Novel patient-derived xenograft models of acute leukemias

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Background and Aim

Acute leukemias (AML-acute myeloid leukemia or ALL-acute lymphoid leukemia) represent a distinct heterogeneous group of hematological malignancies. Most patients show different responses and have poor prognosis with standard of care (SoC) therapy. Acute leukemias are challenging for the development of new targeted therapies. Therefore, there is a demand for corresponding robust preclinical models capable of testing new therapies. Patient-derived xenograft (PDX) models in mice recapitulate a wide variety of clinical features of cancer and have been proven useful in drug development and evaluation of new therapies.

The aim of the project was establishment and characterization of new PDX models representing AML and ALL.

Clinical data of leukemias

Tumor ID	Classification	Age	Gender
AML 6252	M4	58	male
AML 6256	M5	3	male
AML 6799	M1	14	male
AML11655	M1	43	male
AML11810	M5b	65	male
AML12680	M4	62	female
AML12683	M4/M5	76	female
AML13643	M5	75	male
AML13990	M5	56	female
AML14636	N/A	75	male
AML15117	AML NOS	57	female
AML16966	M0	60	female
AML18555	M1	24	male
ALL-SCID 2	c-ALL	16	male
ALL-SCID 3	T-ALL	3	male
ALL-SCID 4	T-ALL	15	male
ALL-SCID 5	c-ALL	3	male
ALL-SCID 6	T-ALL	3	male
ALL-SCID 7	pre-B-ALL	2	female
ALL-SCID 19	pro-B-ALL	1	male
ALL11656	c-ALL	45	male

