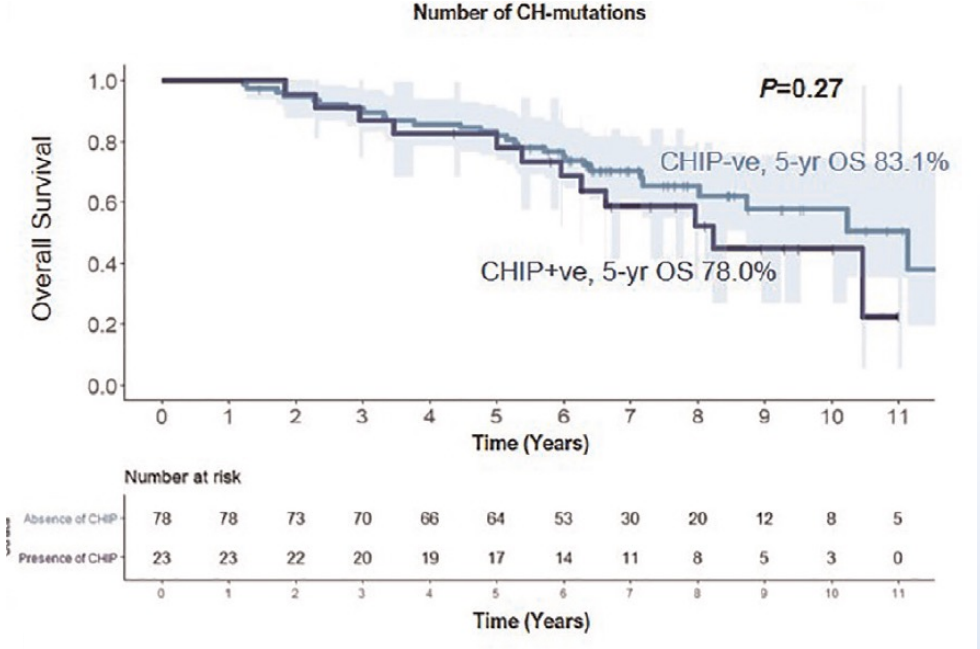
CHIP & MM outcome *(under LEN)*

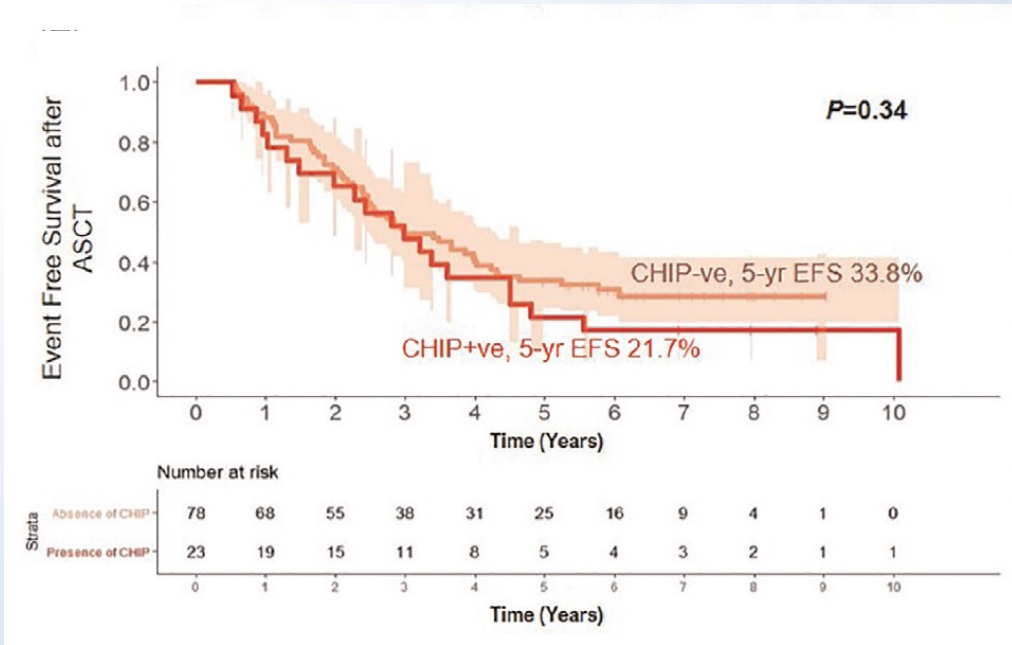


101 MM patients, LEN-maint after ASCT:

- pre ASCT BM CD138-neg cell fractions
- targeted NGS (42 myeloid-related genes)
- CHIP in 23% of patients

