FIFTH
INTERNATIONAL SYMPOSIUM ON
SECONDARY LEUKEMIA
AND LEUKEMOGENESIS

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CLONAL EVOLUTION IN THERAPY-RELATED NEOPLASMS

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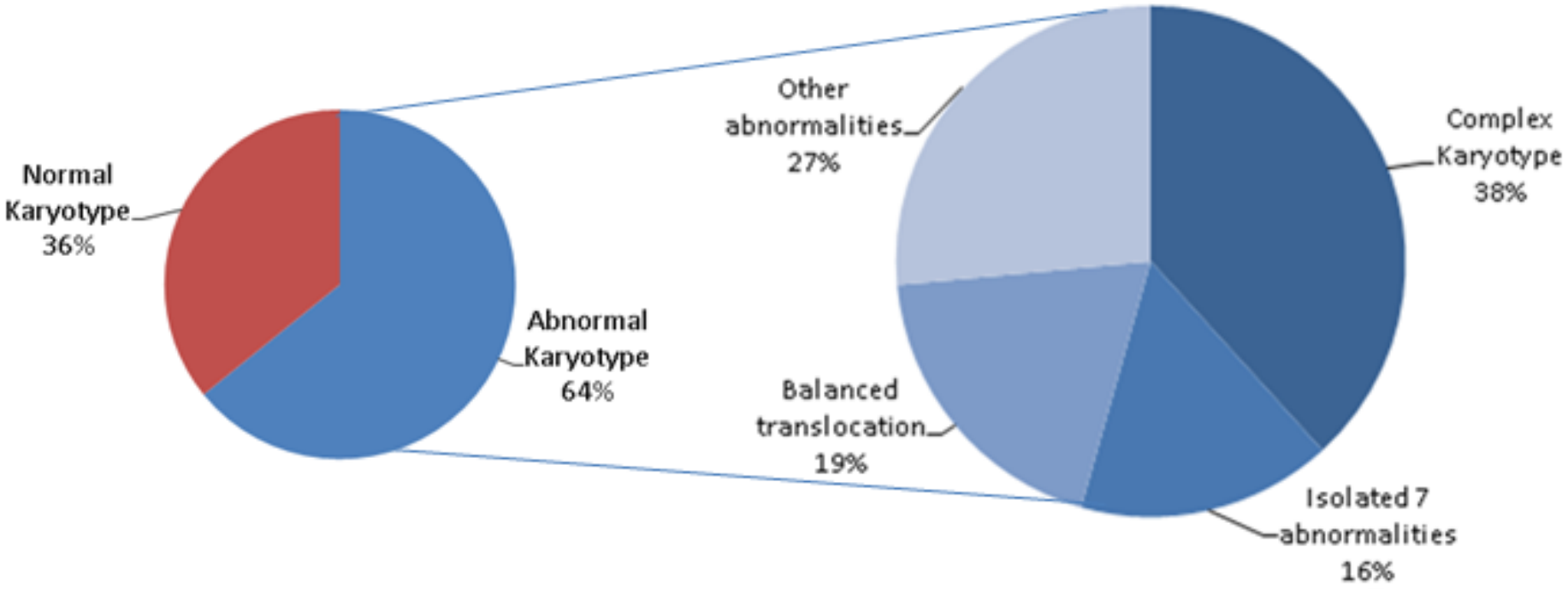
CLONAL EVOLUTION IN THERAPY-RELATED NEOPLASMS

Therapy-related myeloid neoplasms are characterized by high incidence of complex karyotypes, frequent abnormalities of chromosome 7 and/or 5



High prevalence of the t(4;11)(q21;q23) translocation have been commonly reported in t-ALL.