Table 1: Overview of clinical AML and ALL data

PDX establishment

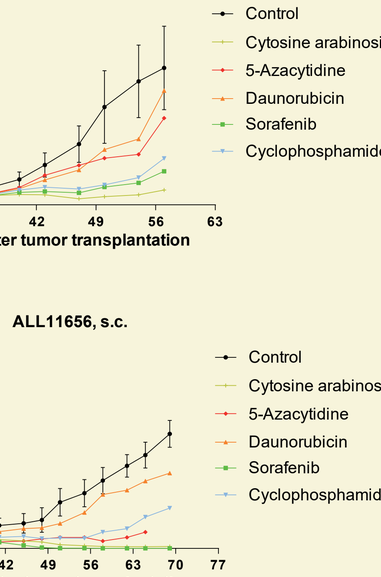

Figure 1: Schematic of PDX generation

Characteristics and drug response of AML PDX and ALL PDX

PDX ID	Transplantation route	Mutations	Read out: Time (days)	Read out: Parameter	Drug response (T/C): Cytosine arabinoside	Cyclo phosphamide	Daunorubicin	5-Azacytidine	Sorafenib	ABT-199	Vincristine	Asparaginase
AML 6252	s.c.	none	50	tumor volume	25*	41*	60	N/A	N/A	N/A	N/A	N/A
	i.v.		30	spleen weight, survival, FACS	21*	N/A	26*	N/A	N/A	N/A	N/A	N/A
AML 6256	s.c.	none	45	tumor volume	53	31	51	N/A	N/A	N/A	N/A	N/A
AML 6799	s.c.	N/A	50	tumor volume	21*	0.1*	91	N/A	N/A	N/A	N/A	N/A
AML 11655	s.c.	IDH2, NPM1,	60	tumor volume	8*	20*	53*	41*	18*	N/A	N/A	N/A
	i.v.	NRAS	80	spleen weight, survival, FACS	60*	34*	N/A	N/A	26*	N/A	N/A	N/A
AML11810	s.c.	N/A	30	tumor volume	157	4*	81	41*	49*	N/A	N/A	N/A
AML12680	s.c.	NRAS	35	tumor volume	69	25*	83	N/A	41	N/A	N/A	N/A
AML12683	s.c.	N/A	60	tumor volume	51	0.1*	56*	50*	39*	N/A	N/A	N/A
AML13643	s.c.	none	35	tumor volume	96	9*	40*	84	80	N/A	N/A	N/A
AML13990	s.c.	N/A	40	tumor volume	65	N/A	57	63	87	30*	N/A	N/A
AML14636	s.c.	N/A	80	tumor volume	0.1*	15*	N/A	8*	23*	26*	N/A	N/A
AML15117	s.c.	ASXL1,	70	tumor volume	2*	54	N/A	63	55	19*	N/A	N/A
	i.v.	NRAS	60	spleen weight, survival, FACS	N/A	90	N/A	18*	51*	62*	N/A	N/A
AML16966	s.c.	none	25	tumor volume	85	45*	N/A	97	81	76	N/A	N/A
AML18555	**i.v.**	N/A	140	spleen weight, survival, FACS	56*	43*	N/A	32*	85	35*	N/A	N/A
ALL-SCID 2	**i.v.**	N/A	45	spleen weight, survival, FACS	136	53	38	N/A	N/A	N/A	N/A	109
ALL-SCID 3	i.p.	CEBPA	20	tumor volume	N/A	N/A	12*	N/A	N/A	N/A	12*	89
ALL-SCID 4	s.c.	none	50	tumor volume	N/A	0.1	2	N/A	N/A	N/A	N/A	1
ALL-SCID 5	s.c.	DNMT3A, IDH1	70	tumor volume	48	30*	45	N/A	N/A	N/A	22	10
ALL-SCID 6	s.c.	IDH2, NPM1	40	tumor volume	1*	36	85	N/A	N/A	N/A	19	57
	i.v.		60	spleen weight	16*	64	62	N/A	N/A	N/A	59	77
ALL-SCID 7	i.v.	N/A	30	spleen weight, survival, FACS	95	30*	148	N/A	N/A	N/A	44	75
ALL-SCID 19	s.c.	none	40	tumor volume	17*	0.1*	42*	N/A	N/A	N/A	0.1*	0.1*
ALL11656	s.c.	DNMT3A,	60	tumor volume	2*	0.1*	54	11*	18*	N/A	N/A	N/A
	i.v.	IDH2, RUNX1	55	spleen weight, survival, FACS	23*	6*	N/A	77*	56*	N/A	N/A	N/A

Table 2: Overview of established AML/ALL PDX, including clinically relevant mutations in leukemia (RNAseq analysis), in vivo growth features, read out parameters and drug response. PDX reveal a wide range of responses towards clinically relevant drugs (optimal T/C % values). N/A=not analysed, *p<0.05 compared with control.

Examples of drug response in systemically and subcutaneously growing PDX

Figure 2: Examples of therapy response in AML11655 and ALL11656 PDX. Mice were treated starting either from palpable tumor size (s.c.-models) or three days after i.v. inoculation of tumor cells (i.v.-models). Response was comparable in s.c.- and i.v.-models.

In vivo growth kinetics of individual PDX

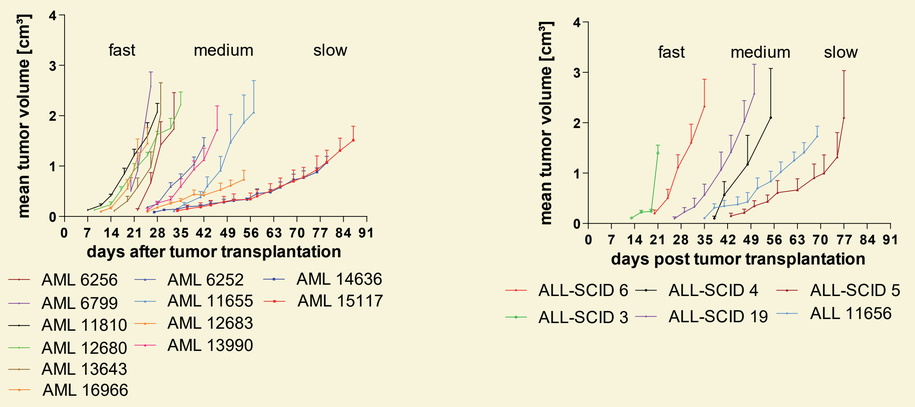

Figure 3: Individual tumor growth of different PDX, including fast (appr. 20 days), medium (appr. 60 days) and slow (up to 90 days) growing tumors. Tumor fragments were subcutaneously transplanted at day 0. The width and length of the tumors were measured 2-3x/week.

Monitoring of therapy effects in blood -adjuvant therapy setting in AML11655 i.v.

