

Supplementary Material

1 Supplementary Tables

Supplementary Table 1: Detailed overview of basic patient characteristics. Data for 25 patients with Multiple myeloma was available. Based on the presence of new clones emerging in the course of disease, we defined two subgroups: 1) without new clone (patients UPN05 to 16), 2) with new clone (patients UPN17 to 25). Due to presence of only 1 time point, patients UPN01 to 04 are excluded from subgroup analysis; SCT – stem cell transplantation.

Patient ID	Sex	Age at first diagnosis	Follow-up [years]	#FISH	SCT	Died
UPN01	F	60	1.33	1	no	no
UPN02	M	65	1.41	1	no	no
UPN03	F	64	1.75	1	no	no
UPN04	M	67	12.5	1	no	no
UPN05	M	73	1.41	2	no	no
UPN06	M	67	2.41	2	no	no
UPN07	M	53	3.33	3	no	no
UPN08	F	65	3.41	7	yes	no
UPN09	F	38	3.62	4	yes	yes
UPN10	M	48	3.92	2	no	no
UPN11	M	53	4.25	6	yes	no
UPN12	M	45	4.50	2	no	no
UPN13	F	53	4.67	2	no	no
UPN14	F	57	5.00	7	yes	no
UPN15	F	70	5.22	2	no	yes
UPN16	M	50	17.76	2	no	no
UPN17	F	56	1.76	5	yes	yes
UPN18	F	58	2.00	3	yes	no
UPN19	M	48	2.01	3	no	yes
UPN20	M	59	2.25	3	no	no
UPN21	M	60	3.41	3	no	no
UPN22	M	58	5.00	11	yes	no
UPN23	F	59	5.01	2	no	yes
UPN24	M	53	5.50	11	yes	no
UPN25	M	49	6.42	6	yes	no

Supplementary Table 2: Summarized main characteristics of the study population and the two subgroups analyzed; IQR – interquartile range; SCT – stem cell transplantation.

	Study population UPN01 to 25	Subgroup 1 UPN05 to 16	Subgroup 2 UPN17 to 25
n	25	12	9
Male:Female	15:10	7:5	6:3
Age at first diagnosis (median [IQR])	58 [53-64]	53 [49.5-65.5]	58 [53-59]
Follow-up [years] (median [IQR])	3.62 [2.01-5]	4.09 [3.39-4.75]	3.41 [2.01-5.01]
#FISH (median [range])	3 [1-11]	2 [2-7]	3 [3-11]
#Patients with SCT	9	4	5
#Patients died	5	2	3

Supplementary Table 3: Overview of cytogenetic aberrations, their CCFs and associated clones for every patient and time point. Presence and absence of cytogenetic aberrations was analyzed by FISH. Absence is indicated by a negative result (neg.). CCF is based on the number of cells with aberration divided by the total number of cells analyzed. In case of no analysis being performed, the corresponding cell in the table is left empty. Every aberration is associated with a clone. A parent defines the precursor clone. A missing precursor, i.e. development from normal cells, is indicated by ‘-’.

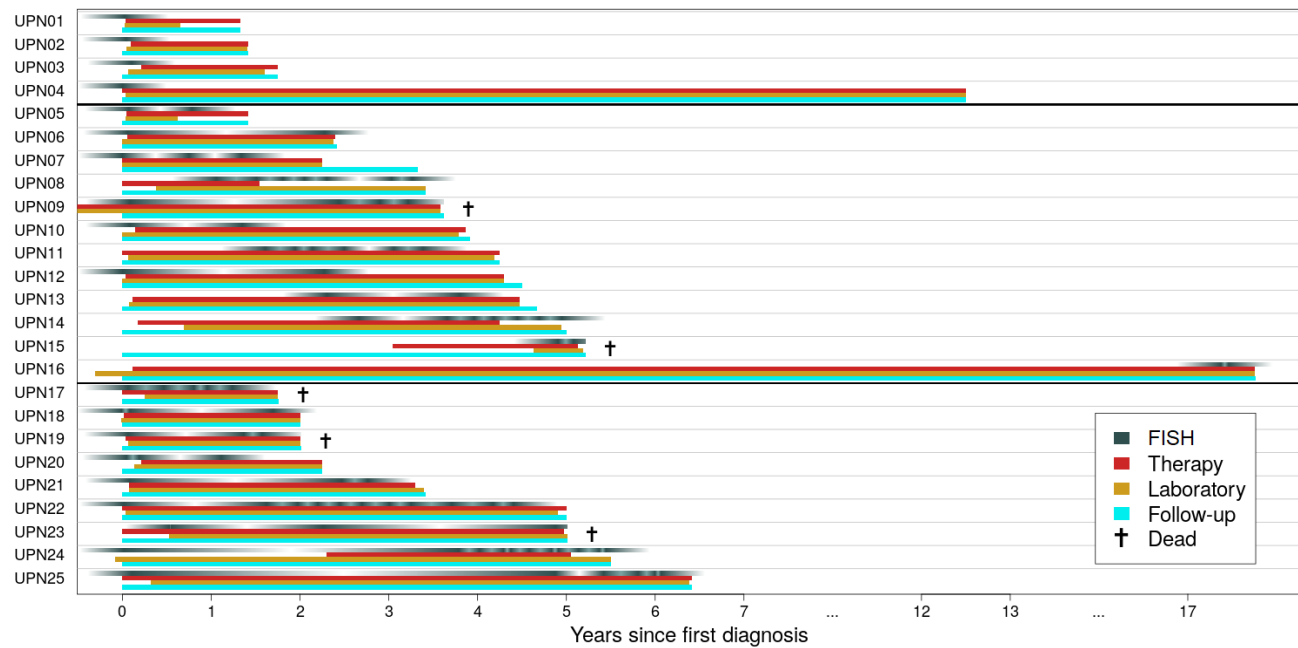
UPN01									
Aberration	TP1		Clone	Parent					
del(17)(p13)	98%		1	-					
del(4)(p16)	96%		2	1					
del(16)(q23)	96%		2	1					
dup(1)(q21)	96%		2	1					
del(1)(p32.3)	96%		2	1					
+3	93%		3	2					
dup(1)(q21)x2	87%		4	3					
UPN02									
Aberration	TP1		Clone	Parent					
del(13)(q14)	99%		1	-					
dup(15)(q22)	99%		1	-					
dup(1)(q21)x2	99%		1	-					
dup(17)(p13)	92%		2	1					
rear(14)(q32.3)	90%		2	1					
dup(19)(q13)	77%		3	2					
UPN03									
Aberration	TP1		Clone	Parent					
t(11;14)	83%		1	-					
rear(14)(q32)	69%		2	1					
+15	13%		3	2					
UPN04									
Aberration	TP1		Clone	Parent					
-	-		-	-					
UPN05									
Aberration	TP1	TP2	Clone	Parent					
del(20)(q12)	81%	neg.	1	-					
dup(11)(q22.3)	90%	neg.	2	1					
dup(11)(q13)	80%	neg.	2	1					
dup(11)(q22.3)x4	15%	neg.	3	2					
dup(11)(q13)x4	13%	neg.	3	2					
dup(17p)x2	15%	neg.	3	2					
dup(16)(q23)x2	11%	neg.	3	2					
dup(14)(q32)x2	11%	neg.	3	2					
dup(4)(p16)x2	10%	neg.	3	2					
UPN06									
Aberration	TP1	TP2	Clone	Parent					
dup(1)(q21)	98%	neg.	1	-					
t(11;14)	99%	neg.	1	-					
del(13q)	98%	neg.	1	-					
rear(14)(q32.33)	98%	neg.	1	-					
del(16)(q22)	97%	neg.	1	-					
UPN07									
Aberration	TP1	TP2	TP3	Clone	Parent				
dup(1)(q21)	97%	neg.	95%	1	-				
t(14;20)	98%	neg.	96%	1	-				
dup(7)(p11.1q11.1)	89%	neg.	95%	2	1				
rear(14)(q32.33)	98%	neg.	neg.	3	2				
UPN08									
Aberration	TP1	TP2	TP3	TP4	TP5	TP6	TP7	Clone	Parent
dup(1)(q21)	85%	neg.	neg.	neg.	neg.	neg.	neg.	1	-
t(4;14)	84%	neg.	neg.	neg.	neg.	neg.	neg.	1	-
del(13q)	80%	neg.	neg.	neg.	neg.	neg.	neg.	2	1
UPN09									
Aberration	TP1	TP2	TP3	TP4		Clone	Parent		
del(1)(p32)	80%	neg.	80%	67%		1	-		
del(1)(q21)	80%	neg.	80%	67%		1	-		
del(14)(q32)	80%	neg.	78%	68%		1	-		
-17	79%	neg.	85%	56%		2	1		
del(13)(q14)	74%	neg.	78%	56%		3	2		

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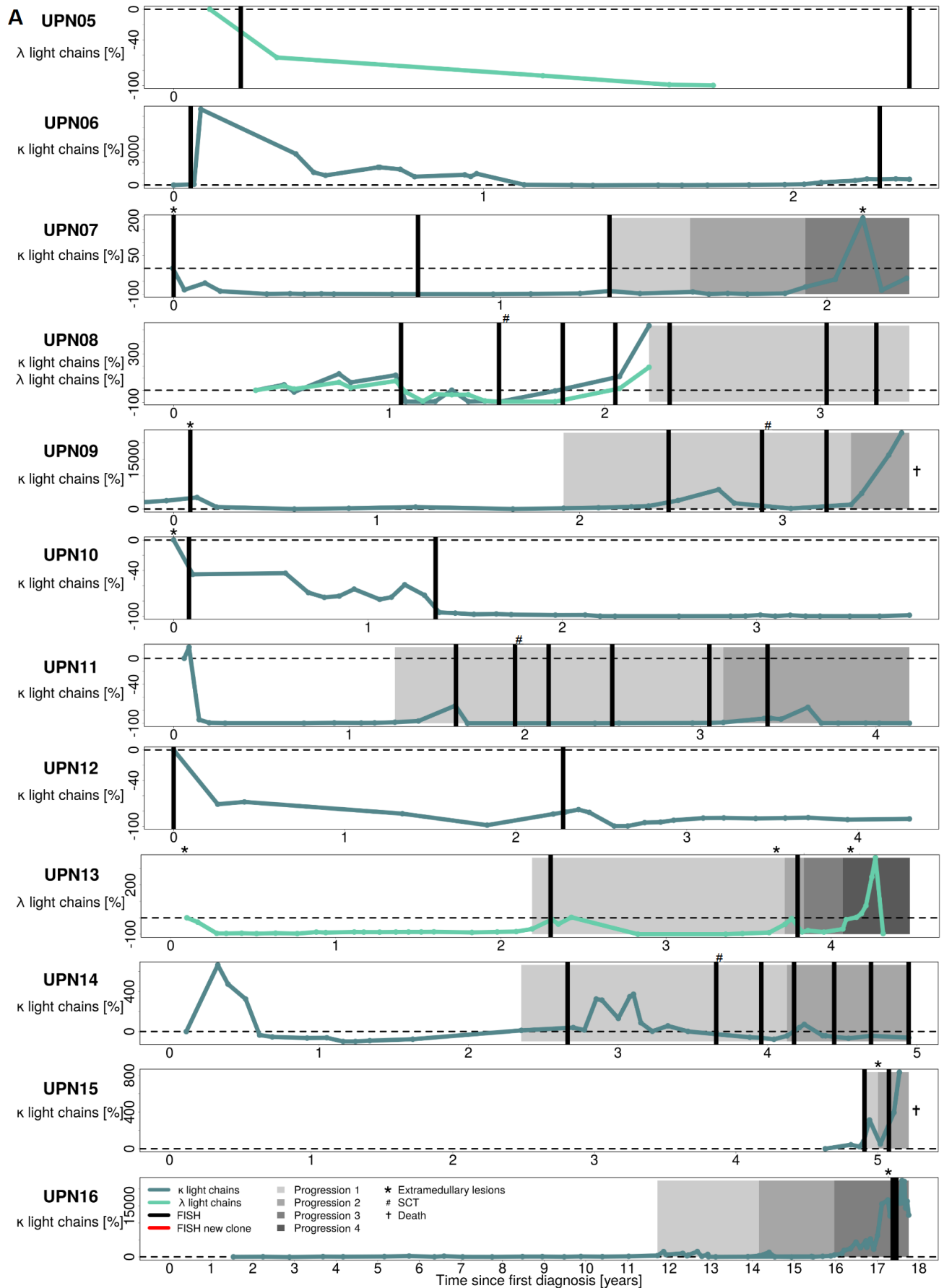
del(13)(q34)	74%	neg.	78%	56%				3	2
UPN10									
Aberration	TP1	TP2						Clone	Parent
dup(3)(p11.1q11.1)	7%	15%						1	-
dup(9)(q12)	14%	13%						2	-
del(15)(p11.1q11.1)	15%	neg.						3	-
UPN11									
Aberration	TP1	TP2	TP3	TP4	TP5	TP6		Clone	Parent V1 V2
del(13q)	95%	6%	3%	neg.	9%	74%		1	- -
del(17)(p13.1)	96%	neg.	4%	neg.	8%	73%		1	- -
dup(9)(q12)	94%	8%	neg.	neg.	3%	80%		2	1 1
dup(15)(p11.1q11.1)	94%	8%	neg.	neg.	3%	71%		2	1 1
dup(7)(p11.1q11.1)	89%	8%	neg.	neg.	7%	68%		3	2 2
dup(15)(p11.1q11.1)x2	20%	neg.	neg.	neg.	neg.	33%		4	3 3
dup(1)(q21)	9%	neg.	neg.	neg.				5	4 3
UPN12									
Aberration	TP1	TP2						Clone	Parent
dup(11)(q22)	70%	15%						1	-
t(11;14)	68%	10%						2	1
rear(14)(q32)	64%	neg.						3	2
UPN13									
Aberration	TP1	TP2						Clone V1 V2	Parent V3 V4
dup(1)(q21)	45%	neg.						1 - -	- -
rear(14)(q32.33)	40%	neg.						2 1 1	- -
del(13)(q14q32)	32%	neg.						3 2 -	2 1
UPN14									
Aberration	TP1	TP2	TP3	TP4	TP5	TP6	TP7	Clone	Parent
dup(9)(q12)	20%	neg.	neg.					1	-
dup(15)(p11.1q11.1)	20%	neg.	neg.					1	-
del(17)(p13.1)	18%	74%	62%	neg.	neg.	neg.	neg.	2	-
dup(17)(q11.2)	19%	80%	77%					3	2
dup(17)(q22)	17%	80%	65%					3	2
dup(17)(q22)x3	10%	80%	35%					4	3
dup(3)(p11.1q11.1)	12%	61%						4	3
UPN15									
Aberration	TP1	TP2						Clone	Parent
del(13q)	33.3%	neg.						1	-
+17, +17	23.8%	16%						2	-
UPN16									
Aberration	TP1	TP2						Clone	Parent
dup(11)(q13.3)x2	39%	98%						1	-
dup(14)(q32.33)x1-2		94%						2	1
dup(1)(q21)		16%						3	2
UPN17									
Aberration	TP1	TP2	TP3	TP4	TP5			Clone	Parent
t(4;14)(p16.3q32.33)	96%	90%	80%	neg.	neg.			1	-
-13	neg.	neg.	80%	3%	neg.			2	1
dup(1)(q21)	2%	neg.	54%	14%	neg.			3	-
UPN18									
Aberration	TP1	TP2	TP3					Clone	Parent
del(13)(q14)	98%	95%	neg.					1	-
dup(9)(q34)	84%	96%	neg.					2	1
dup(15)(q22)	84%	97%	neg.					2	1
dup(5)(p15q35)		94%	neg.					3	2
dup(19)(q13)	77%	89%	neg.					4	3
dup(4)(p16)	73%	89%	neg.					5	4
rear(8)(q24)	16%	21%	neg.					6	5
dup(14)(q32)	8%	1%	neg.					7	6
dup(1)(q21.3)	4%	neg.	neg.					8	7
del(16)(q23)	neg.	10%	neg.					9	6
del(17)(p13)	neg.	4%	neg.					10	9
UPN19									
Aberration	TP1	TP2	TP3					Clone	Parent
t(4;14)	98%	100%	28%					1	-
del(13q)	100%	90%	28%					1	-
rear(14)(q32.33)	96%		neg.					2	1
+3	79%		neg.					3	2
-15	21%	14%						4	3