=> NO difference in OS or PSF

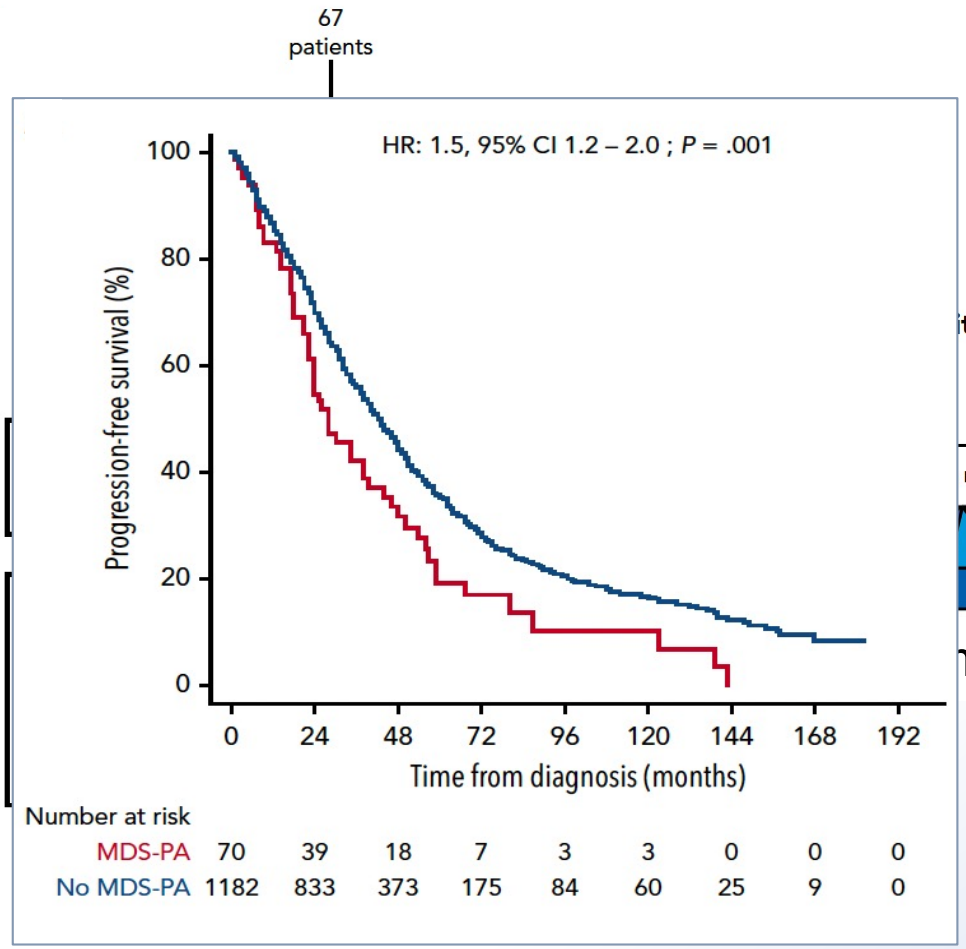


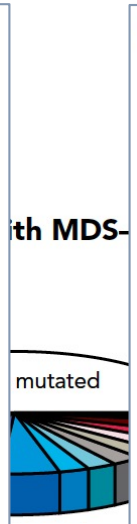


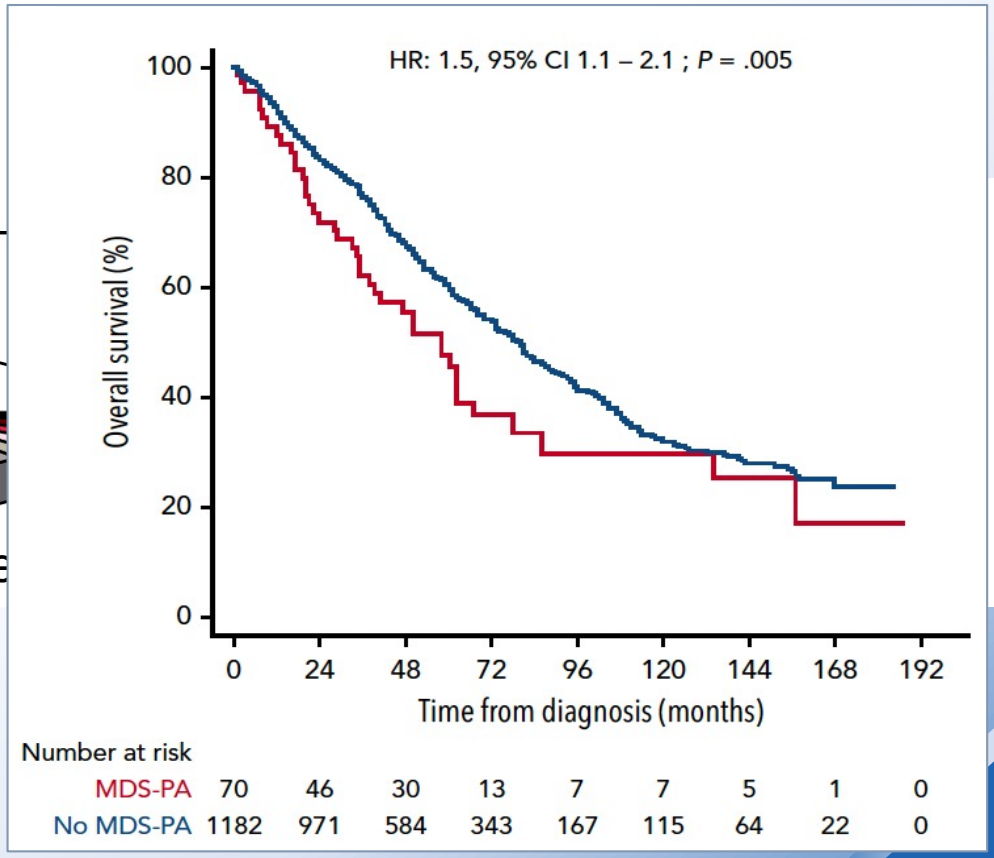
K..Wudhikarn et al., Am.Journal Hematol. (2021)

MDS-PA in MM

33/256 (11.6%) NDMM (treated with triplets including LEN) with MDS-PA



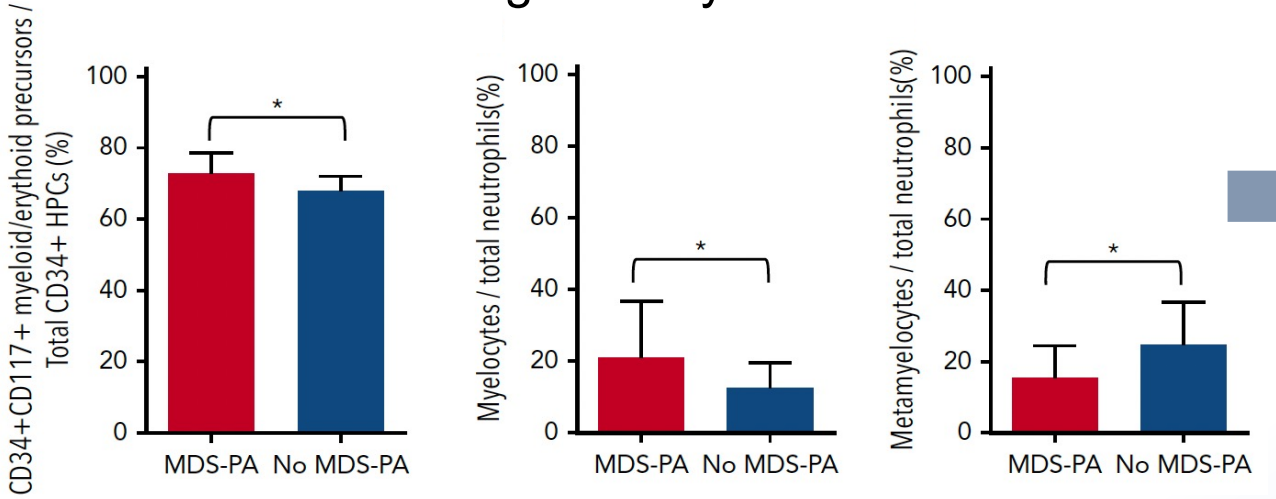




C..Maia et al., Blood (2020)