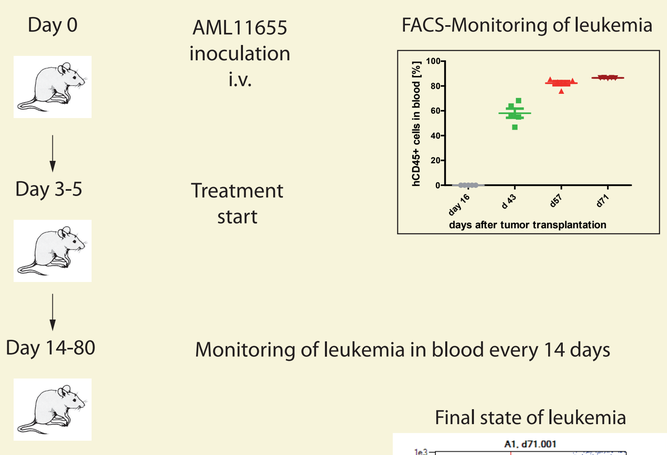

Figure 5: Schematic of leukemia and therapy monitoring in blood

Different read out parameters for drug response in AML15117i.v. PDX

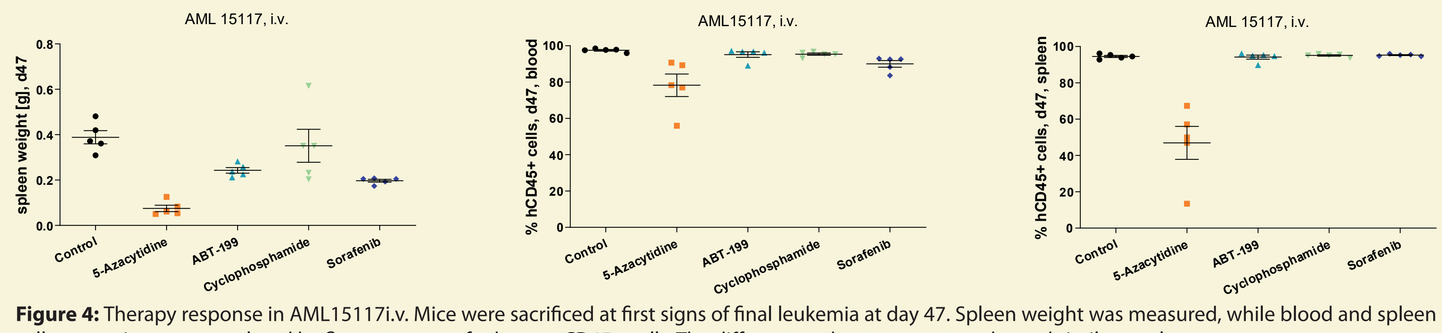

Figure 4: Therapy response in AML15117i.v. Mice were sacrificed at first signs of final leukemia at day 47. Spleen weight was measured, while blood and spleen cell suspensions were analysed by flow cytometry for human CD45+ cells. The different read out parameters showed similar results.

Summary of results

- 13 AML PDX were established, representing 7 various subtypes –1x M0, 3x M1, 2x M4, 1x M4/M5, 3x M5, 1x M5b, 1x NOS (**table 1**)
- 8 ALL PDX of varied subtypes were established, 1x pre-ALL, 1x pro-ALL, 3x c-ALL, 3x T-ALL (**table 1**)
- PDX panel includes models with clinically relevant mutations in acute leukemias like IDH1, IDH2, NPM1, NRAS, ASXL1, CEBPA, DNMT3A, RUNX1 (**table 2**)
- PDX exhibit different in vivo growth kinetics in a range from 20 days to 80 days (**figure 3**)
- PDX display heterogeneous response to SoC, reflecting the clinical situation (**table 2**)
- Response to SoC was similar and independent from PDX growth type - systemically or subcutaneously (**figure 2**)
- In systemically growing PDX, spleen weight correlated with flow cytometric analysis of human CD45 positive cells in blood and spleen (**figure**

Conclusion

The established PDX models of AML and ALL represent suitable tools for preclinical drug development. They provide an exceptional platform for the identification and validation of new targets, while allowing screening of new compounds and testing of new therapies, including immuno-oncological and cell therapies.

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