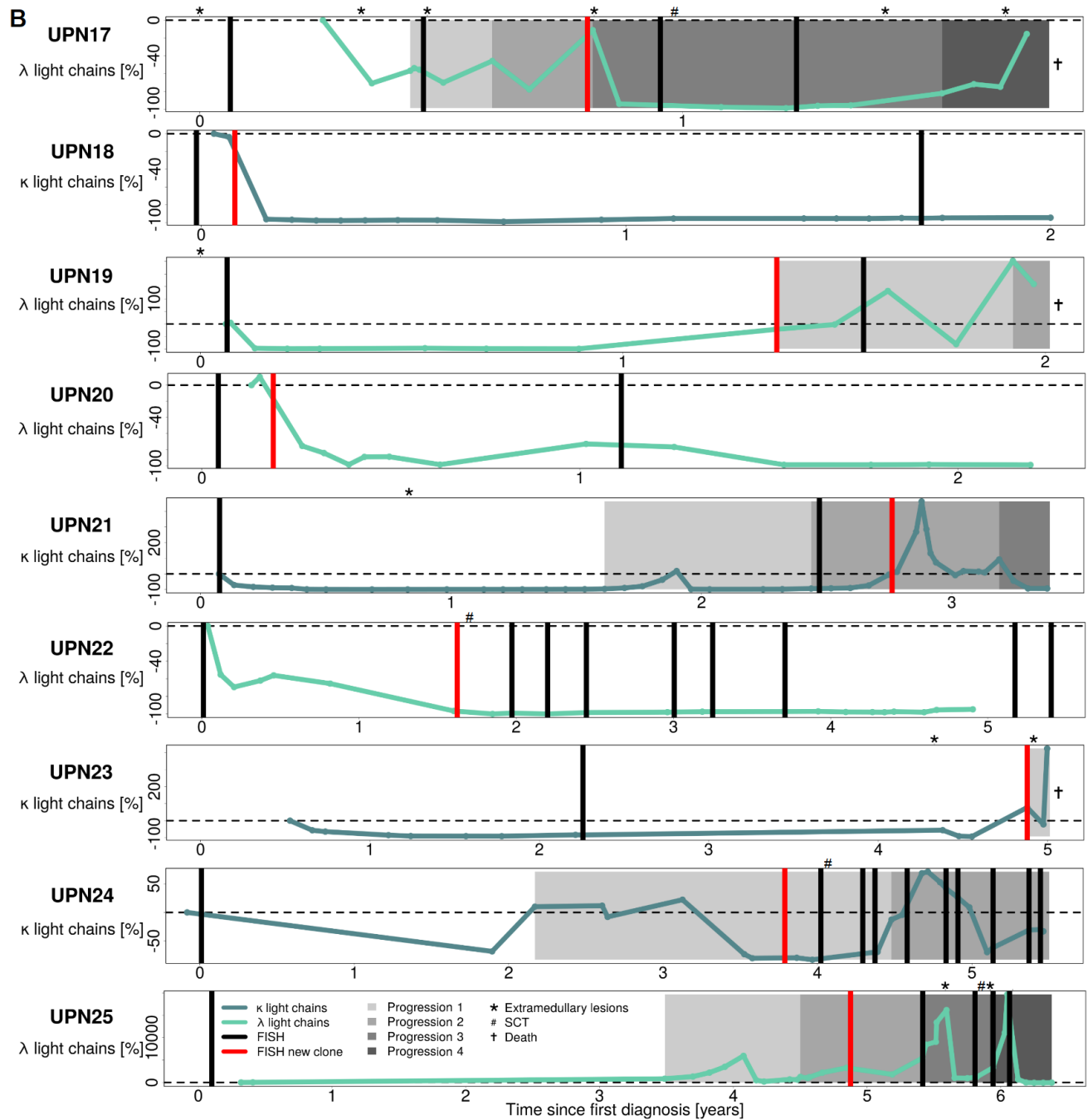
+9	neg.	15%											5	4
del(17p)	neg.	neg.	28%										6	1
del(1)(p32)	neg.	neg.	18%										9	8
dup(1)(q21)	neg.	neg.	15%										7	6
dup(1)(q21)x2	neg.	neg.	3%										8	7
UPN20														
Aberration	TP1	TP2	TP3										Clone	Parent
del(13)(q14)	30%	93%	neg.										1	-
t(4;14)	20%	89%	neg.										2	1
rear(14)(q32)		90%	neg.										2	1
del(16)(q23)	23%	70%	neg.										3	2
dup(9)(q34)	neg.	72%	neg.										4	3
del(17)(p13)	neg.	10%	neg.										5	4
UPN21														
Aberration	TP1	TP2	TP3										Clone	Parent
rear(14)(q32)	89%	neg.	neg.										1	-
dup(1)(q21)	81%	neg.											2	1
del(13q)	15%	neg.	93%										3	-
dup(16)(q23)	neg.	neg.	96%										4	3
t(11;14)		neg.	84%										5	4
dup(1)(q32)	neg.	neg.	82%										5	4
UPN22														
Aberration	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8	TP9	TP10	TP11		Clone	Parent
t(11;14)	99%	4%	neg.	neg.	neg.	neg.	neg.	neg.	neg.				1	-
del(17p)	80%		neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		2	1
-15		4%	neg.	neg.	neg.	8%	5%	6%	neg.	3%	11%		3	-
UPN23														
Aberration	TP1	TP2											Clone	Parent
t(11;14)	neg.	91%											1	-
dup(1)(q21)	neg.	80%											2	1
dup(1)(q21)x2	neg.	66%											3	2
UPN24														
Aberration	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8	TP9	TP10	TP11		Clone	Parent
t(11;14)	13%	6%	26%	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		1	-
dup(11)(q13)	13%	0%	26%	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		2	-
rear(14)(q32)	8%	8%		neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		3	-
del(13)(q14)	neg.	10%	36%	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		4	-
dup(13q)	neg.	10%	36%	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		4	-
del(17)(p13)	neg.	4%	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.		5	-
UPN25														
Aberration	TP1	TP2	TP3	TP4	TP5	TP6							Clone	Parent V1 V2
del(14)(q32.33)	86%	23%	neg.	83%	96%								1	- -
del(13q)	91%		neg.	77%	93%								2	1 1
dup(1)(q21)	34%	12%	neg.	65%	90%								3	2 2
dup(1)(q21)x2	neg.	3%	neg.	23%	30%								4	3 3
dup(11)(q13q22.3)	neg.	2%				22%							5	4 -

2 Supplementary Figures

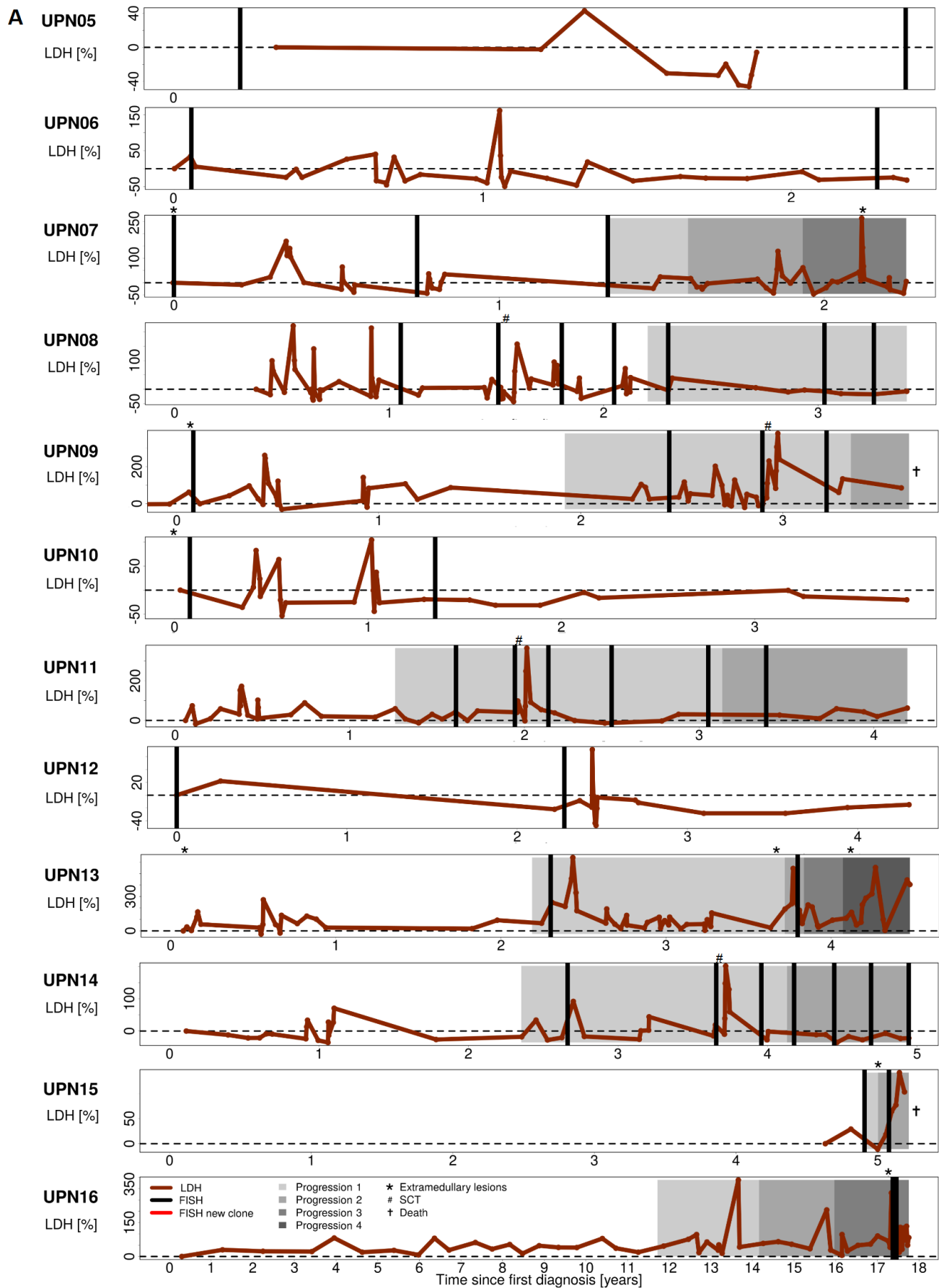


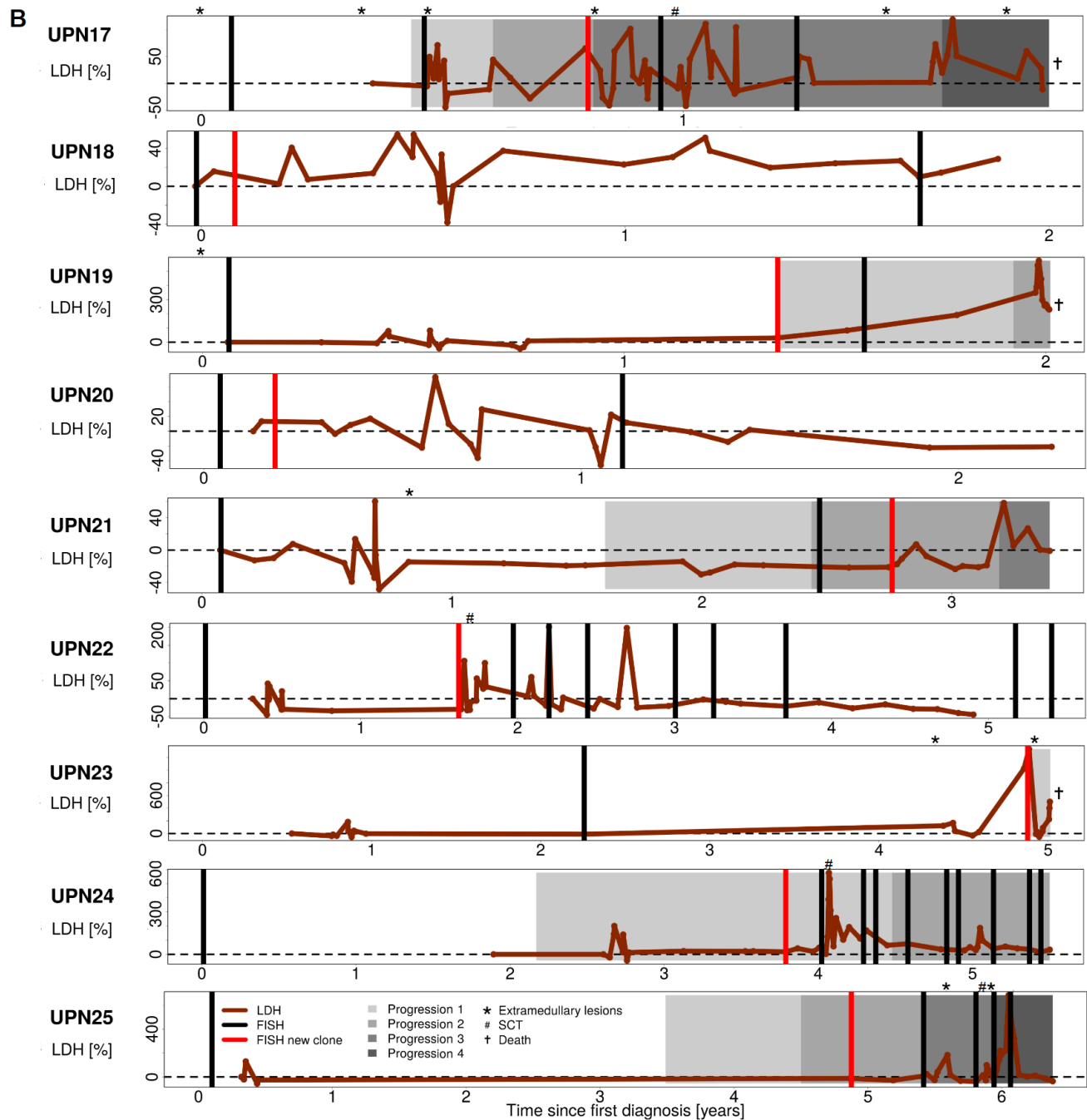
Supplementary Figure 1: Data available for every patient, including information on cytogenetic aberrations detected by FISH (green), therapy (red), laboratory parameters (yellow) and follow-up (light blue). For 4 out of 25 patients (UPN01 to 04), information on cytogenetic aberrations was available at only 1 time point. Remaining 21 patients were characterized by 2 to 11 FISH analyses. The group was split into patients without a new clone emerging in the course of disease (patients UPN05 to 16) and patients with a new clone (patients UPN17 to 25).