Italian Registry on Secondary Leukemias. Fianchi et al., Am J Hematol 2015

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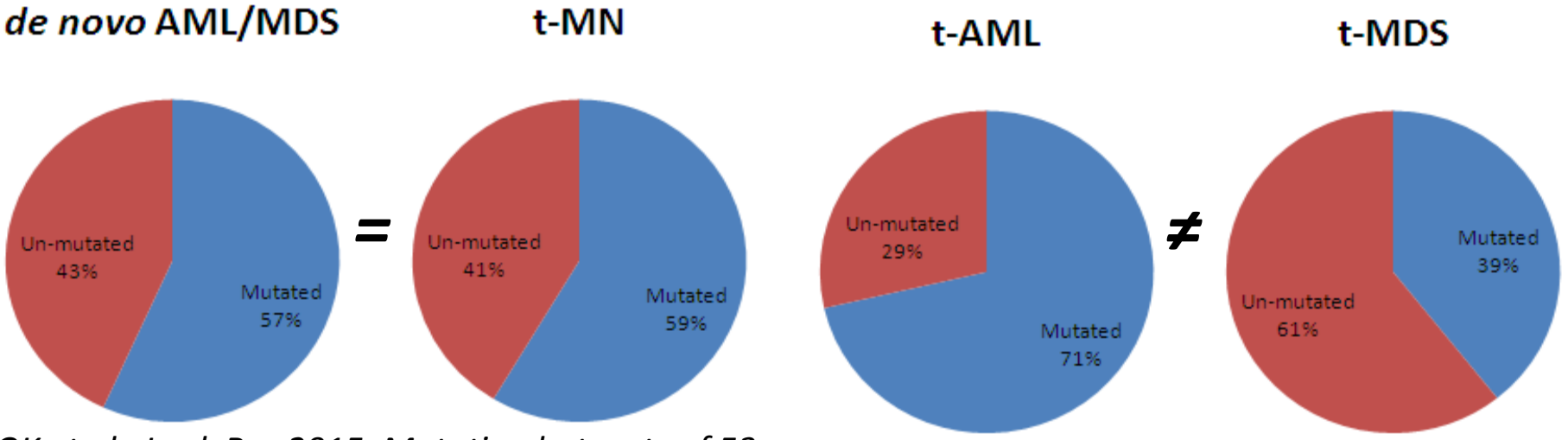
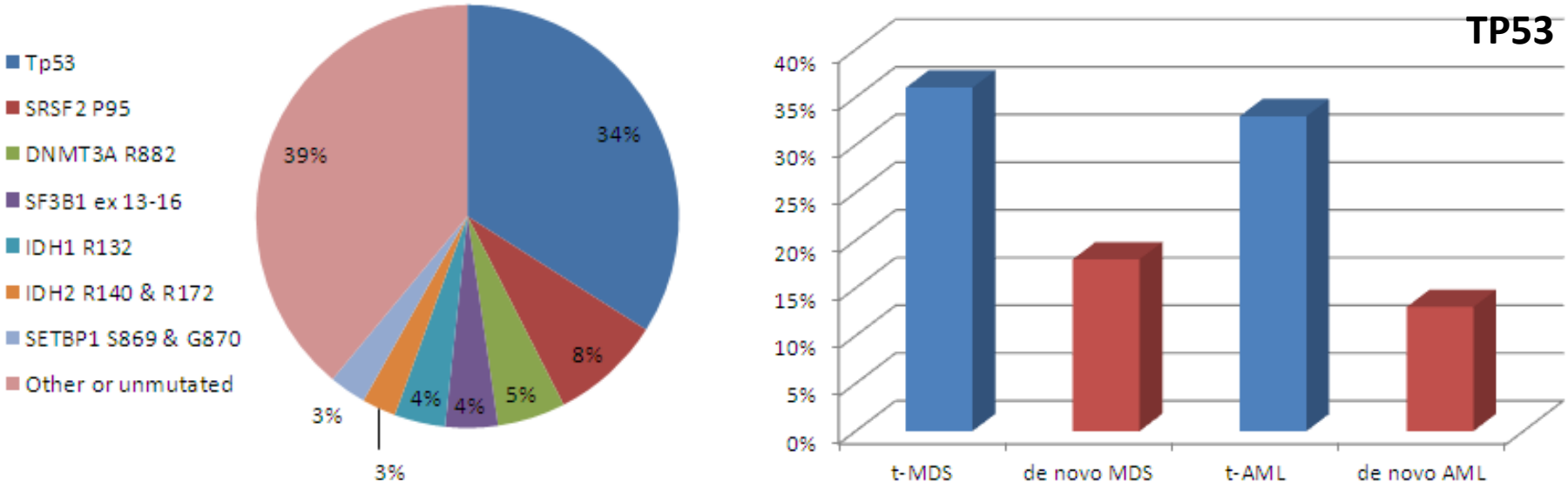
Prevalence of Somatic Mutations in t-MN



OK et al., Leuk Res 2015. Mutation hotspots of 53 genes



Voso et al., Leukemia 2013 and Fabiani et al., Haematologica 2014.