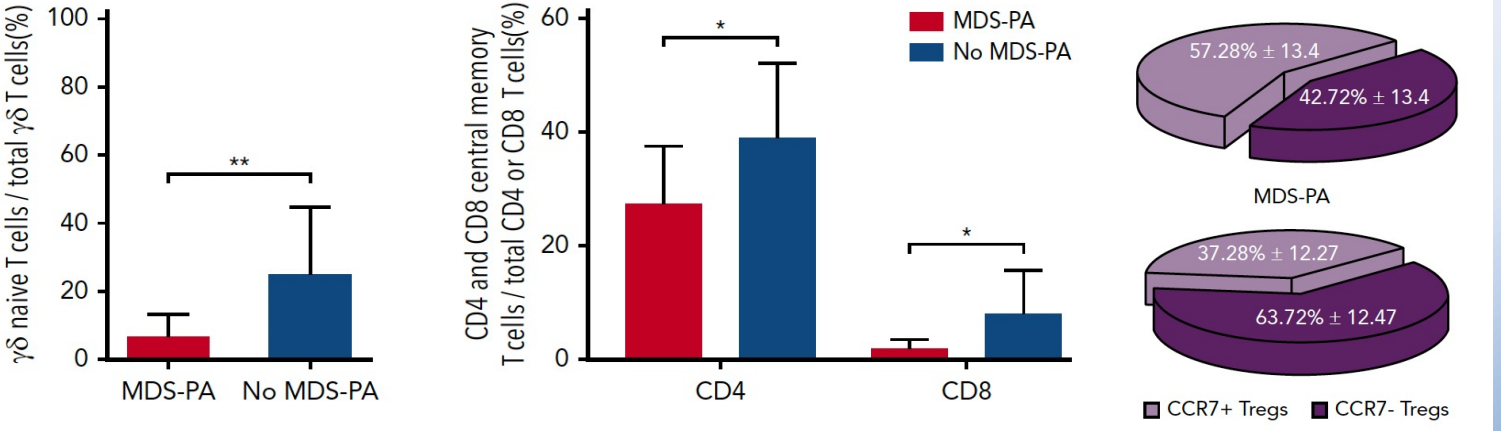
impact of MDS-PA on the tumor microenvironment

1. alteration in the granulocytic differentiation



expansion of myeloid/erythroid precursors & maturation ***arrest***

2. differences in the T-cells compartment after ASCT

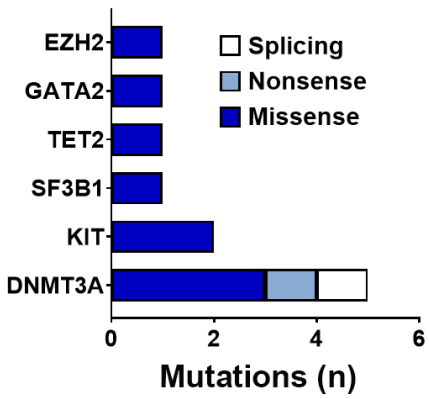


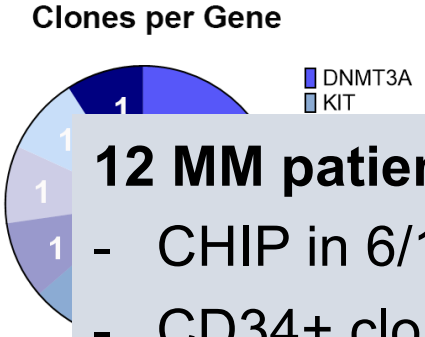
immune alterations due to ***altered*** distribution of γ/δ T cells & Tregs

C..Maia et al., Blood (2020)

scDNA seq to assess CHIP

Number of Mutations	Patient (n)	Patient (%)
1	2	33
2	3	50
3	1	17
Number of Clones	**Patient (n)**	**Patient (%)**
1	2	33
2	1	17
3	3	50