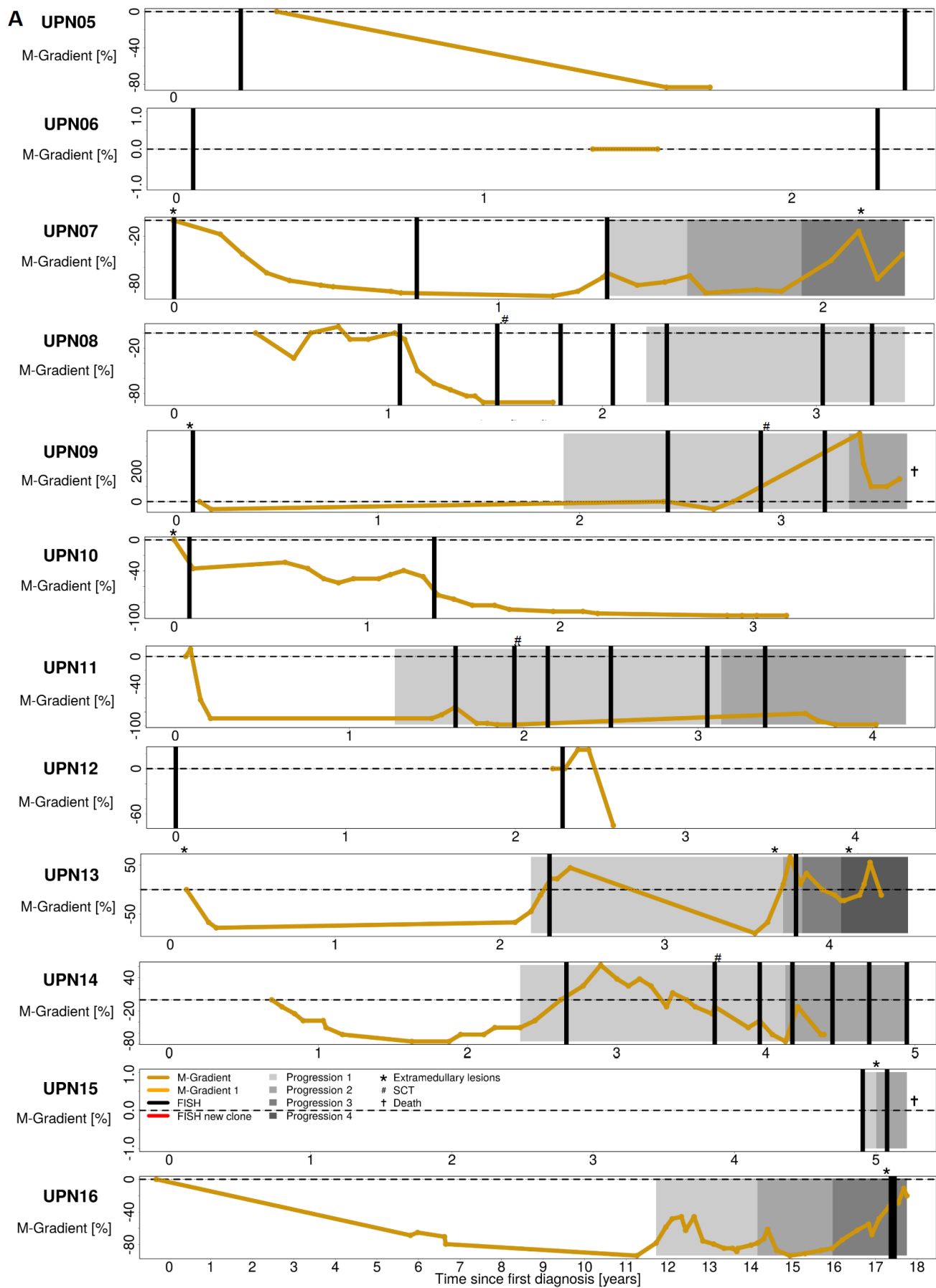


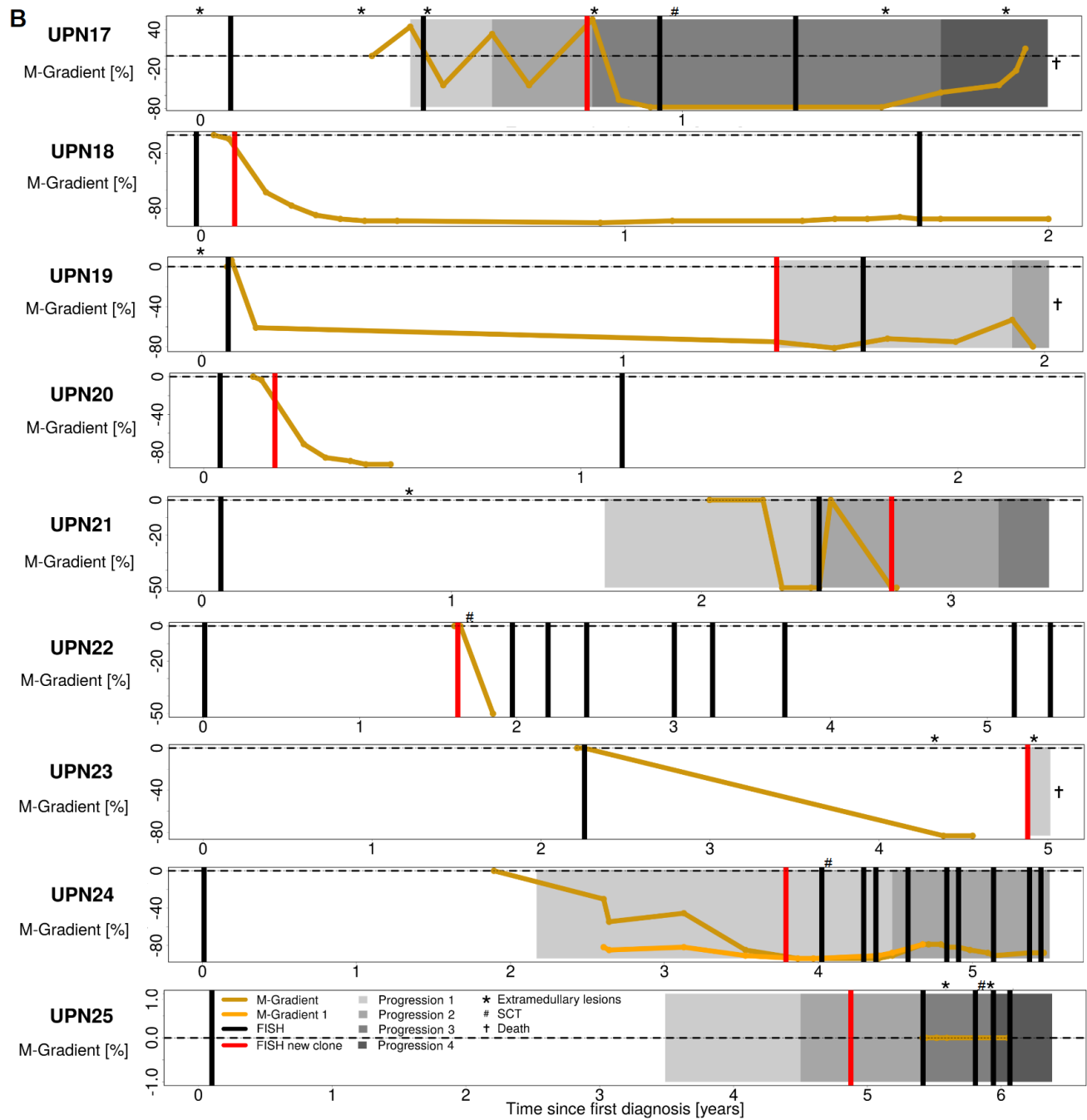
Supplementary Figure 2: Relative development of κ (dark green) and λ (light green) light chains in serum from first diagnosis until the end of follow-up. Horizontal dashed lines indicate the level of light chains at first measurement. A) Patients without a new clone emerging in the course of disease (patients UPN05 to 16). B) Patients with a new clone emerging in the course of disease (patients UPN17 to 25).



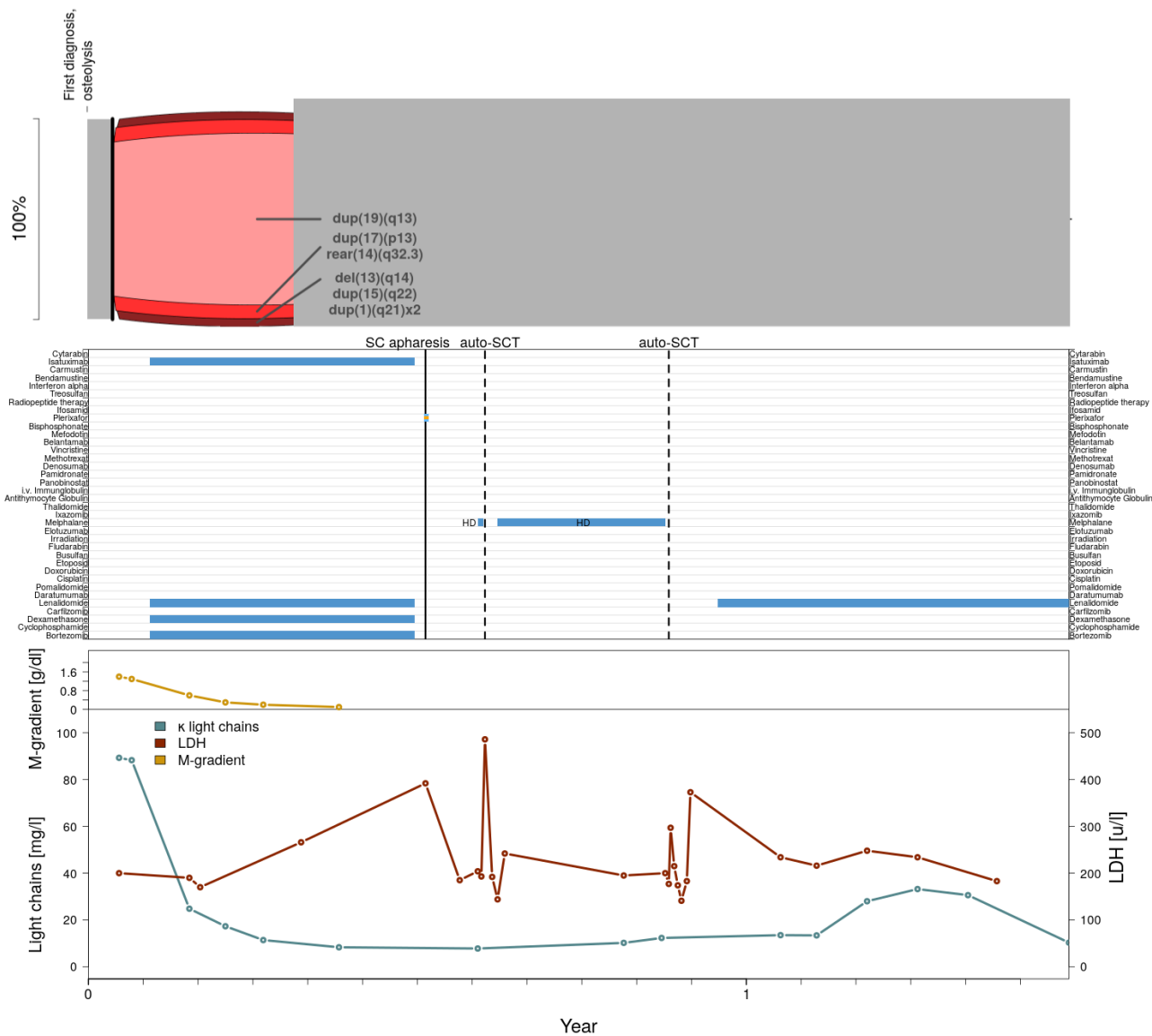


Supplementary Figure 3: Relative development of LDH activation from first diagnosis until the end of follow-up. Horizontal dashed lines indicate the level of LDH activation at first measurement. A) Patients without a new clone emerging in the course of disease (patients UPN05 to 16). B) Patients with a new clone emerging in the course of disease (patients UPN17 to 25).