Patient Characteristics

UPN	Age at t-MN diagnosis (yrs)	Gender	Primary Malignancy	Treatment of primary malignancy	BM-blasts (%)	Diagnosis	Latency (months)	Karyotype
1	73	F	NHL	ProMACE-Cytabom + RT	12	t-MDS	100	46,XX [25]
2	62	F	APL	AIDA 2000 [17]	14	t-MDS	30	46,XX [9] / 42,45,ring [13]
3	63	M	NHL	R-CHOP, R-MICMA, autoSCT; bortezomib/lenalidomide	4	t-MDS	83	47,XY,+21 [12]
4	62	F	NHL	CHOP, MICMA, autoSCT, fludarabin	8.5	t-MDS	124	46,XX [12] / 45,XX,-7 [6]
5	30	M	NHL	CODOX-M/IVAC, MICMA, autoSCT	10	t-MDS	54	46,XY,-7 [10]
6	63	F	NK-AML and Breast Ca	AML-12 [18], Carboplatin, taxotere, herceptin + RT	10	t-MDS	83	46,XX [24] / 46,XX,del(5)(q14q34) [1]
7	74	F	NHL	CHOP, R-FC, chlorambucil	19	t-MDS	66	46,XX [25]
8	60	F	APL	AIDA + AutoSCT	6	t-MDS	100	46,XX,-7, +21 [11] / 45,XX, -7 [2]
9	81	M	NHL	Chlorambucil + RT	13	t-MDS	100	Not available
10	74	M	NHL	R-FND, R-MICMA	4.5	t-MDS	74	46,XY [19] / 44,X,-7, del(1)(p35), del(5)(q13), del(11)(q14) [6]
11	37	F	HL	BEACOPP escalated	18	t-MDS	83	47-49,-7,ring [10]/ 46,XX [1]
12	50	F	HL	BEACOPP escalated	43	t-AML	18	46,XX [25]
13	43	M	ALL	GMALL 05/93 [19]	7	t-MDS	32	45,XY,-7 [6]
14	40	F	APL	AIDA 2000 [17]	80	t-ALL	18	46,XX, t(4;11)(q21;q23)[5]

CLONAL EVOLUTION IN THERAPY-RELATED NEOPLASMS

1. Methods

Mutational screening of common somatic mutations and other gene alterations in therapy-related leukemia patients

Sanger Sequencing

IDH1 R132
IDH2 R140/R172
DNMT3A R882
ASXL1

→ **Epigenetic enzymes**

SF3B1 ex 13-16
SRSF2 P95
U2AF1 S34

→ **Spliceosome machinery**

SETBP1 SKI Domain
N-RAS
K-RAS

Next Generation Sequencing

TP53

Q-RT-Polymerase chain reaction

t(4;11)(q21;q23)
KMT2A/AFF1

Pyrosequencing

Validation & quantification of the mutated clone

2. Methods

Mutations identified in the t-MN sample were then tracked backwards in samples collected during follow-up of the primary tumor, using high sensitivity techniques

High-throughput Next Generation Sequencing

- ✧ Specific homemade designed primers were linked to specific flags
- ✧ Average coverage was >120000x for all corresponding specific amplicon
- ✧ Variant allele frequencies in % (alternative allele count/total depth x100)

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Screening of genetic changes in therapy-related neoplasms

UPN	TP53	IDH1 R132	IDH2 R172	IDH2 R140	DNMT3A R882	U2AF1	SF3B1 13-16	SRSF2	SETBP1	N-RAS	K-RAS	ASXL1
1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	Y591*
2	Y220C	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
3	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	S689*
4	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	R693*
5	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
6	WT	WT	WT	WT	WT	WT	K700E	WT	WT	WT	WT	WT
7	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
8	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
9	WT	R132H	WT	WT	WT	WT	WT	P95H	WT	WT	WT	WT
10	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
11	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
12	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
13	WT	WT	WT	WT	WT	WT	WT	WT	G870R	WT	WT	WT

- ✧ We identified 8 mutations (*IDH1 R132H, SRSF2 P95H, SF3B1 K700E, SETBP1 G870R, TP53 Y220C, ASXL1 Y591*, ASXL1 S689* and ASXL1 R693**) in 7 of 13 t-MN patients
- ✧ UPN 9 carried two mutations (*IDH1* R132H and *SRSF2* P95H)
- ✧ UPN 14, a t-ALL patient, was *KMT2A-AFF1*-positive

Quantification of the identified variants at the time of t-MN diagnosis

- Pyrosequencing analyses, specifically designed to quantify the mutations confirmed the data obtained from Sanger sequencing in all cases.