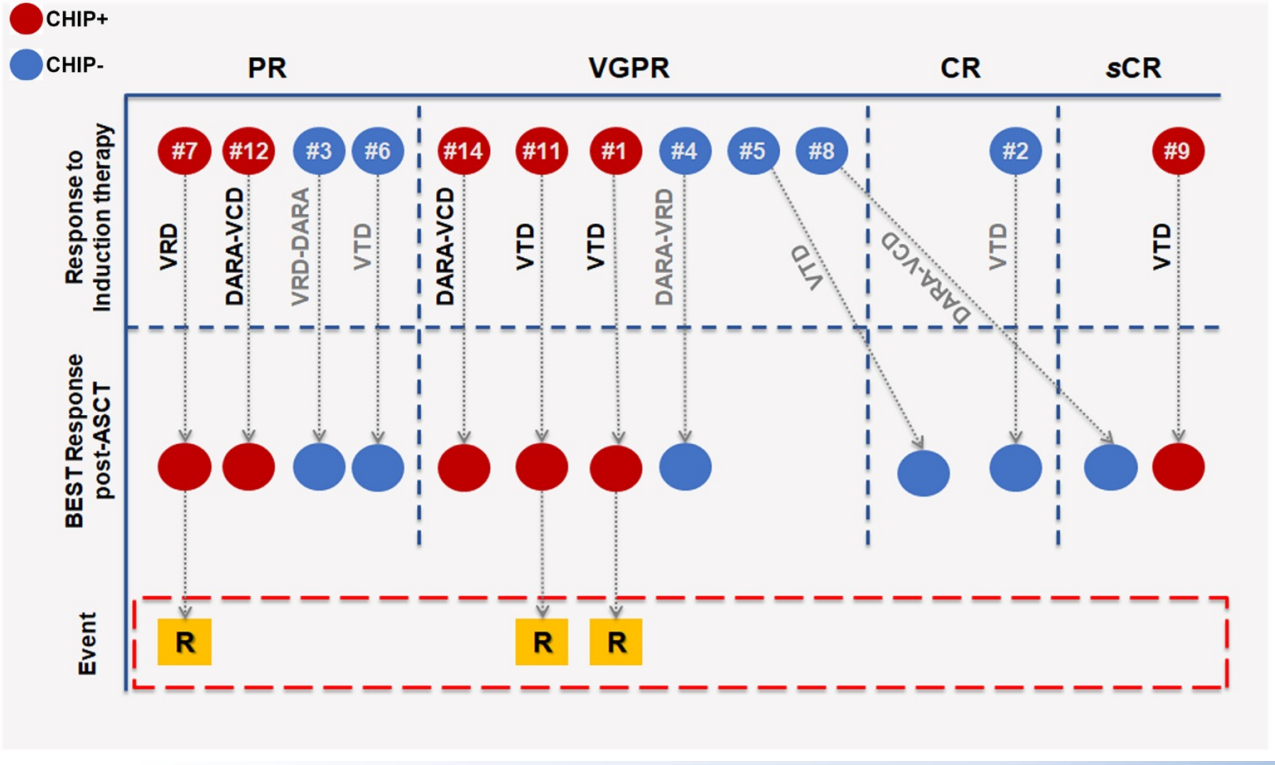




.EN maint.

12 MM patients:

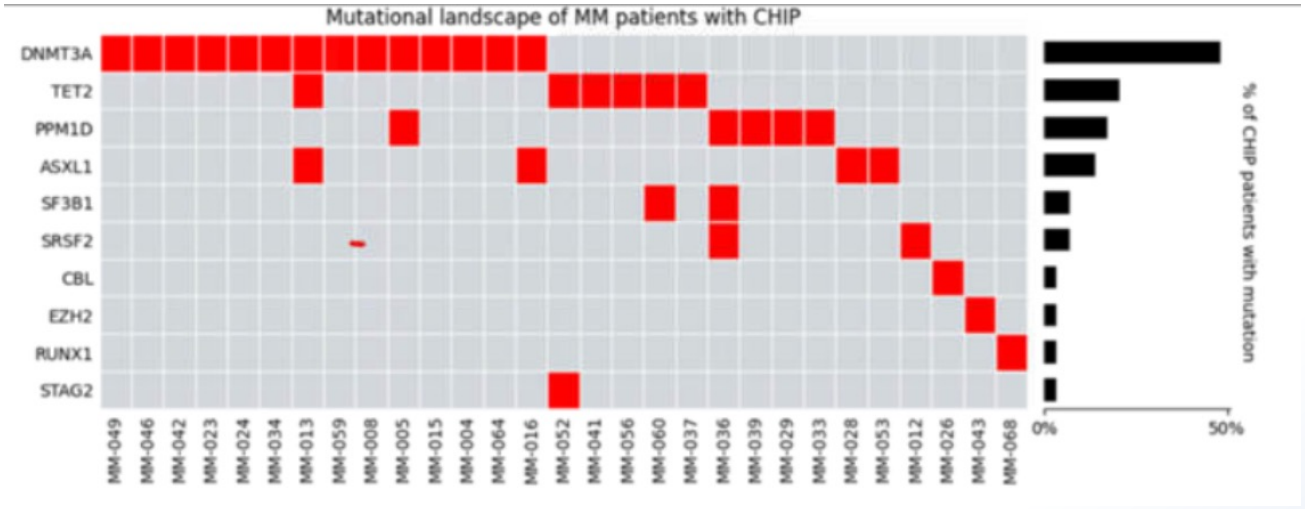
- CHIP in 6/12 (**50%**)
- CD34+ clonal composition
- sub-clonal mutations



E.Borsi et al., manuscript in preparation

ASH2023

760 Characterization of Clonal Hematopoietic of Indeterminate Potential (CHIP) Mutations in an Imid-Naïve Multiple Myeloma (MM) Autologous Stem Cell Transplant (ASCT) Population: First Results from a Pre-Transplant Time Point in a Prospective, Longitudinal Study



66 MM patients:

- CHIP in 66 (**43%**)

(NGS 4000X seq depth)

4814 Clonal Hematopoiesis Is Associated with Severe Cytokine Release Syndrome in Patients Treated with Chimeric Antigen Receptor T-Cell (CAR-T) Therapy

62 MM/NHL patients pre CAR-T:

- CHIP in 15 (**24%**)
- VAF >2%

(1000X seq depth, targeted NGS of 108 pre-defined gene panel)

S.Kahn et al., ASH 2023; S.R.Goldsmith et al, ASH 2023

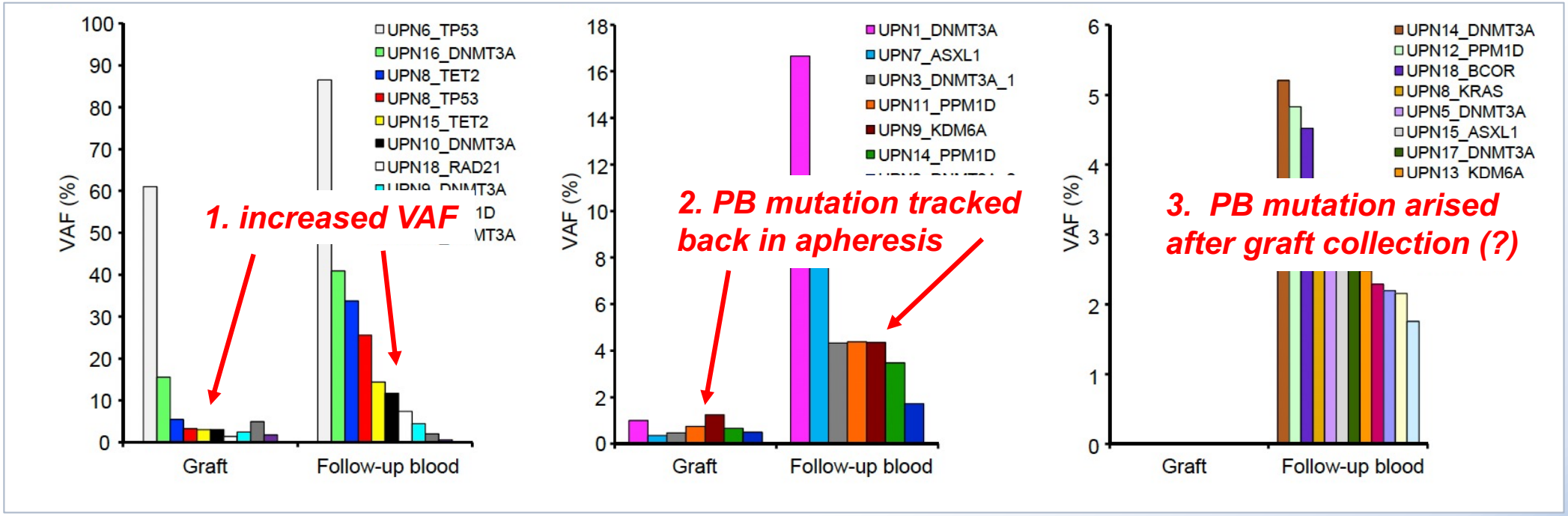
CHIP is common in MM (more common than previously observed?), is associated with altered ME features, causes dismal patients outcomes