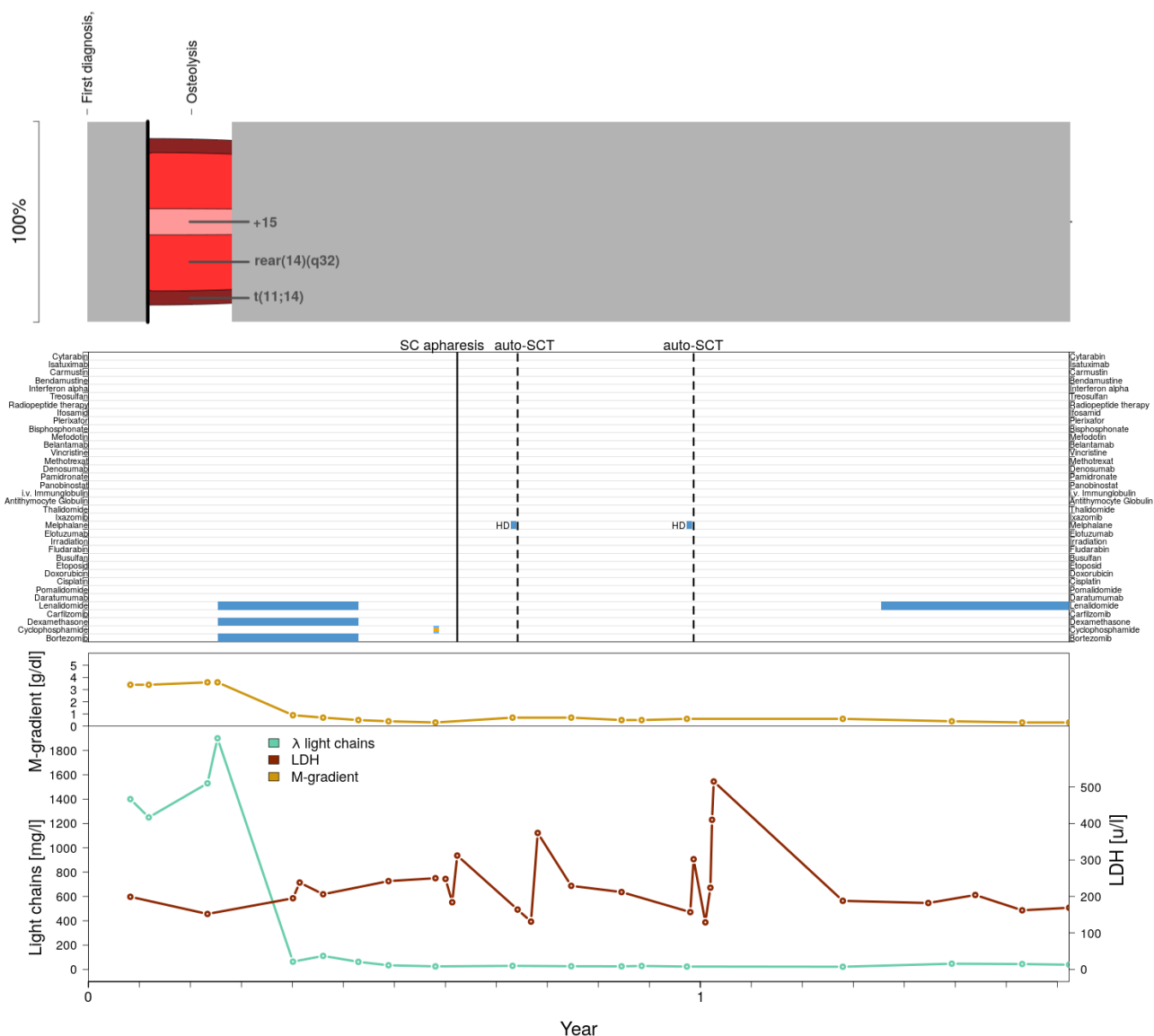




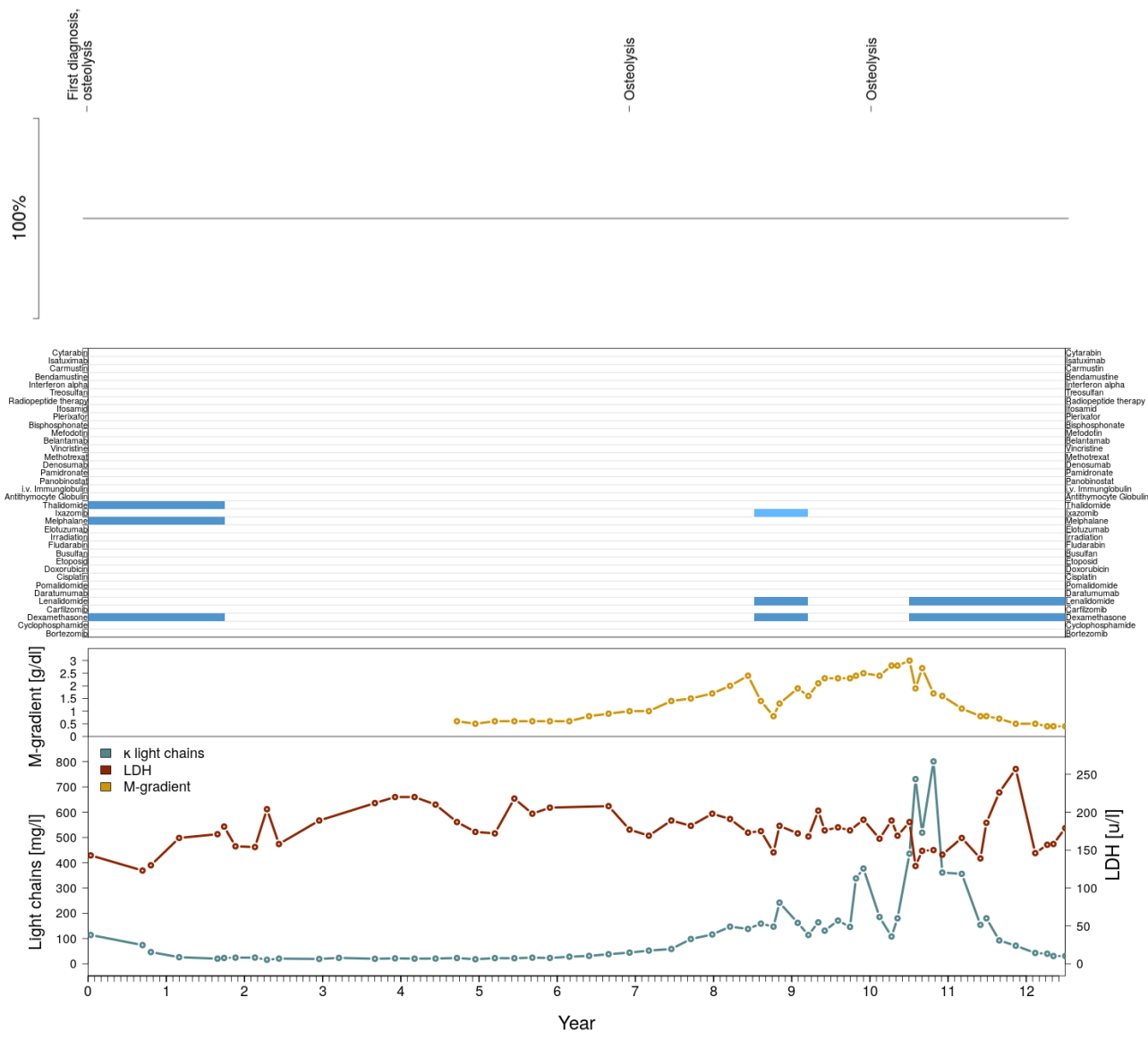
Supplementary Figure 4: Relative development of monoclonal protein (M-Gradient) from first diagnosis until the end of follow-up. Horizontal dashed lines indicate the level of LDH activation at first measurement. A) Patients without a new clone emerging in the course of disease (patients UPN05 to 16). B) Patients with a new clone emerging in the course of disease (patients UPN17 to 25).



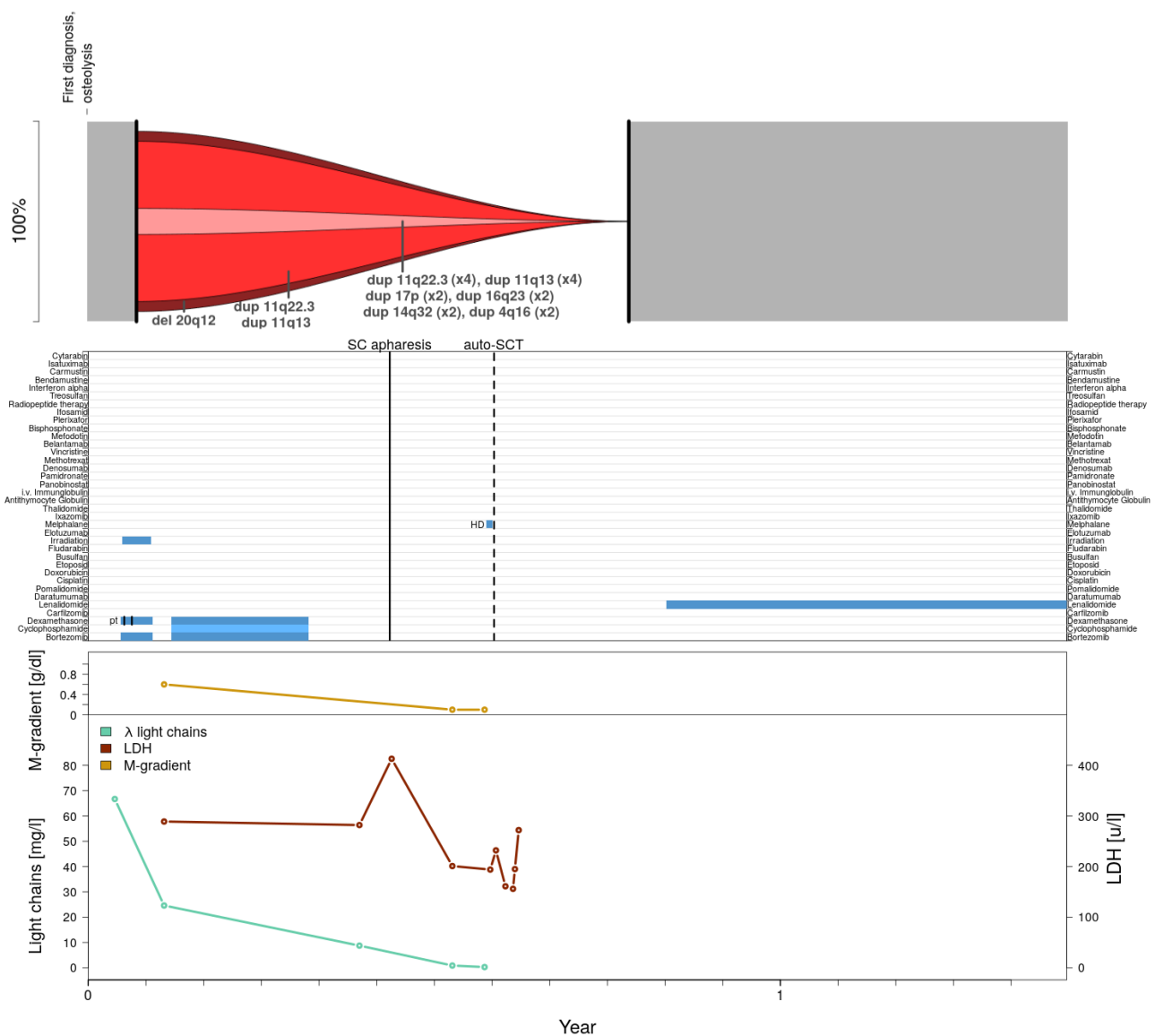
Supplementary Figure 6: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN02. Vertical black line in the clonal evolution plot indicates the time point of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



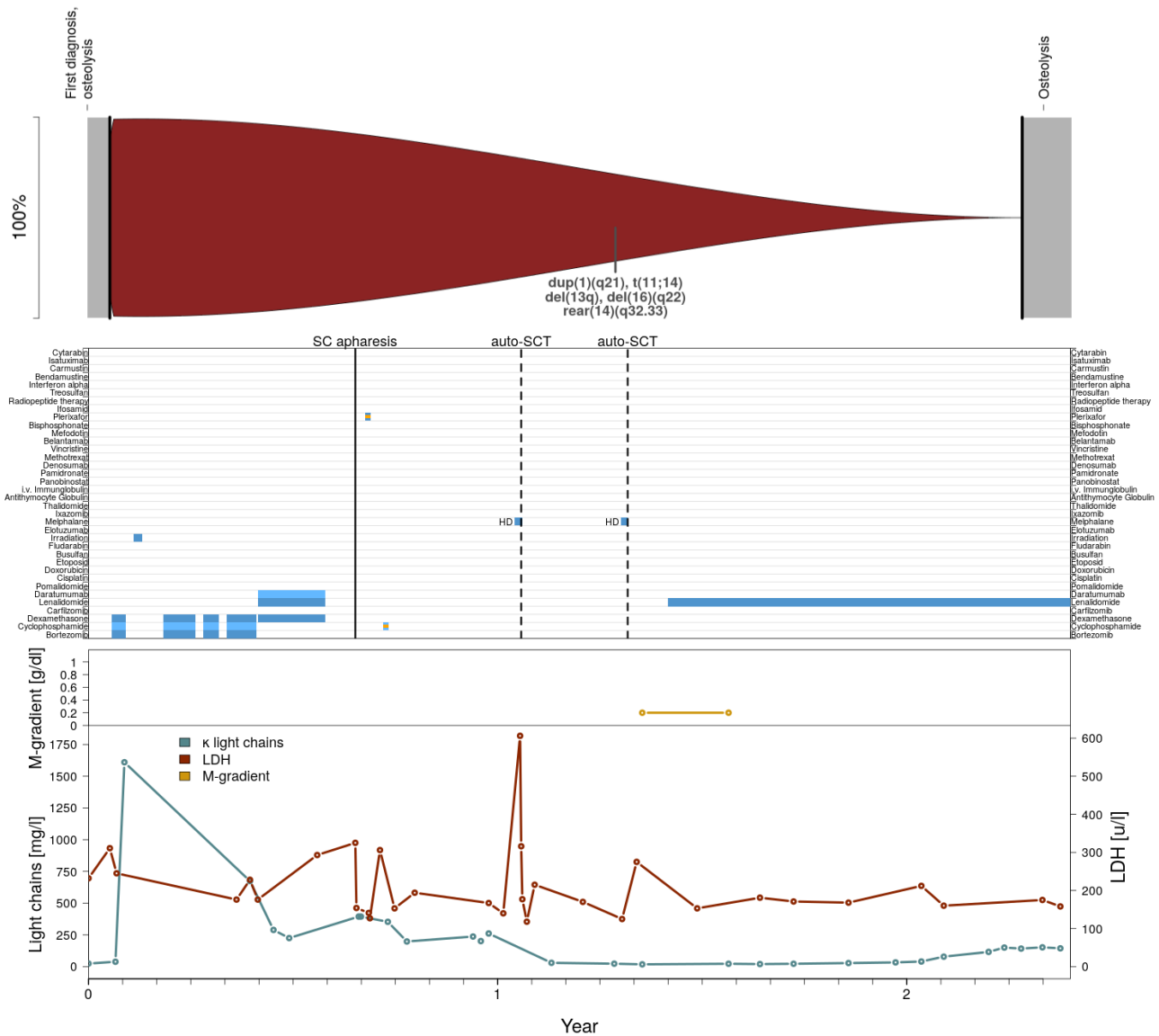
Supplementary Figure 7: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN03. Vertical black line in the clonal evolution plot indicates the time point of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



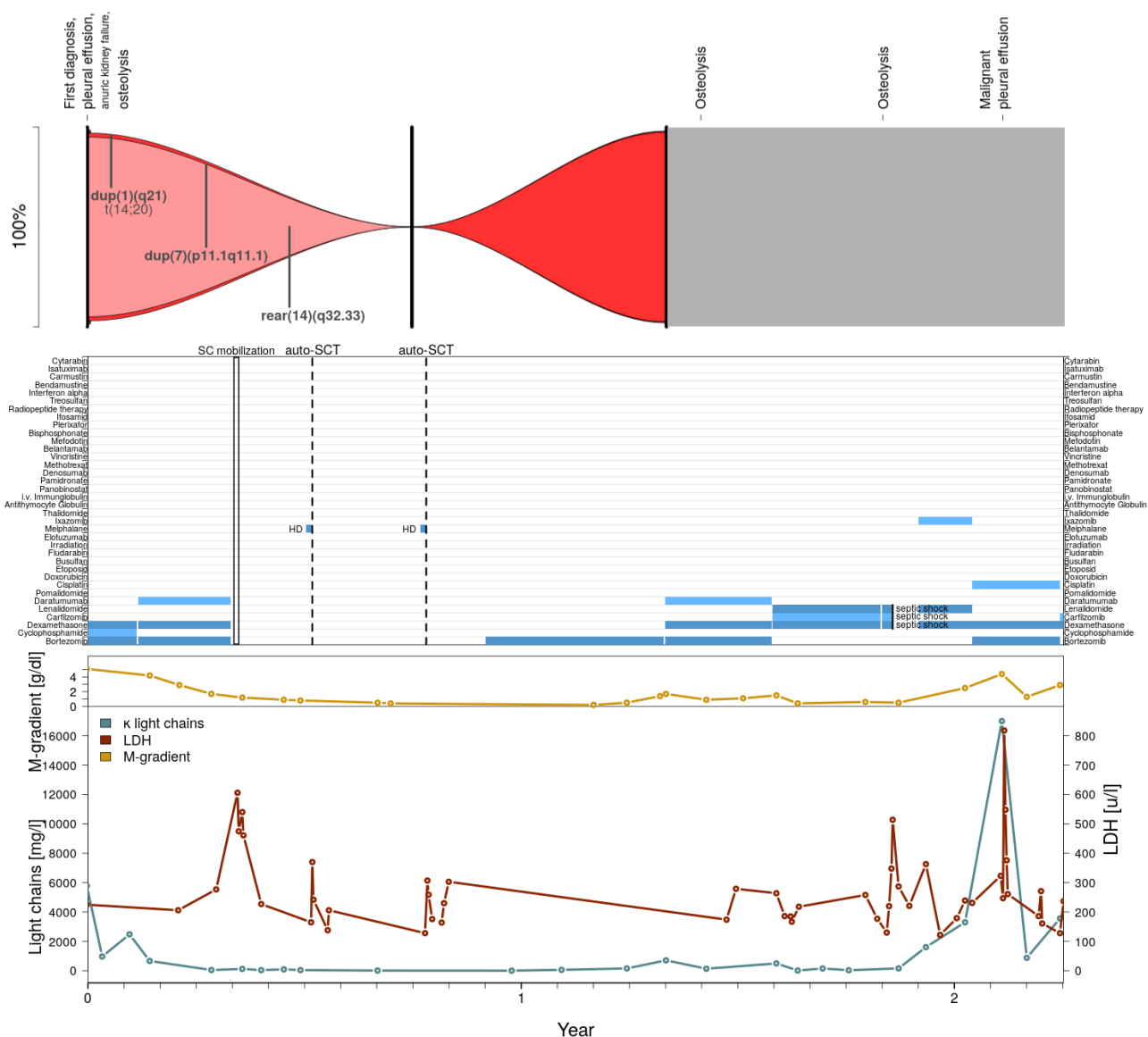
Supplementary Figure 8: Applied therapies and development of laboratory parameters of patient UPN04. No aberrations have been detected.