- Mutations identified in epigenetic regulators, spliceosome enzymes and *SETBP1* were detectable at a variant allele frequency (VAF) of 20 to 42% at the time of t-MN diagnosis

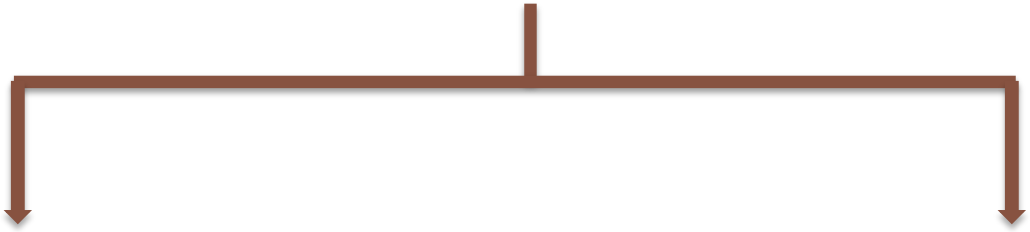
- TP53 mutation was detectable at a lower rate (6,75%)

- UPN9 carried two mutations (*IDH1* R132H and *SRSF2* P95H), at a similar VAF (38% and 35%, respectively) suggesting that the two mutations were present in the same leukemic clone.

- Quantitative evaluation of the *KMT2A-AFF1* transcript in UPN 14 revealed strong positivity for the chimeric transcript at the time of t-ALL diagnosis (3500 copies /10^4 ABL)

Disease markers and clonal evolution

Ultra-deep NGS reveals at least two different patterns of clonal evolution



First scenario

Somatic mutations characterizing the t-MN clone were not detectable at primary cancer diagnosis and appeared after cytotoxic treatment

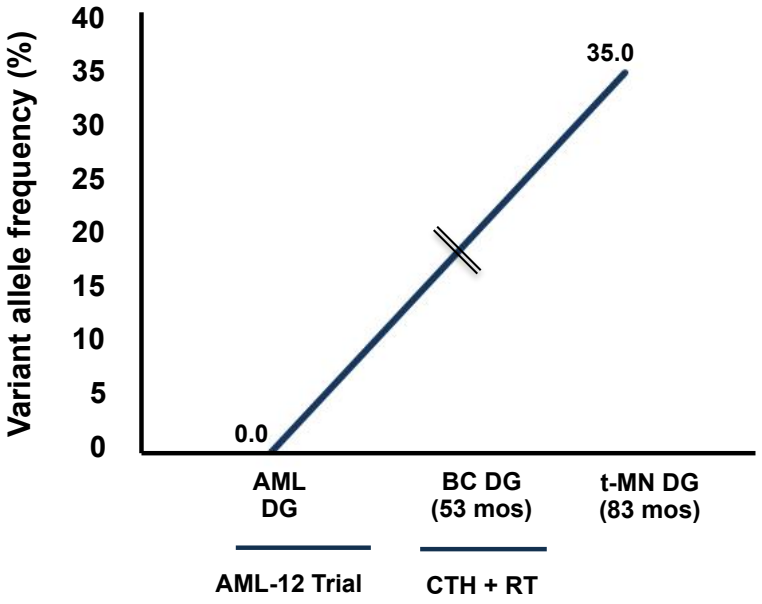
Second scenario

Somatic mutations were detectable at primary cancer diagnosis, prior to any cytotoxic treatment

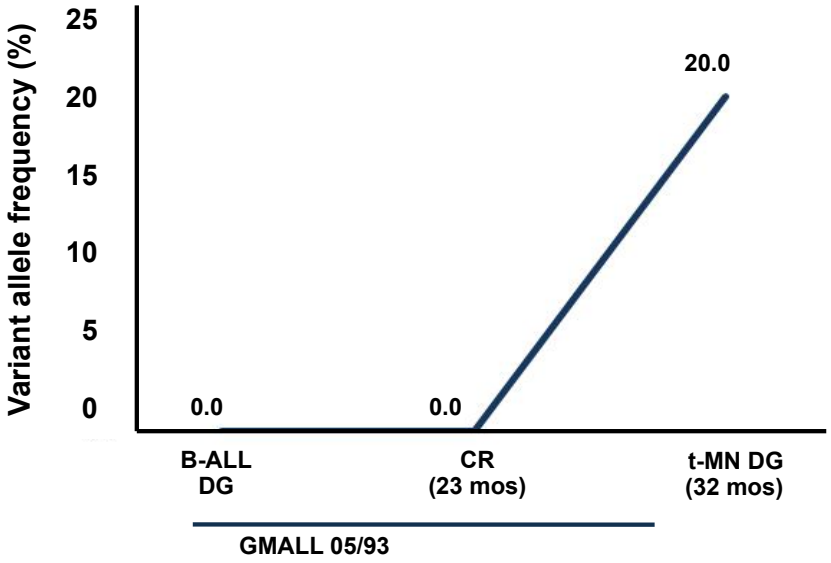
1. First scenario

Somatic mutations characterizing the t-MN clone were not detectable at primary cancer diagnosis and appeared after cytotoxic treatment