*does **therapy** influence CH **evolution**?*

RATIONALE

1. MDS-associated cytogenetic abnormalities were observed in MM patients after HD-CT
2. hematologic stress (induced by cytotoxic therapy, chronic infections, myeloablative regimens…) might support the ***clonal dominance of CHIP clones***, that might possibly outcompete non-mutated HSCs upon ASCT

can CHIP lead to ***clonal evolution***?



⇒ development of CHIP in 81 patients (59 MM, 18 lymphoma, 4 solid tumours) upon ASCT

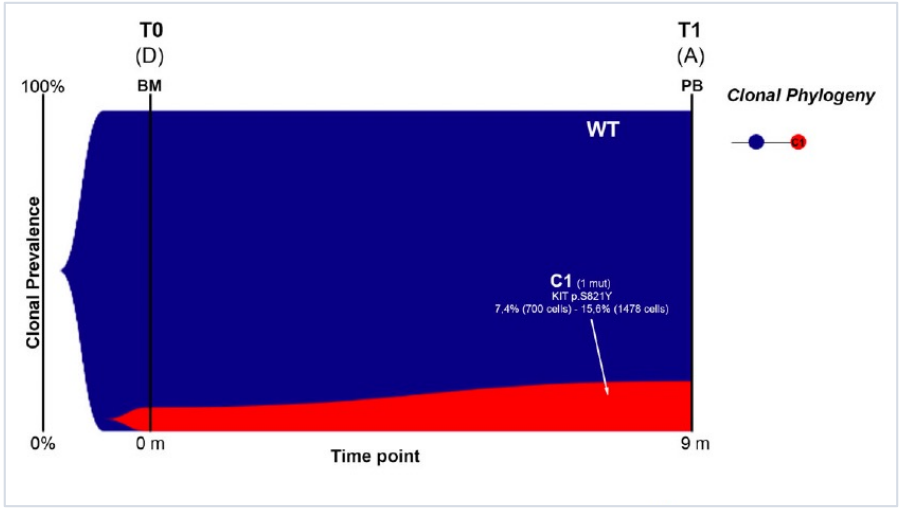
- apheresis & FUP PB samples
- NGS 55 genes associated with CHIP

=>18/81 patients where CHIP carriers (VAF > 2%)

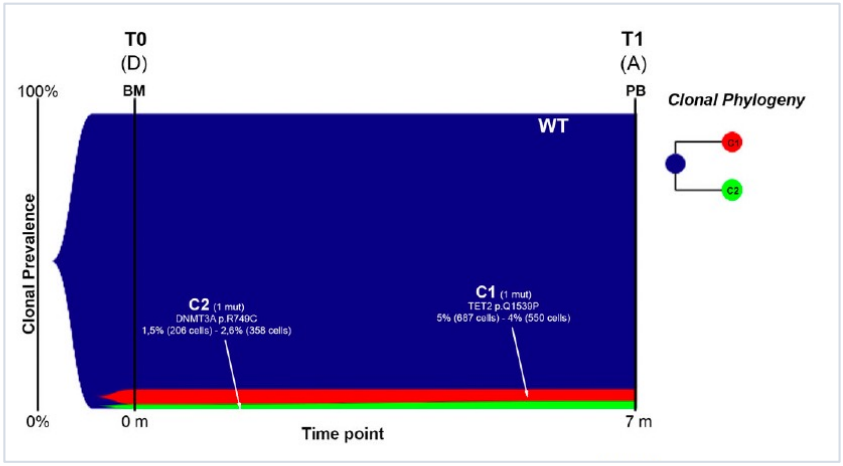
mutations *were not* induced by HD-CT, but pre-exist in patients at the time of graft collection and conferred a reconstitution advantage to mutated HSCs

C.A..Ortmann et al., Cell Reports (2022)

CHIP in longitudinal samples

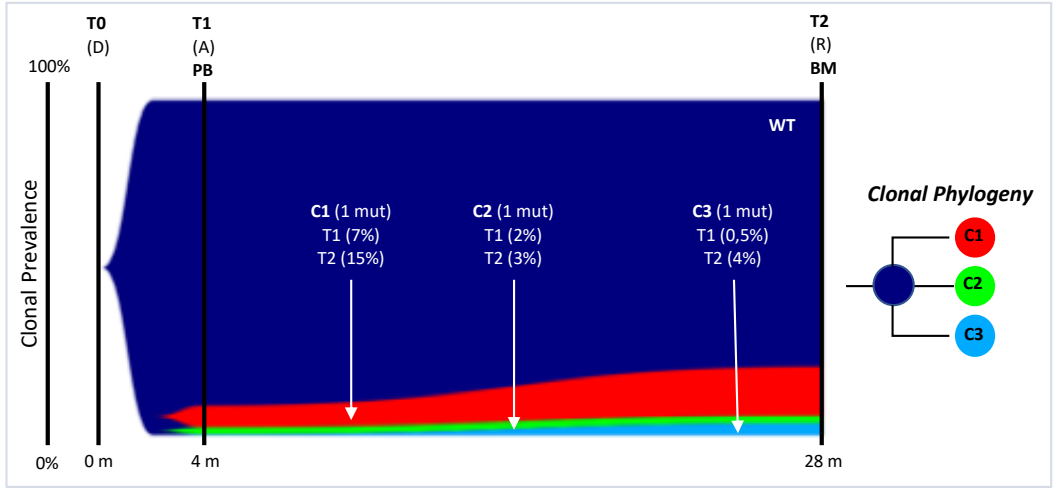


Comparison	Sample pair	Clone	Time point	Mut cells	Other cells	Total cells	Propotion	Change magnitude	Fisher *p* value	*p* val code
1	CHIP#14 CD34+ BM pre-ASCT *vs* CD34+ Apheresis pre-ASCT	C1	T1	298	3731	4029	7,40%	8,21%	**>0,00001**	***
			T2	860	4650	5510	15,61%			