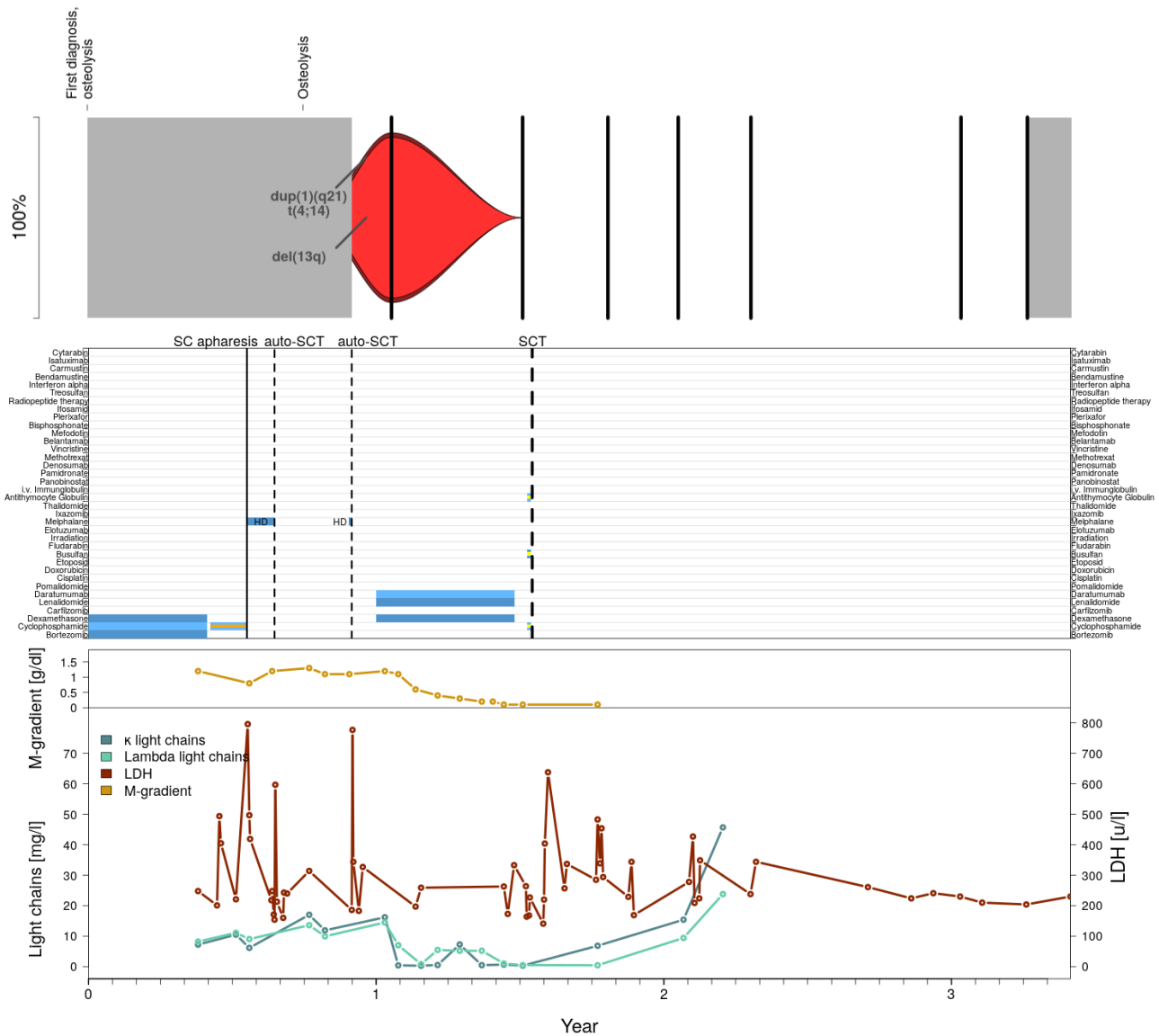
Supplementary Figure 9: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN05. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, pt – pulse therapy, SC – stem cell.



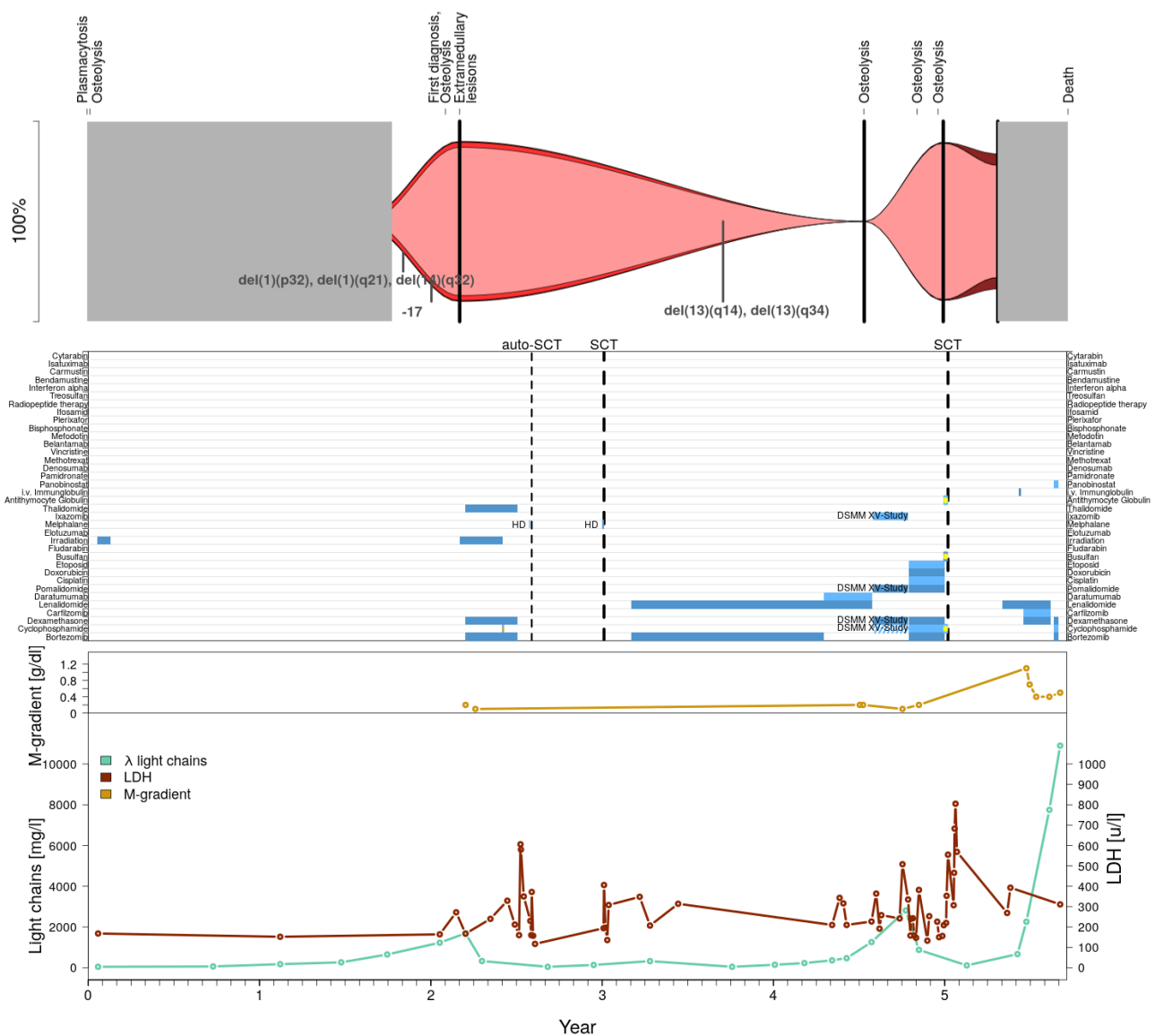
Supplementary Figure 10: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN06. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



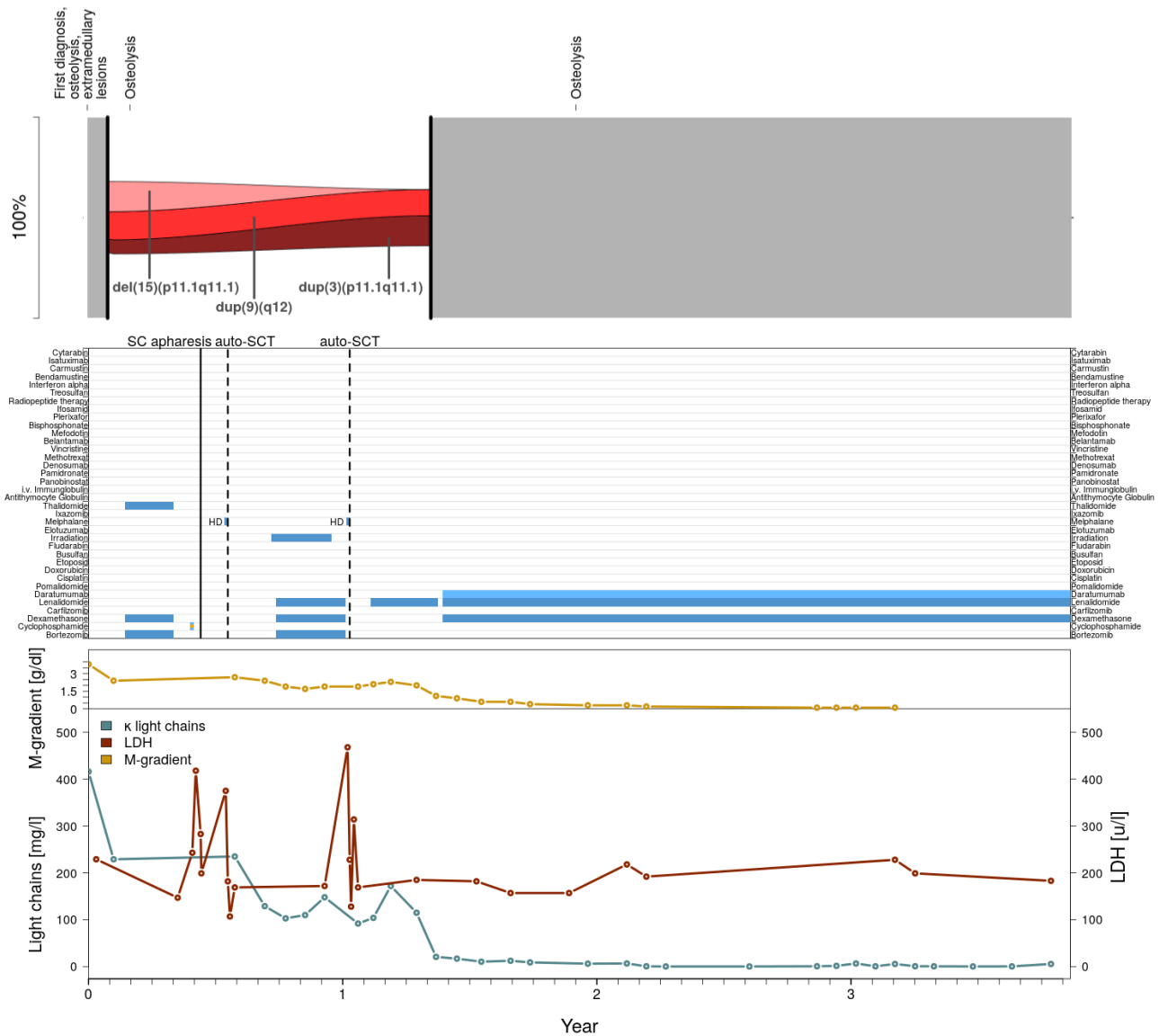
Supplementary Figure 11: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN07. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



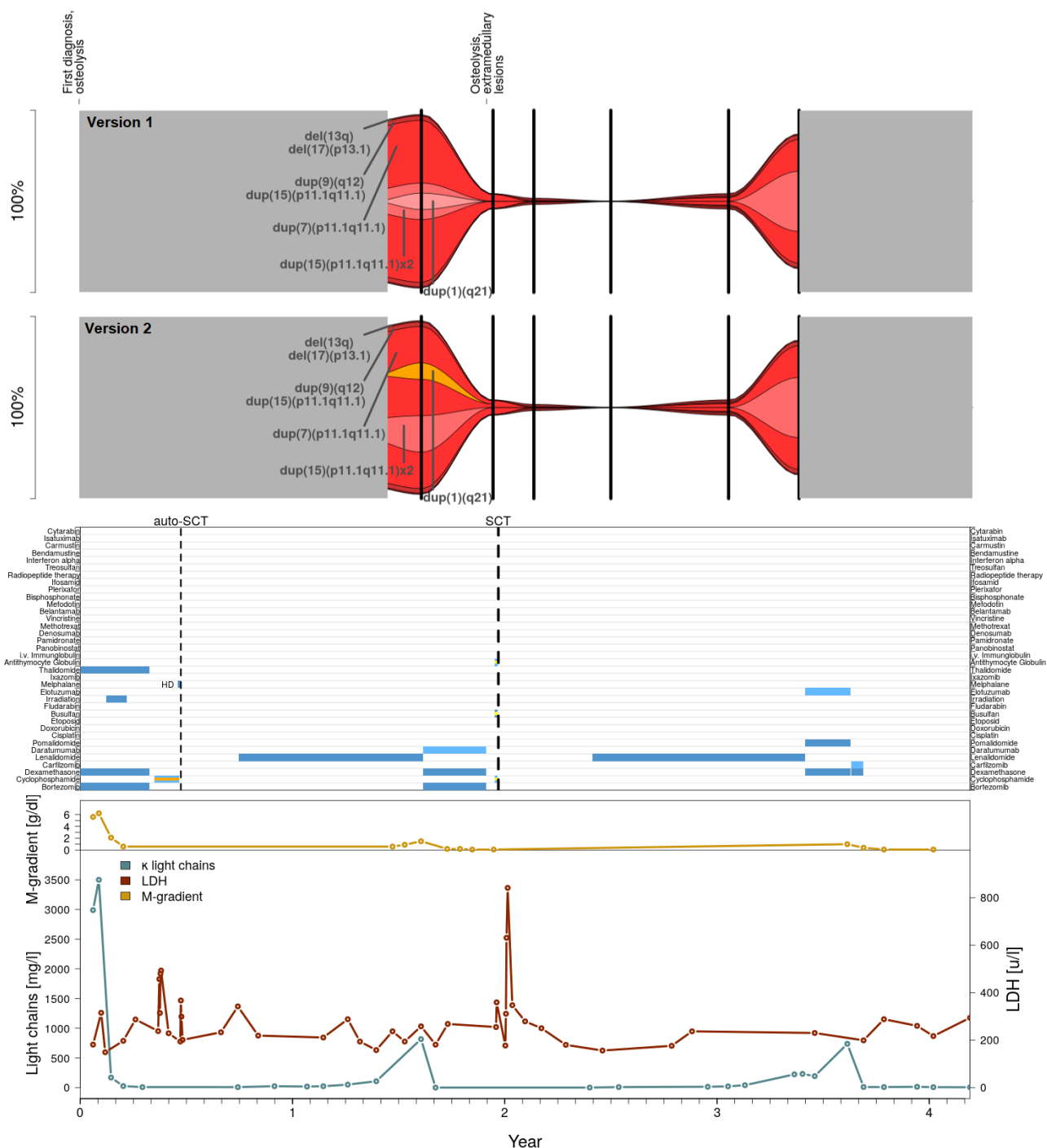
Supplementary Figure 12: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN08. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT: autologous stem cell transplantation, HD – high dose, SC – stem cell, SCT – stem cell transplantation.