UPN6: *SF3B1 K700E*



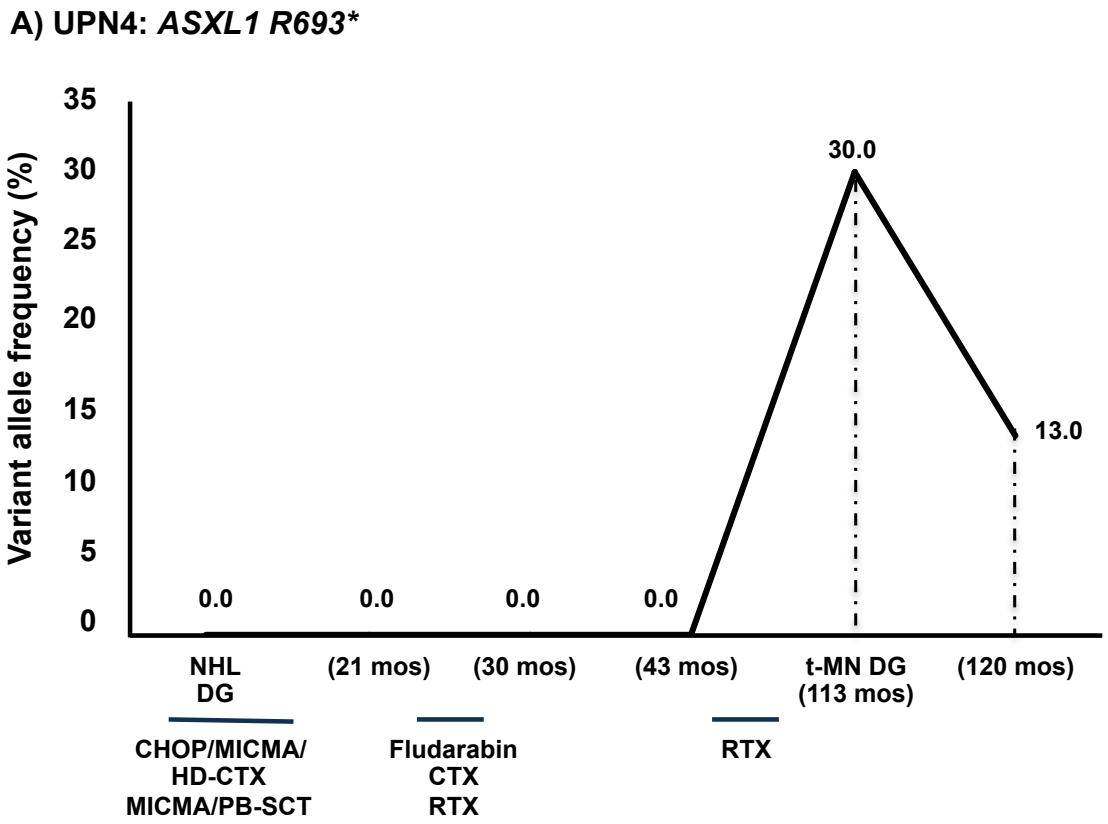
UPN13: *SETBP1 G870R*



Fabiani et al., submitted

2. First scenario

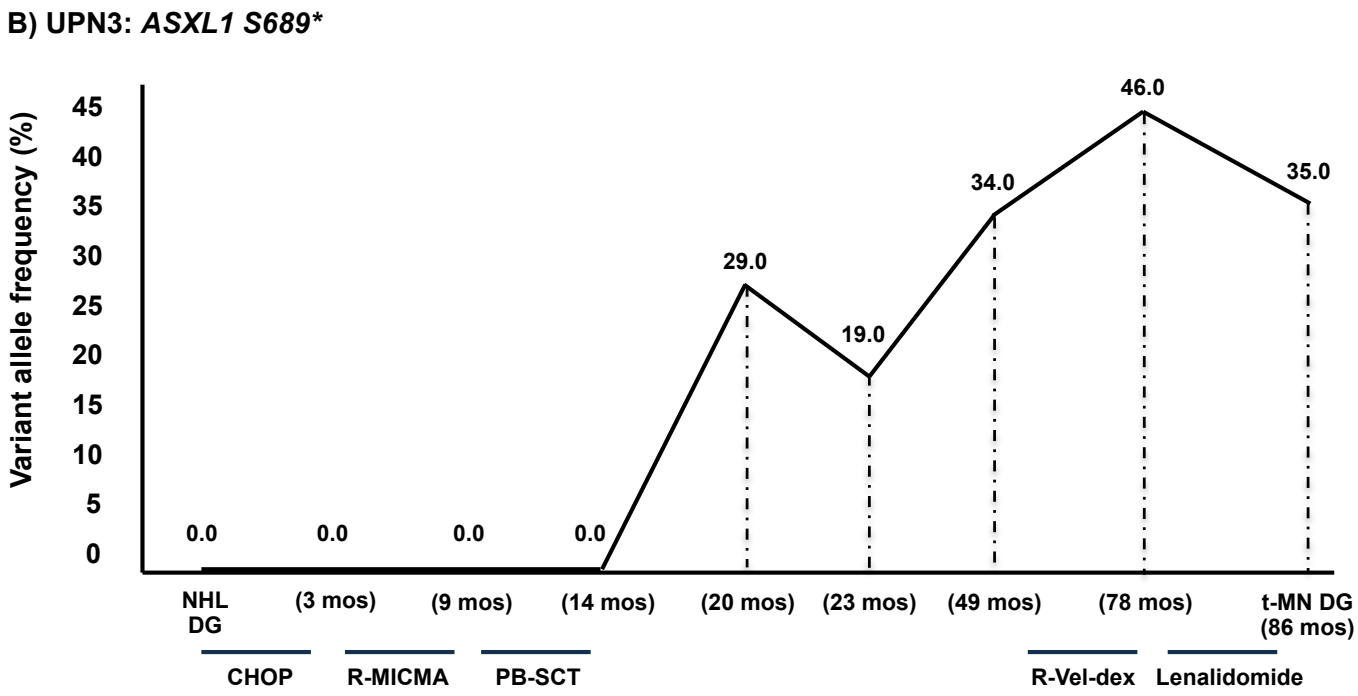
Somatic mutations characterizing the t-MN clone were not detectable at primary cancer diagnosis