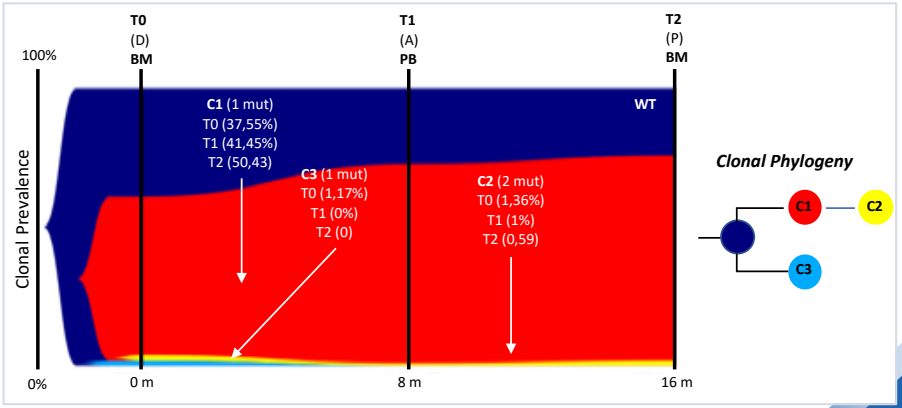


Comparison	Sample pair	Clone	Time point	Mut cells	Other cells	Total cells	Propotion	Change magnitude	Fisher *p* value	*p* val code
1	CHIP#14 CD34+ BM pre-ASCT *vs* CD34+ Apheresis pre-ASCT	C1	T1	419	7966	8385	5,00%	-1,00%	**0,00444**	**
			T2	249	5982	6231	4,00%			
2		C2	T1	126	8259	8385	1,50%	1,10%	**>0,00001**	***
			T2	162	6069	6231	2,60%			

E.Borsi et al., manuscript in preparation



Comparison	Sample pair	Clone	Time point	Mut cells	Other cells	Total cells	Propotion	Change magnitude	Fisher *p* value	*p* val code
1	CHIP1 CD34+ Apheresis pre-ASCT *vs* CD138- BM Relapse	C1	T1	234	3391	3625	6,46%	8,54%	**>0,00001**	***
			T2	1336	7574	8910	14,99%			
2		C2	T1	73	3552	3625	2,01%	0,98%	**0,00194**	**
			T2	267	8643	8910	3,00%			
3		C3	T1	18	3607	3625	0,50%	3,50%	**>0,00001**	***
			T2	356	8554	8910	4,00%			