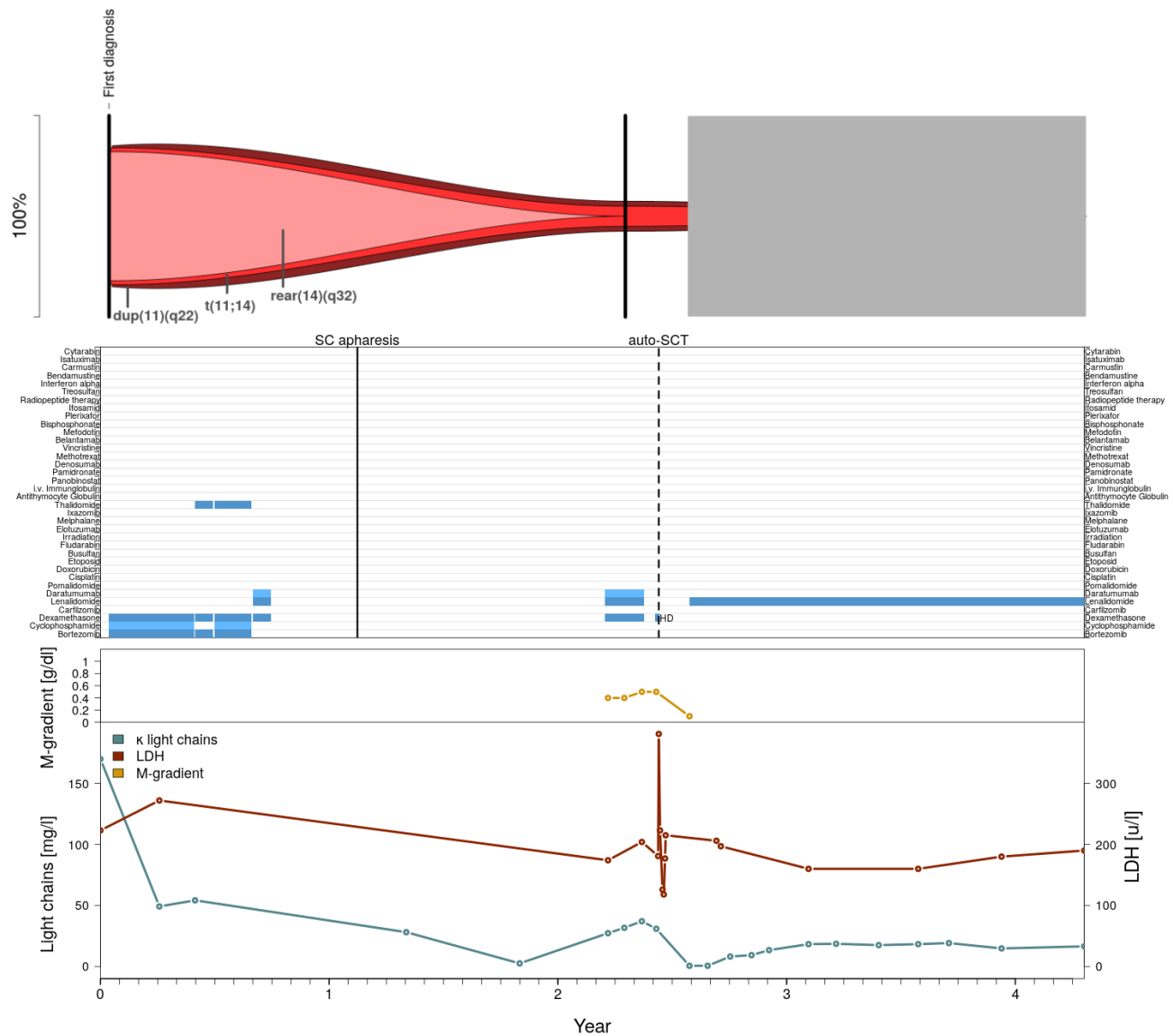
Supplementary Figure 13: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN09. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell, SCT – stem cell transplantation.



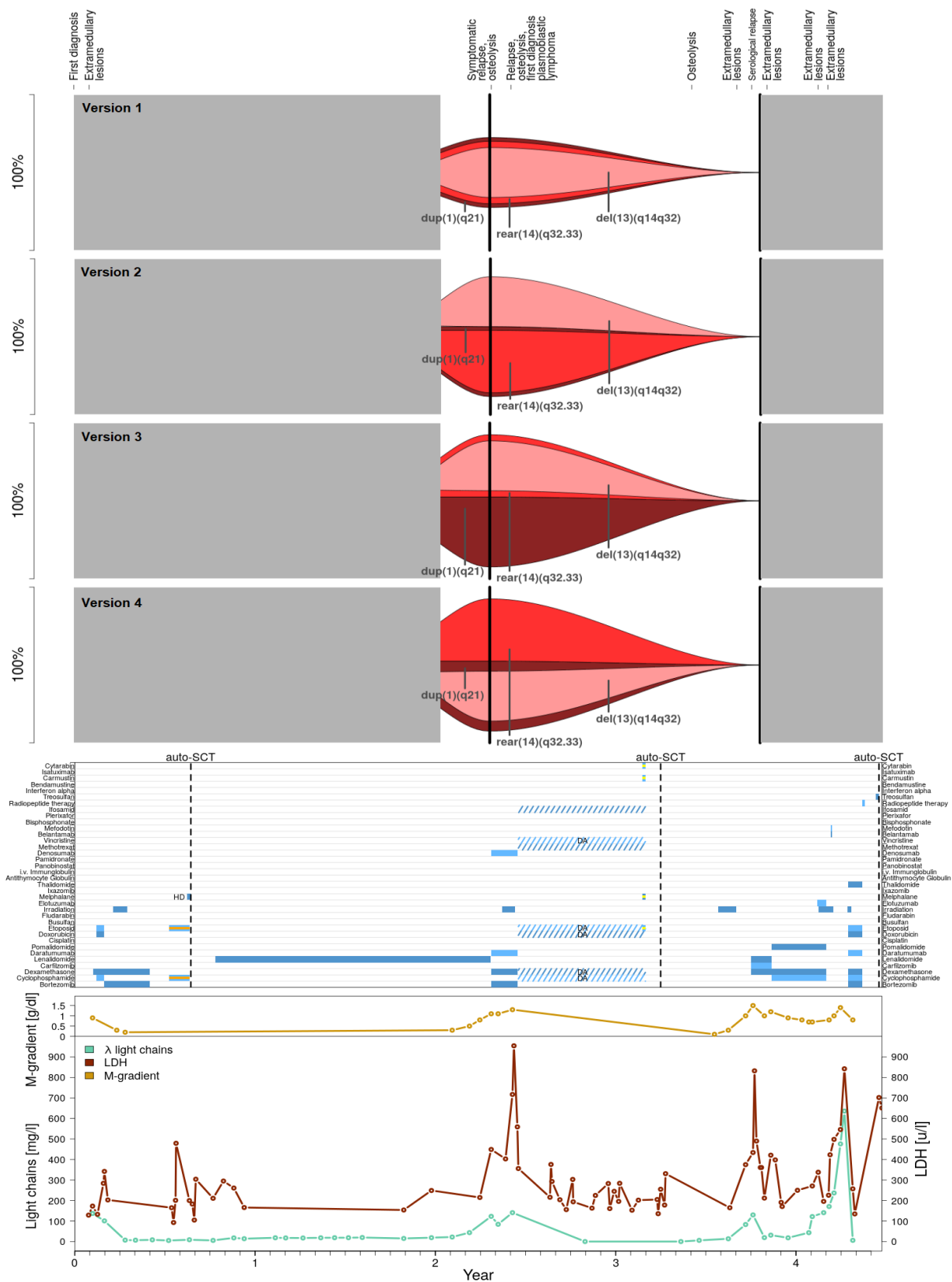
Supplementary Figure 14: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN10. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



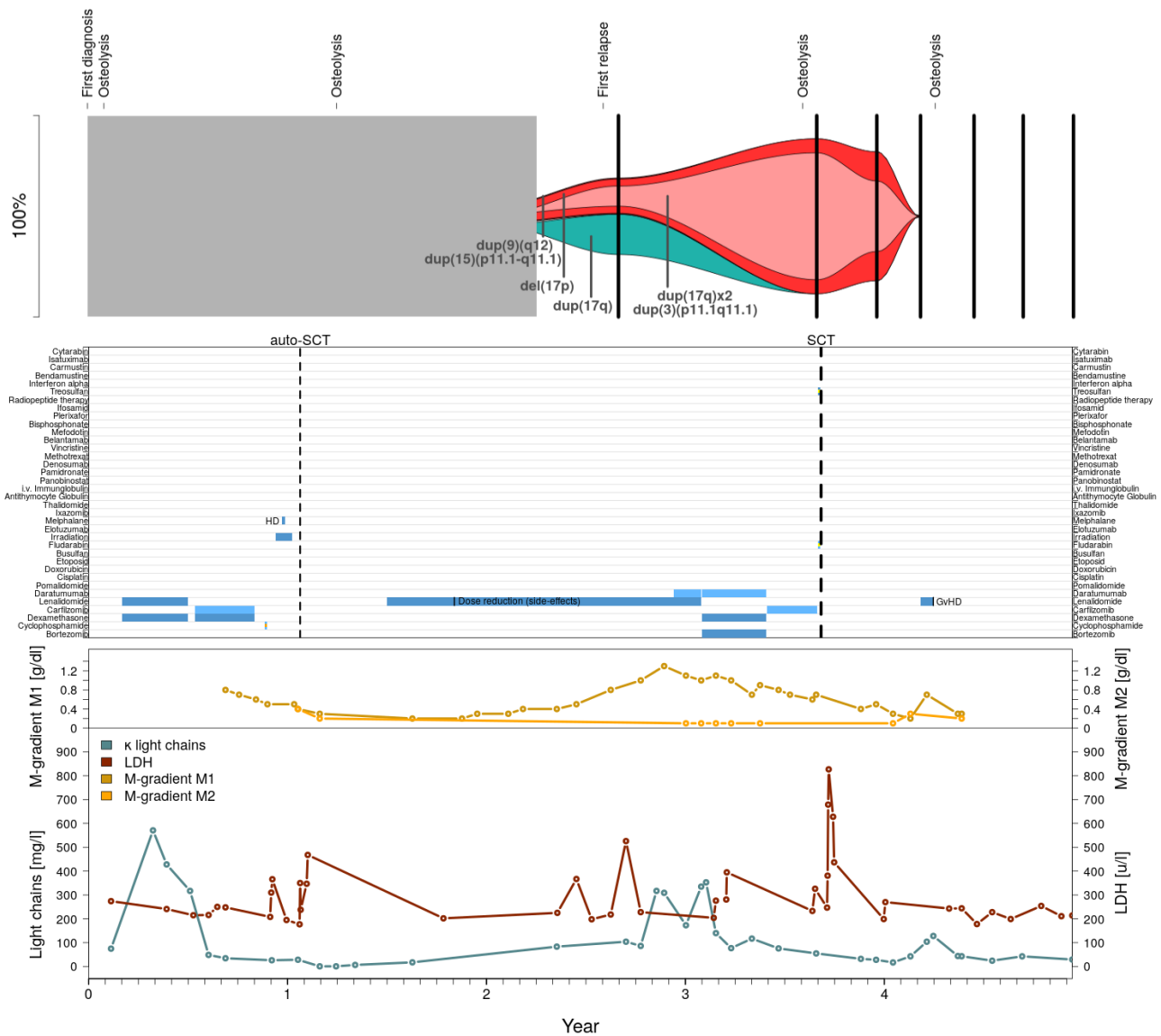
Supplementary Figure 15: Clonal evolution (2 possible versions), applied therapies and development of laboratory parameters of patient UPN11. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SCT – stem cell transplantation.



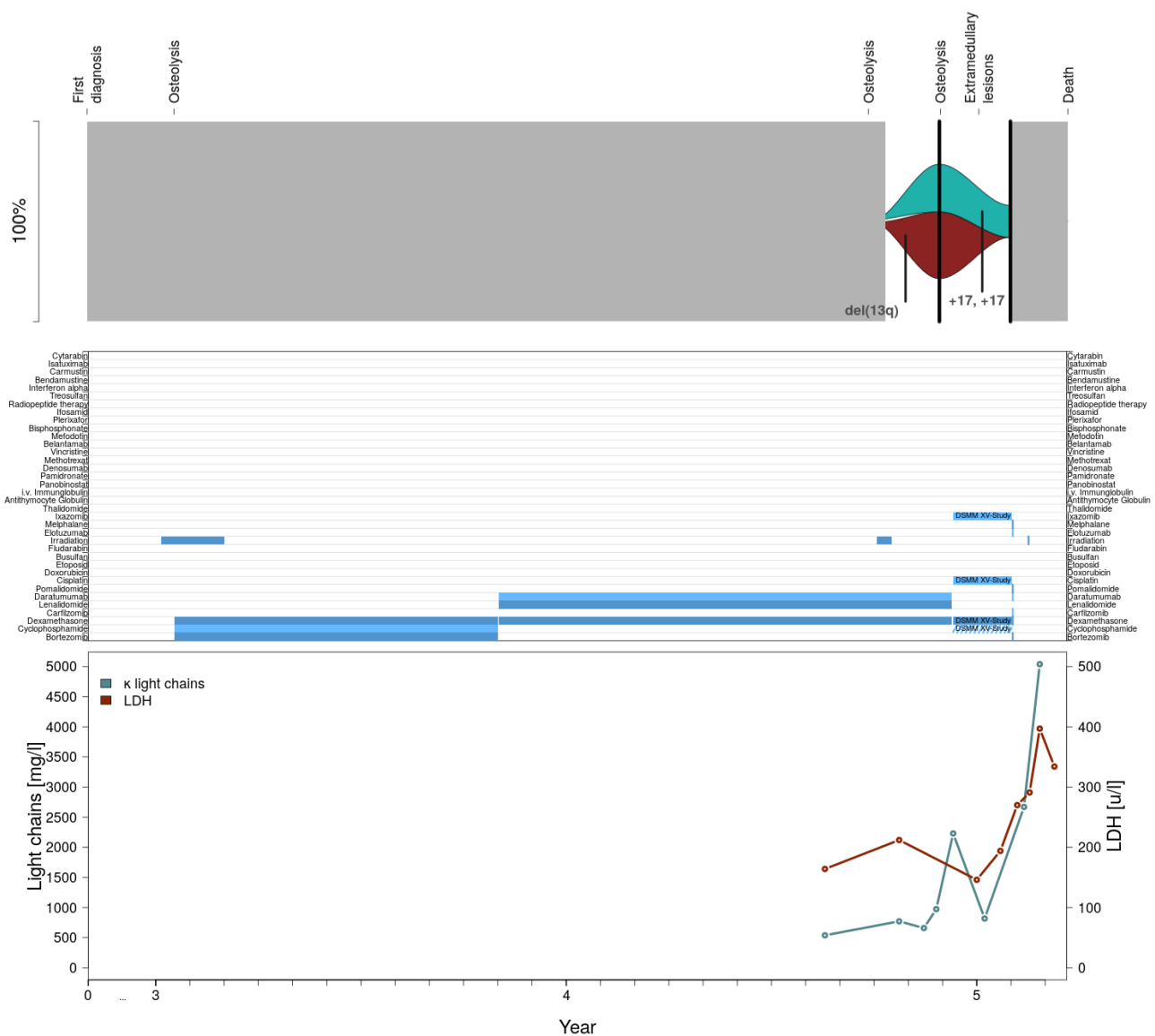
Supplementary Figure 16: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN12. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