and appeared after cytotoxic treatment



Fabiani et al., submitted

3. First scenario

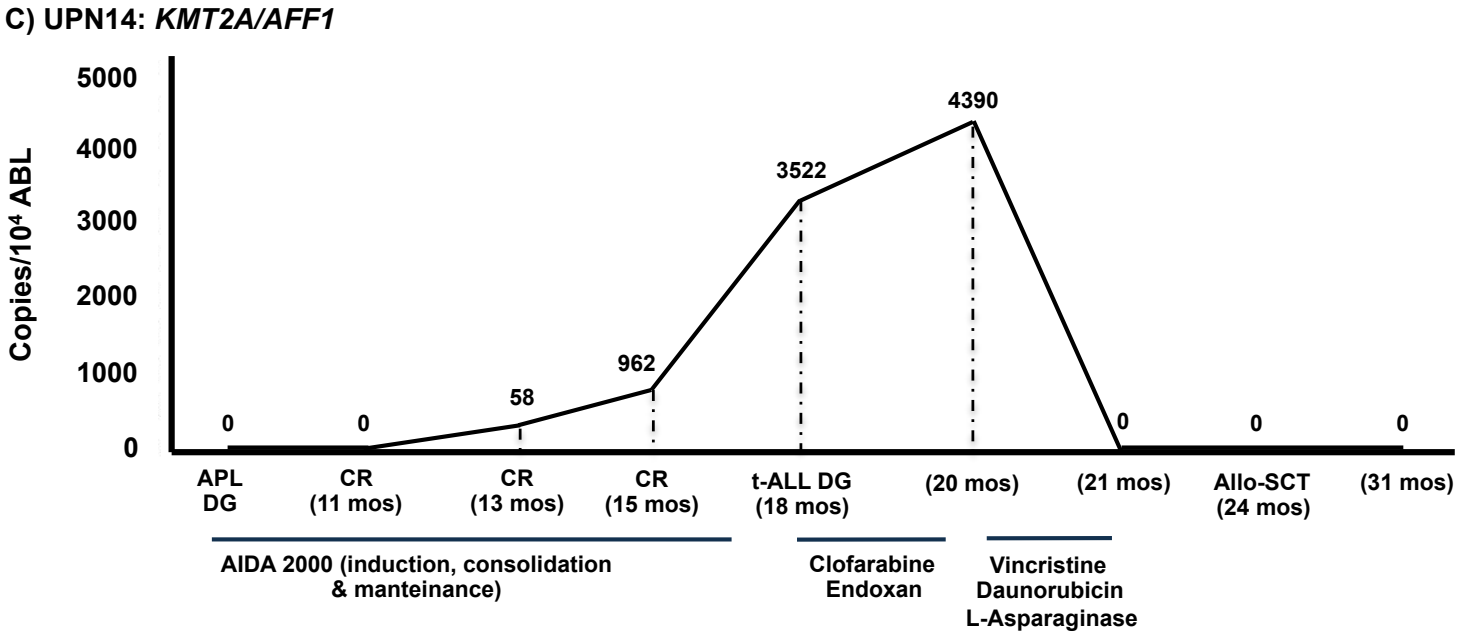
Somatic mutations characterizing the t-MN clone were not detectable at primary cancer diagnosis and appeared after cytotoxic treatment