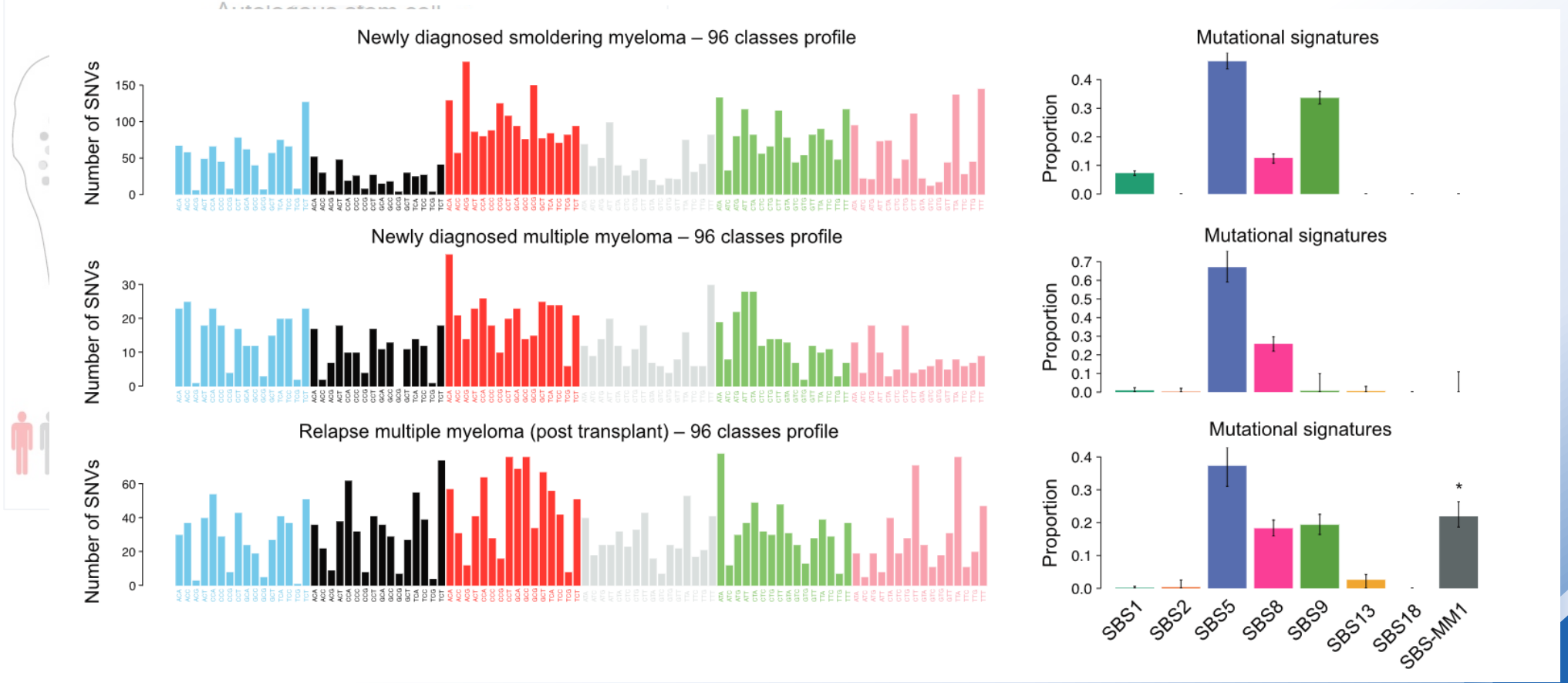


Comparison	Sample pair	Clone	Time point	Mut cells	Other cells	Total cells	Propotion	Change magnitude	Fisher *p* value	*p* val code
1	CHIP#11 CD34+ BM vs CD34+ Apheresis pre-ASCT	C1	T0	386	642	1028	37,55%	3,90%	**0,0154**	*
			T1	4418	6242	10660	41,44%			
2		C2	T0	14	1014	1028	1,36%	-1,00%	**0,00014**	***
			T1	39	10621	10660	0,37%			
3		C3	T0	12	1016	1028	1,17%	-1,17%	**>0,00001**	***
			T1	0	10660	10660	0,00%			
1	CHIP#11 CD34+ Apheresis pre-ASCT vs MNCs post-ASCT	C1	T2	4418	6242	10660	41,44%	8,90%	**>0,00001**	***
			T3	3362	3306	6668	50,42%			
2		C2	T2	39	10621	10660	0,37%	1,24%	**>0,00001**	***
			T3	107	6561	6668	1,60%			
3		C3	T2	0	10660	10660	0,00%	0,00%	**1,0000**	***ns***
			T3	0	6668	6668	0,00%			

two paths from CH to clonal expansion

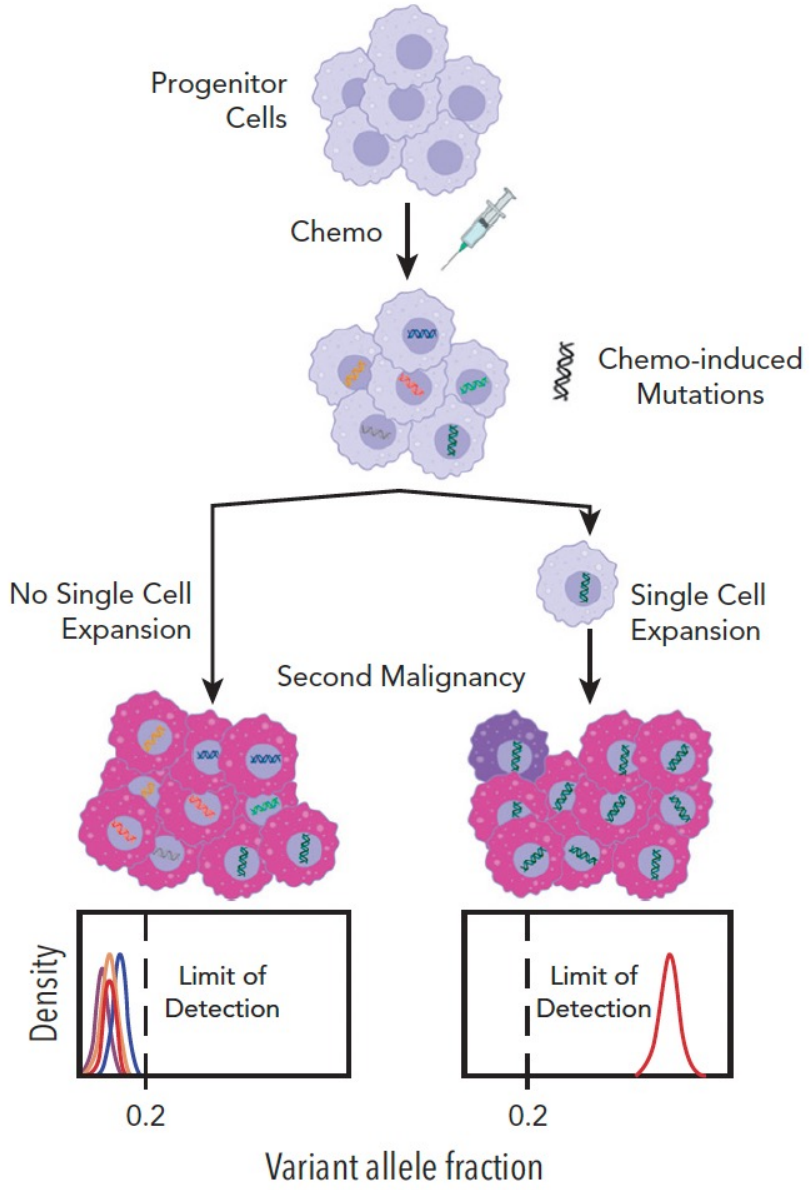
pre-transplantation → post-transplantation

mutational signature as «***molecular barcode***»