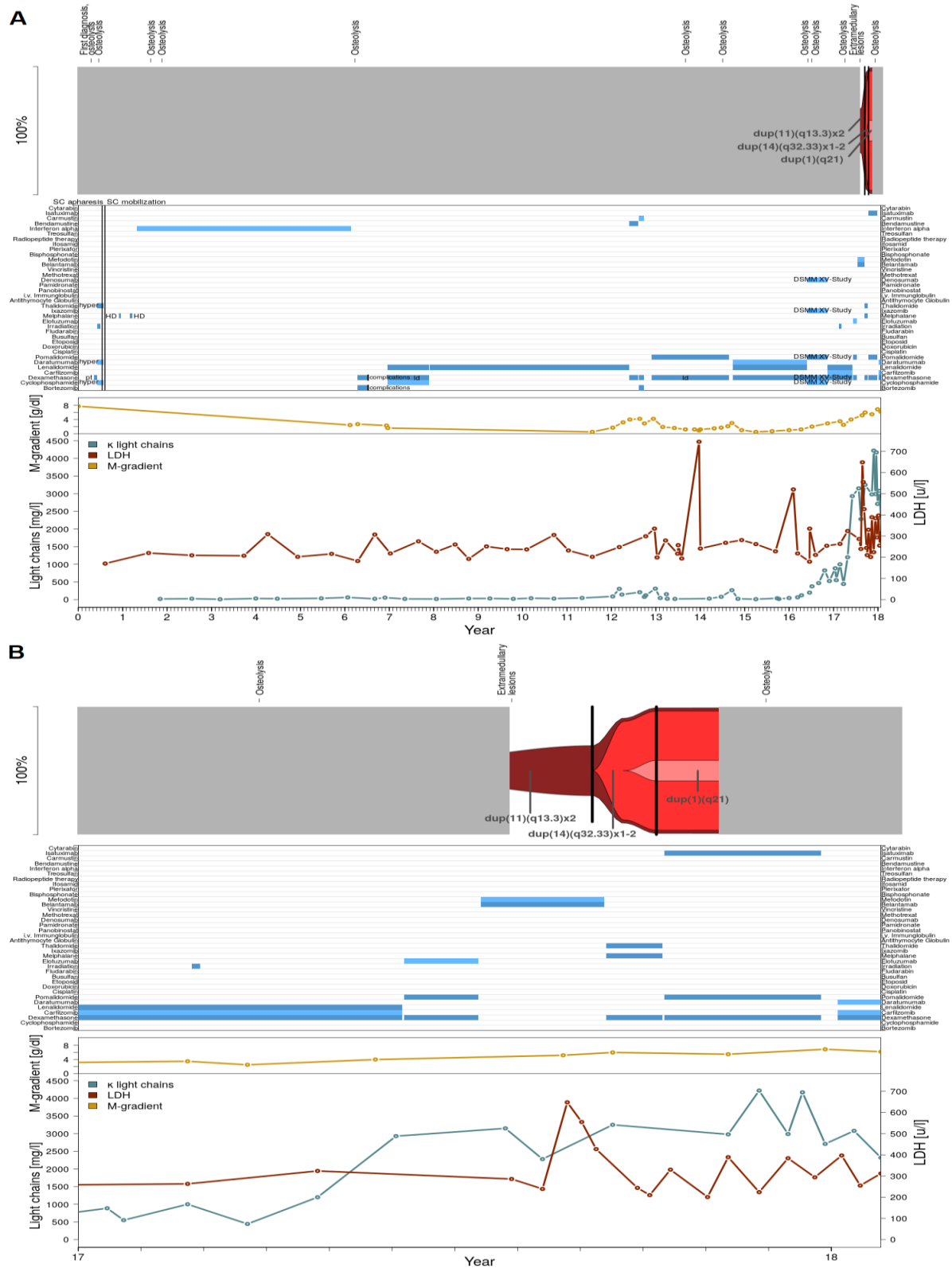
Supplementary Figure 17: Clonal evolution (4 possible versions), applied therapies and development of laboratory parameters of patient UPN13. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, DA – dose adjusted, HD – high dose, SC – stem cell.



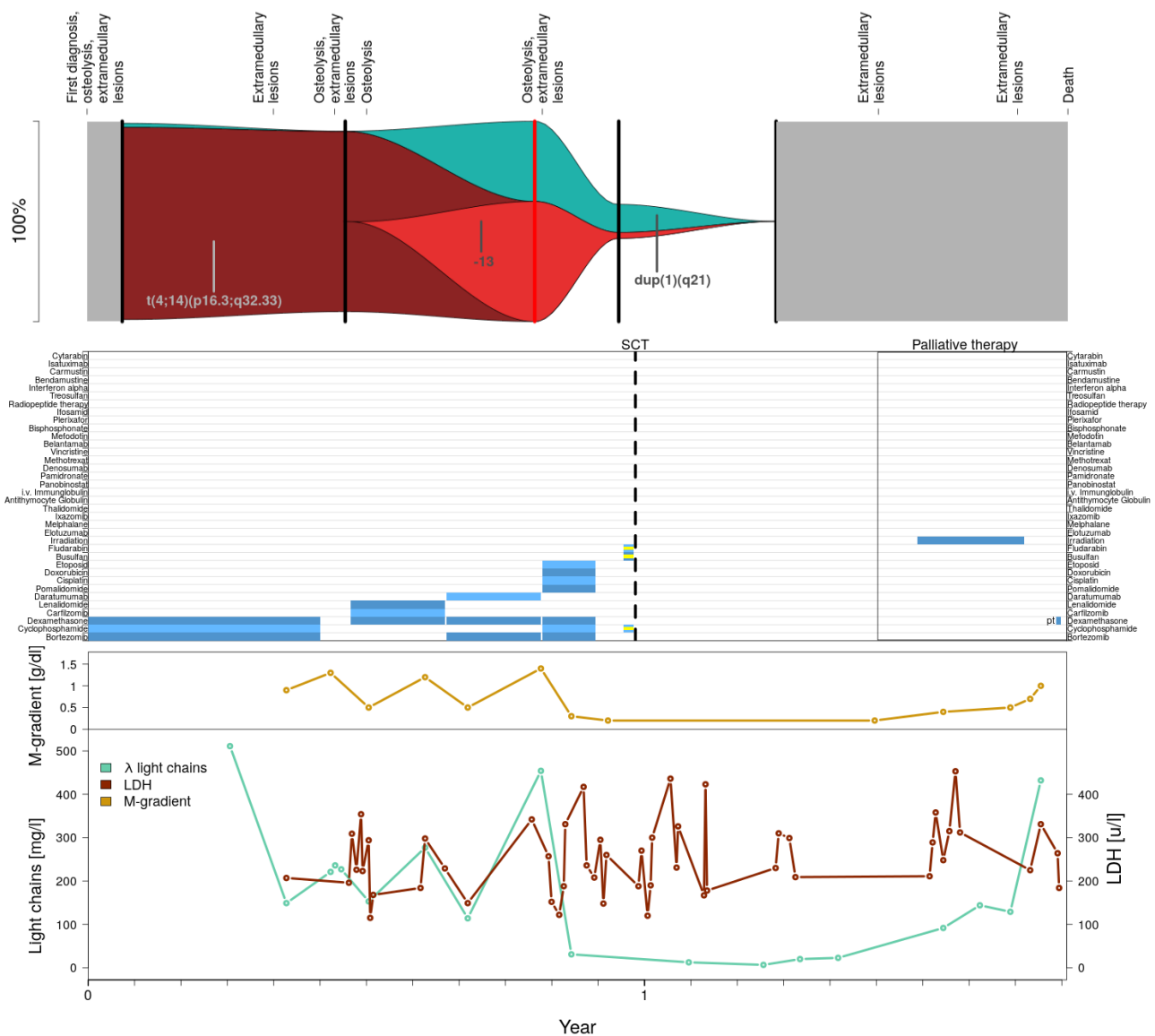
Supplementary Figure 18: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN14. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; auto-SCT – autologous stem cell transplantation, GvHD – graft-versus-host disease, HD – high dose, SCT – stem cell transplantation.



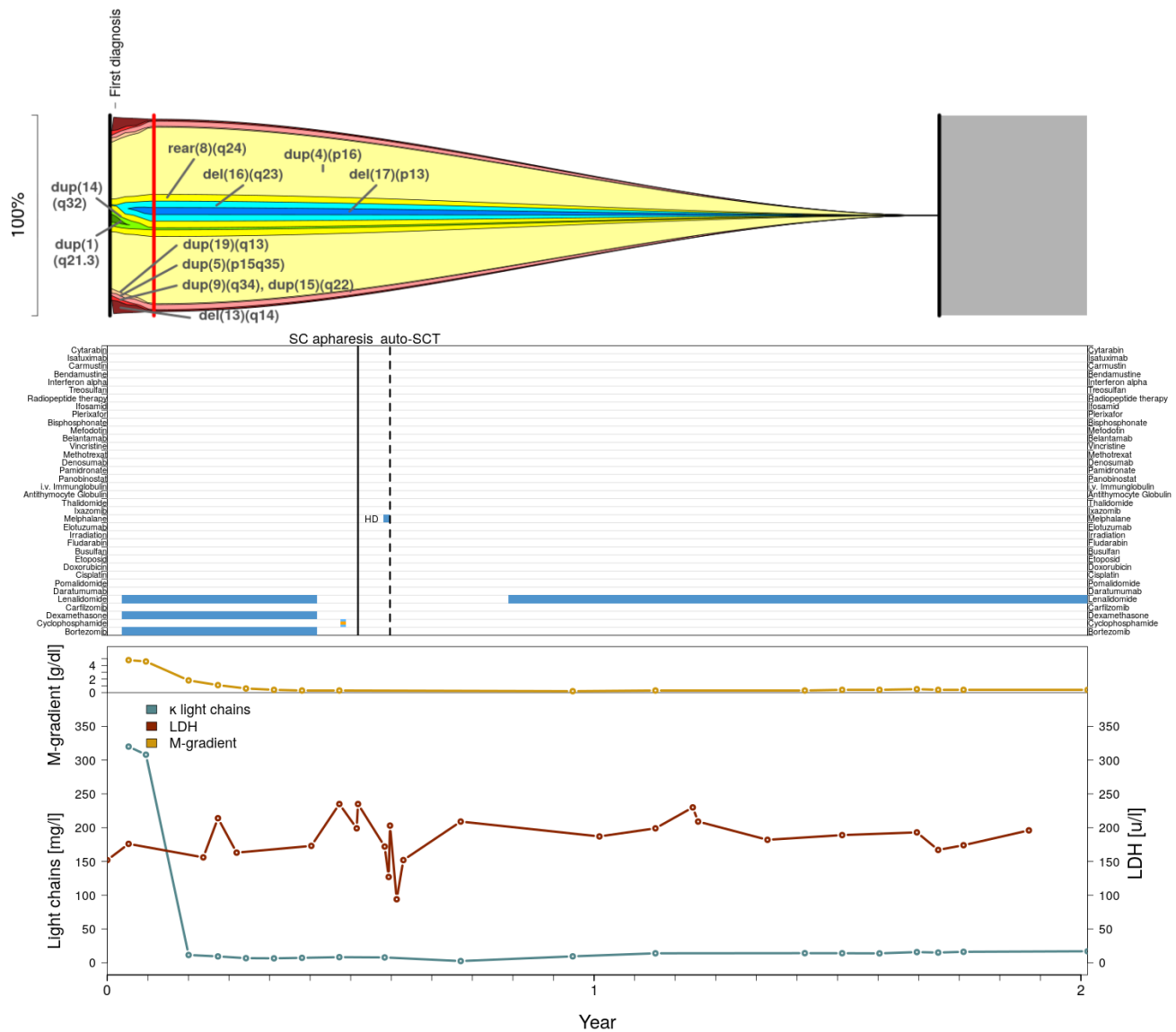
Supplementary Figure 19: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN15. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis.



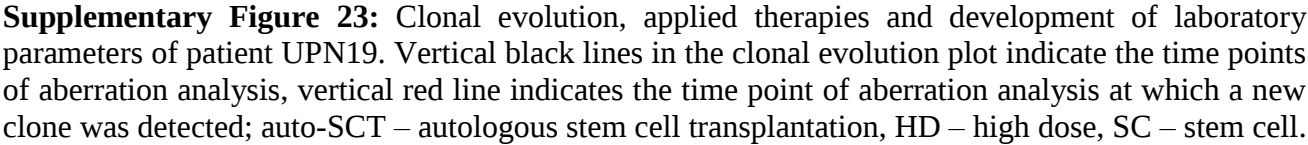
Supplementary Figure 20: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN16. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis; HD – high dose, LD – low dose, pt – pulse therapy, SC – stem cell. A) Full time of follow-up. B) Starting 17 years after first diagnosis.

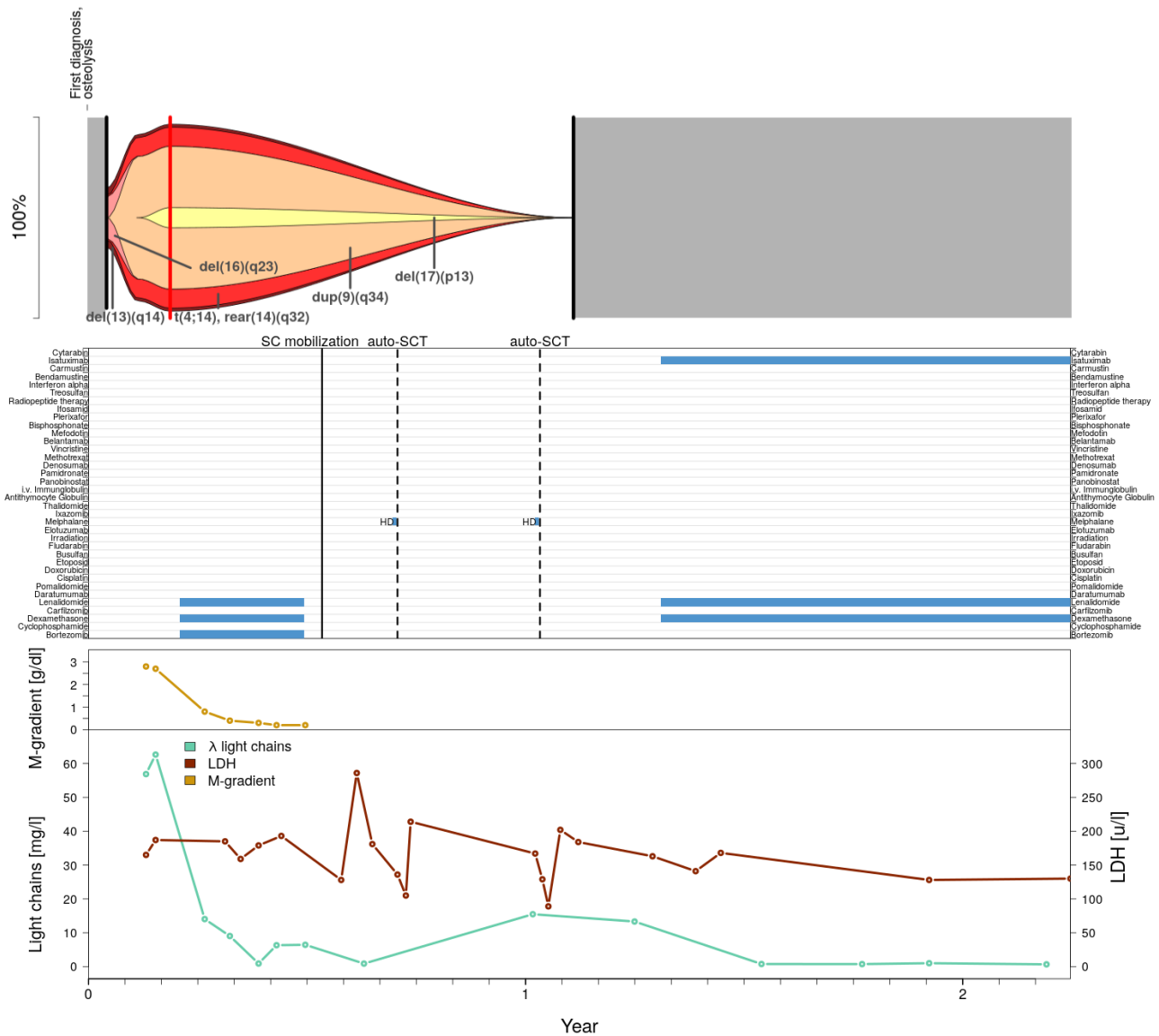


Supplementary Figure 21: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN17. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; SCT – stem cell transplantation.