Fabiani et al., submitted

4. First scenario

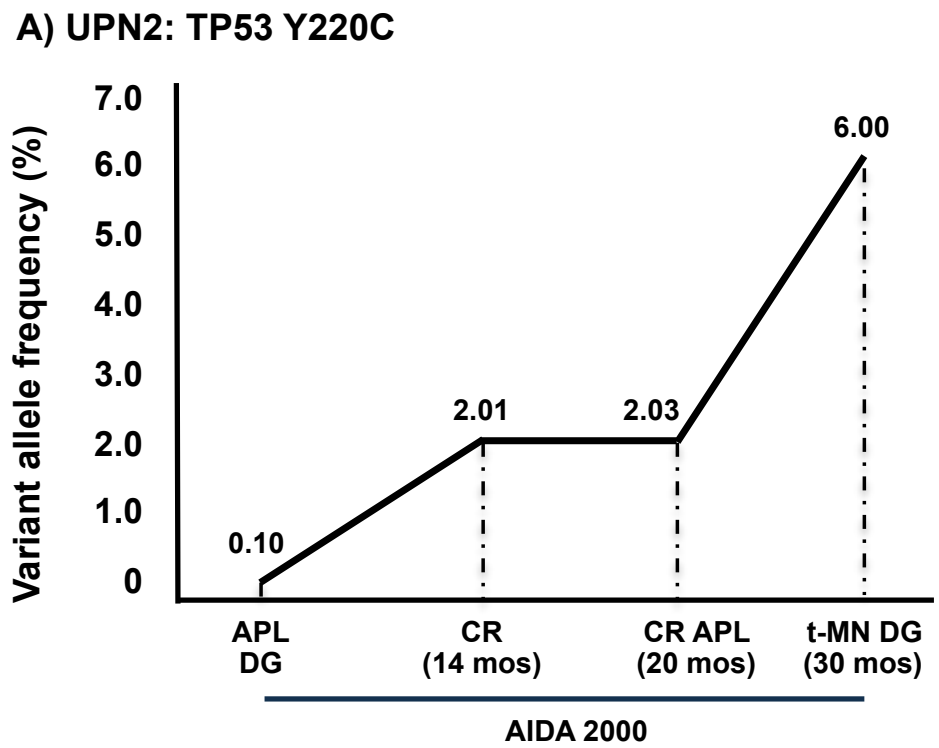
Genomic alteration characterizing the t-ALL clone was not detectable at primary cancer diagnosis and appeared after cytotoxic treatment