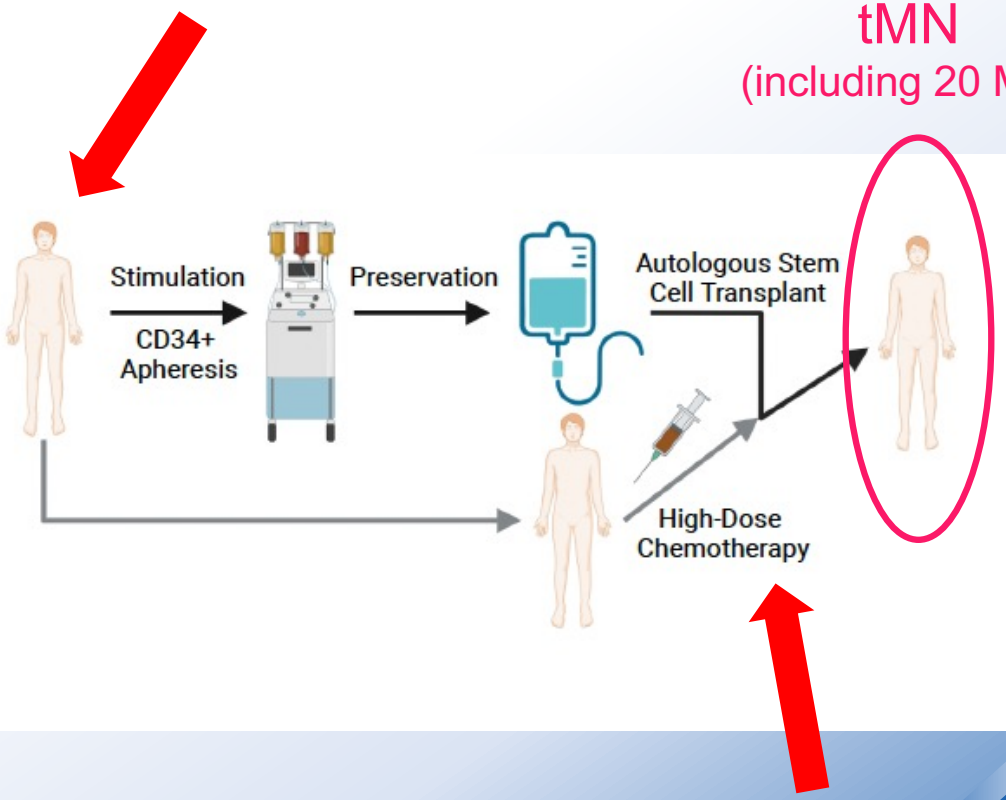
C.A..Ortman et al., Cell Reports (2022
F.Maura et al., Leukemia (2021)

mutational signature to track CH evolution



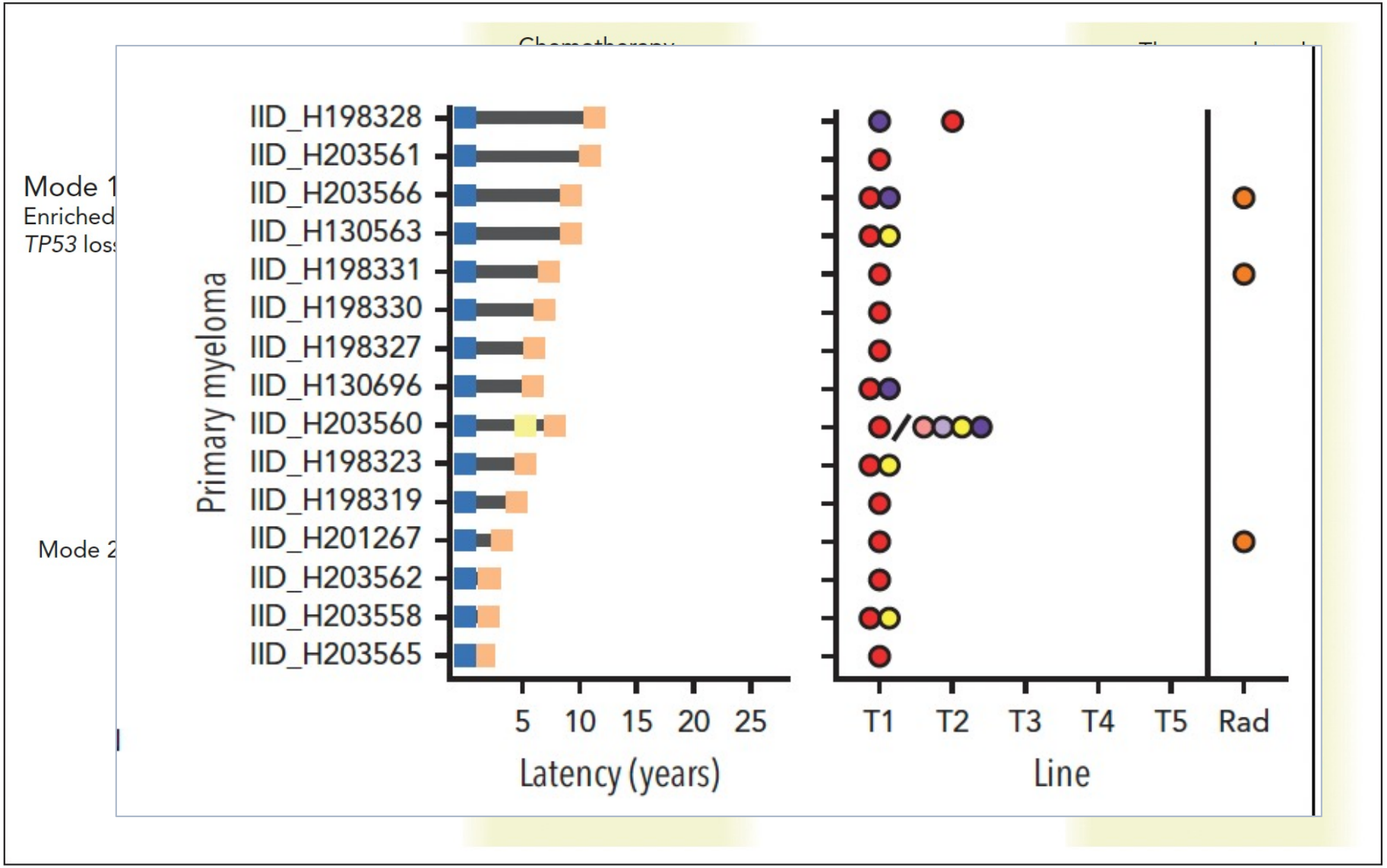
mutational signature = genomic single-cell *barcode*, linked to a mutational process exposure

tMN
(including 20 MM)



B..Diamond et al., Blood (2023)

two path leading to CH clonal evolution



Y.S..Lee and P.van Galen, Blood (2023)

take-home-message

1. CH is ***common*** in MM patients and tends to become more common after treatment

2. ***high-throughput*** technologies are needed to detect very infrequent clones carrying CH-related mutations

3. CH may confer ***worse outcomes*** in patients undergoing ASCT; worse outcome is abrogated by IMiDs maintenance

4. CH is associated with an ***increased risk*** of subsequent hematologic malignancies

5. genomic alterations driving the myeloid clones' expansion can be either already pre-existing at diagnosis or can be acquired in response to DNA damaging therapy

thanks

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