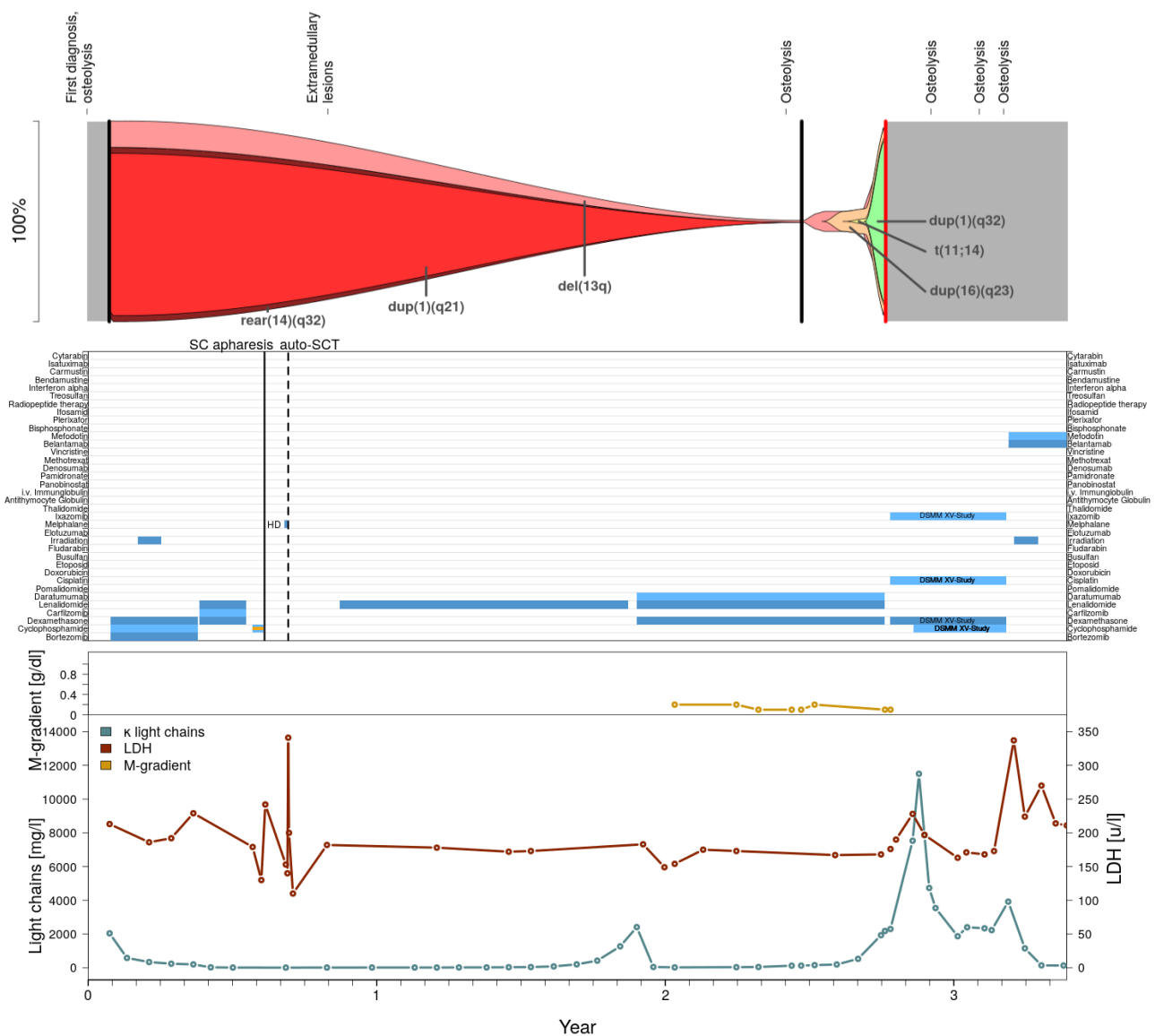


Supplementary Figure 22: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN18. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.

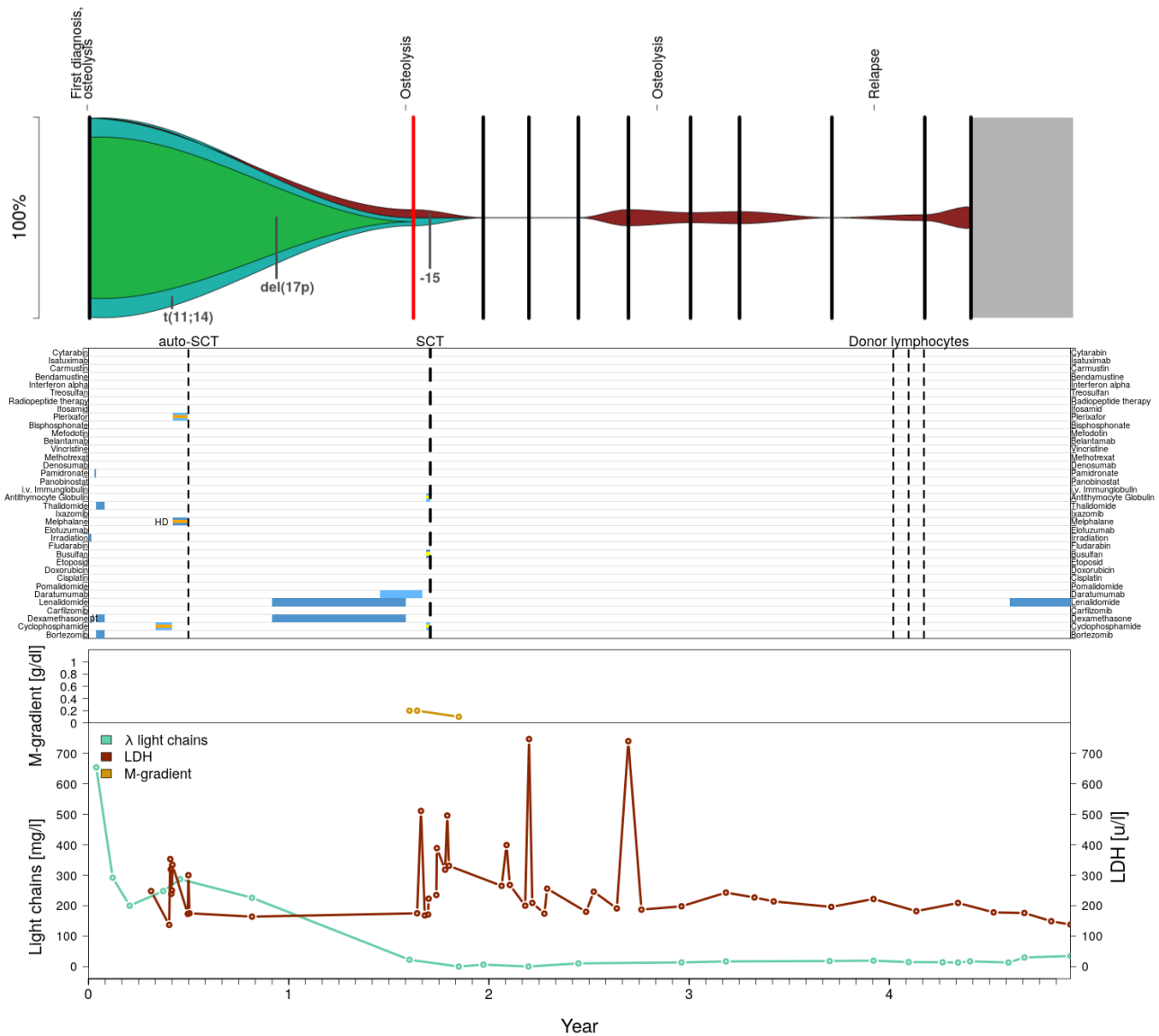




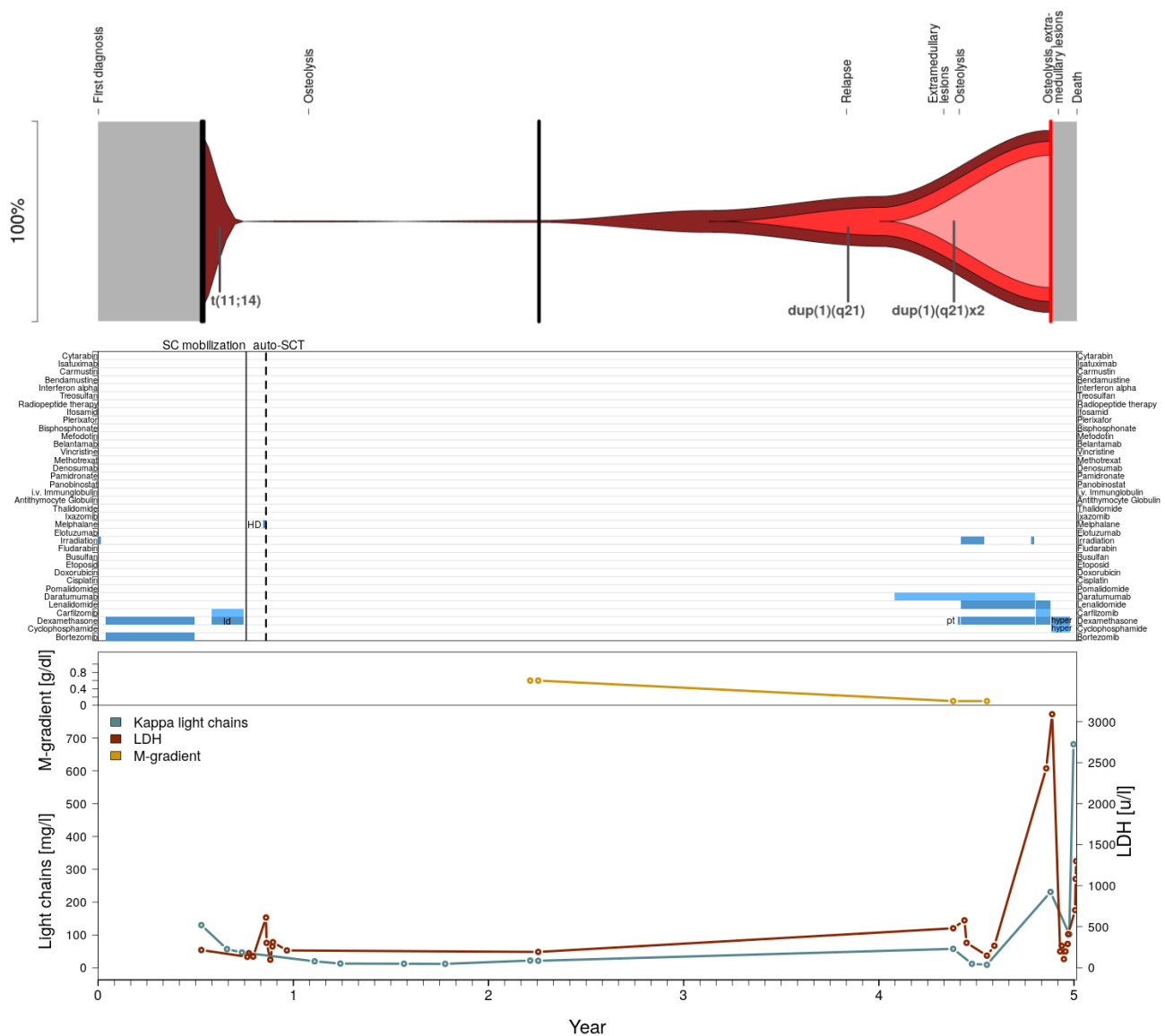
Supplementary Figure 24: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN20. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



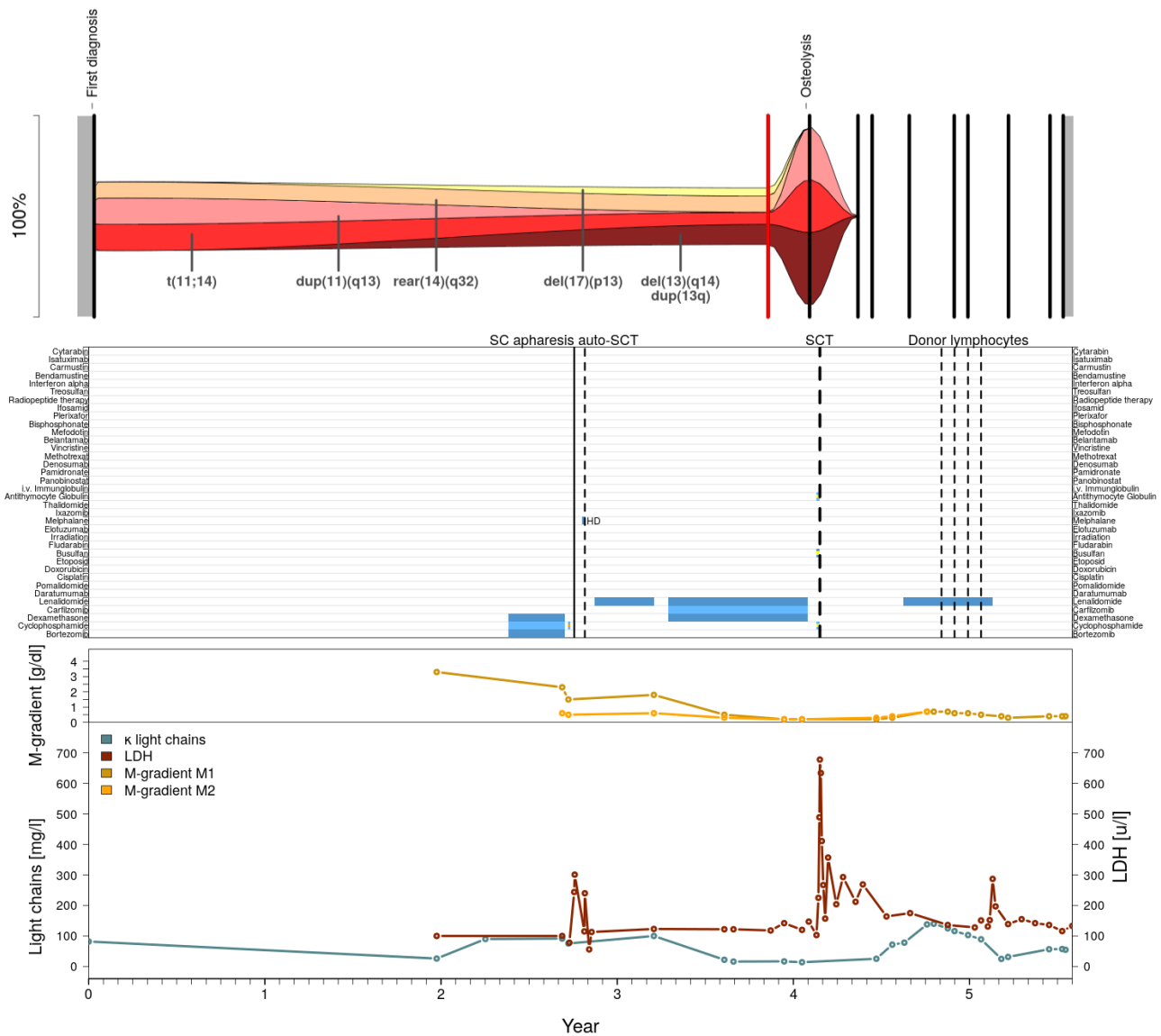
Supplementary Figure 25: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN21. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell.



Supplementary Figure 26: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN22. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, SCT – stem cell transplantation.



Supplementary Figure 27: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN23. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, Id – low dose, pt – pulse therapy SC – stem cell.



Supplementary Figure 28: Clonal evolution, applied therapies and development of laboratory parameters of patient UPN24. Vertical black lines in the clonal evolution plot indicate the time points of aberration analysis, vertical red line indicates the time point of aberration analysis at which a new clone was detected; auto-SCT – autologous stem cell transplantation, HD – high dose, SC – stem cell, SCT – stem cell transplantation.