Fabiani et al., submitted

1. Second scenario

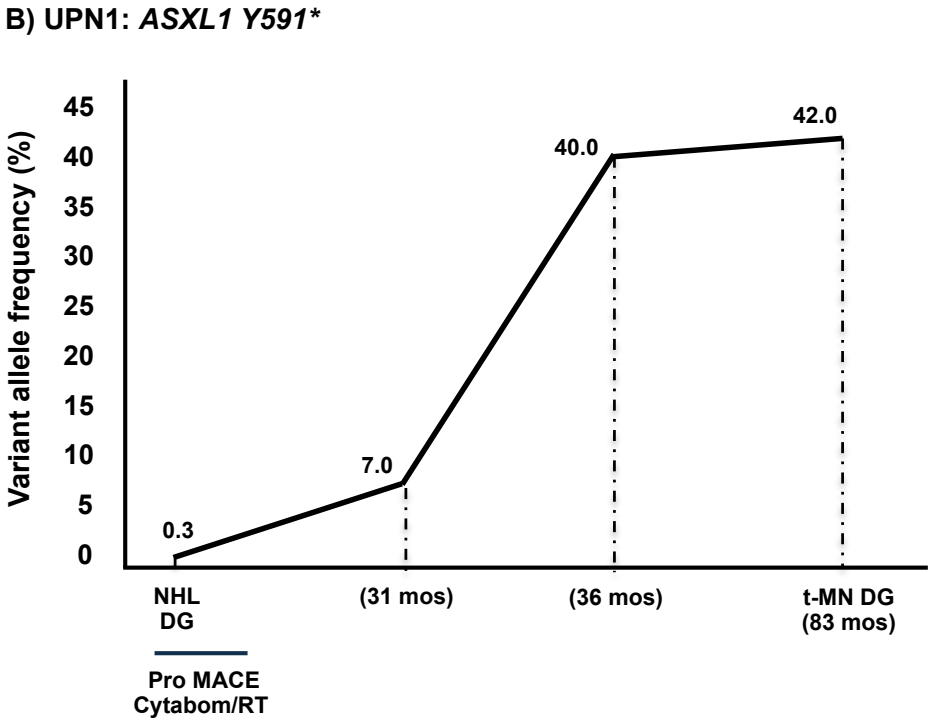
Somatic mutations were detectable at primary cancer diagnosis, prior to any cytotoxic treatment



Fabiani et al., submitted

2. Second scenario

Somatic mutations were detectable at primary cancer diagnosis, prior to any cytotoxic treatment



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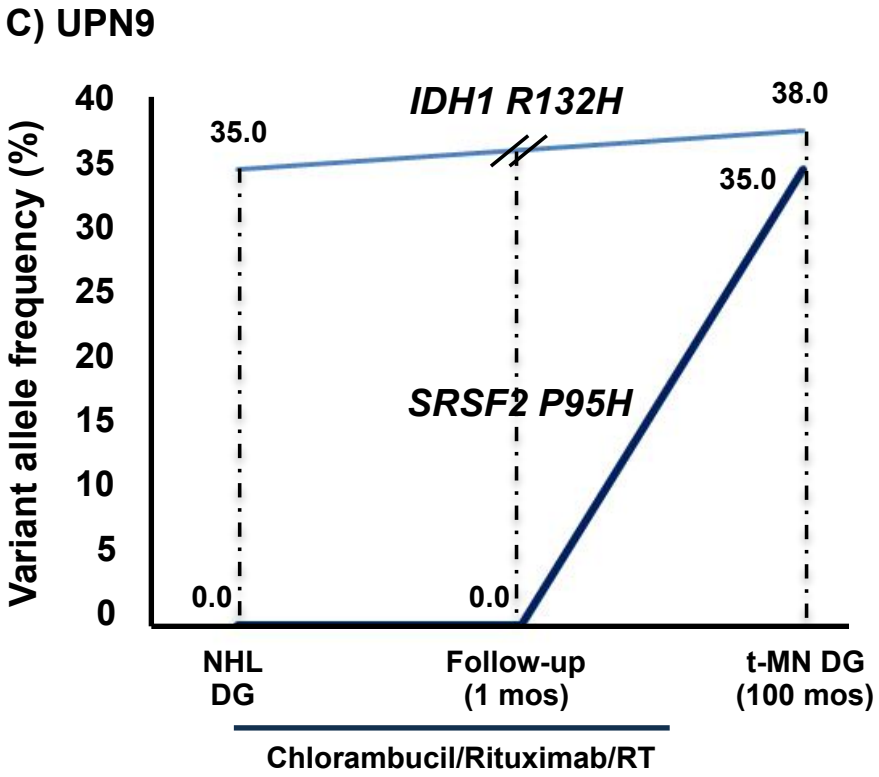
Somatic mutations were detectable at primary cancer diagnosis, prior to any cytotoxic treatment



Fabiani et al., submitted

Conclusions

- ✧ Secondary leukemogenesis is a heterogeneous process
- ✧ Somatic mutations in critical genes may be induced by the cytotoxic treatment (4/13 of studied patients)
- ✧ The t(4;11)(q21;q23) translocation was induced by the cytotoxic treatment
- ✧ Somatic mutations in critical genes may precede and favor leukemic development (3/13 of studied patients)

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