Patient derived AML xenografts for drug evaluation

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Summary

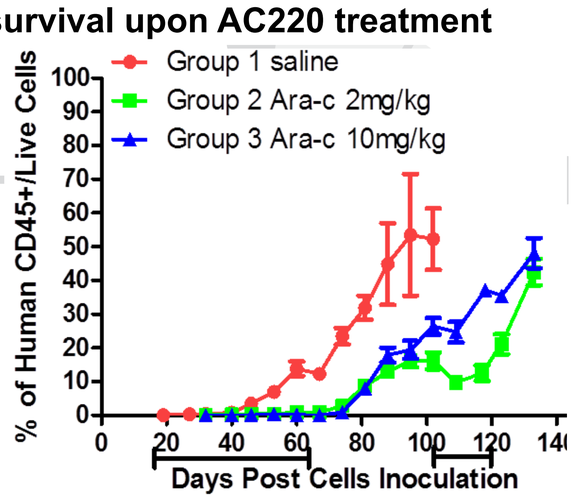
We have recently successfully engrafted leukemic cells from bone marrow of 4 AML patients into immunocompromised NOD/SCID mice, AM7577, AM8096, AM8070 and AM999. They are from patients of different subtypes: M5 (AM7577), M2 (AM8096) and M6 (AM8070). We have examined two of them and revealed they have very distinct genotypes and phenotypes. AM7577 also harbors many of the common AML genotypes of mutations for IDH2-R140Q, FLT3-ITD, DNMT3A R882H and NPM1. It displays typical aggressive M5-AML disease: starts at bone marrow and gradually expand to peripherals (spleen – massively enlarged spleen, lymphonode and peripheral blood). It causes full blown leukemia with severe symptoms (body weight loss, hunched, inactivity, labored breathing, ruffled coat, etc.) and eventual death with 100% mortality. In contrast to AM7577, AM8096 does not harbor any of these mutations. Although causing similarly aggressive disease symptoms and 100% mortality in the end, the disease of AM8090 mainly resides in bone, and only spread to peripherals at terminal stage at lower levels and with only lightly enlarged spleen. The leukemic cell morphology of AM8090 is also less differentiated as compared to AM7577, all together as expected for M2 diseases. For both models, the leukemic cells can serially be passed in mice with 100% take-rate and cause consistent disease (even with < 1e4 cells). This also creates a renewable and potentially unlimited source of leukemia cells. The leukemic cells in mice are identical to those of the original patient leukemic cells ($CD45^+$, $CD33^+$, $CD13^+$, $CD123^+$, and $CD19^-$). We are currently performing RNAseq of AM8096 and AM7577 in order to explore the underlying molecular mechanisms that drive both diseases. Meanwhile, we are also investigating the drug response to standard of care (SOC, AraC) and FTL3 inhibitor, and found both respond well to these treatments in term blood leukemia burden and symptoms despite different genotypes. In addition, we revealed complete difference in relapse, or in the maintenance of the remission, after the drug withdraws between the two treatments, which implicate the potential roles of bone marrow leukemia initiation cells (LICs). We believe these patient derived AML models could serve as useful experimental models to investigate the diverse leukemogenesis and evaluate new treatments for AML.

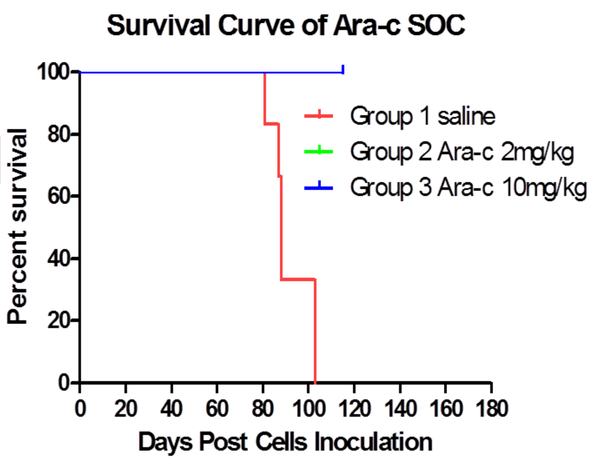
Results

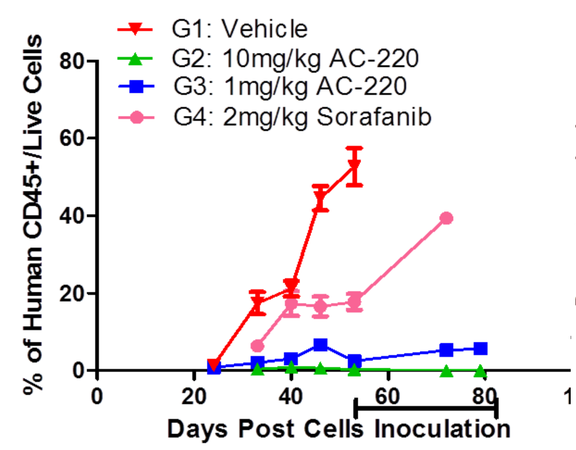
Table 1. Summary of AM7577patient information and model information.

	Patient	Model
	Male, 69-yrs old, M5	
Chromosome	Normal karyotype	
Blood-Rt.	WBC 38.9X109/L， HB 74.2g/L,plt 163X109/L, abnormal : 79.20%, Naive / nuclear:11.47%. Classification of blood: 63%	
Mutations	FLT3(+); DNMT3A(+), IDH2(R140Q); NPM/A, (SNP: CEBP-2'+ins c)	FLT3(+); DNMT3A(+);IDH2(R140Q); CEBP-2'+ins c; NPM/A
Immunological phenotypes	Express:CD13,CD33,HLA-DR,CD117,CD38,CD71. Partially express: MPO, CD15, CD19, CD7. Not express: CD34, CD10, CD20, CD79a, CD3, CD5, CD11b, CD14, CD56, GlyA	Express: CD38, CD123, CD45, CD13, CD33 Not express: CD34, HLA-DR, CD19
Treatment & response	Chemotherapy(ECAG), complete response	Complete responses to AraC, sorafenib & AC220. No relapse for AC220 after drug withdrawal

Figure 1. A. Leukemic burden in peripheral blood of AM7577-mice (immdiate below left) and survival (immediate below right) upon AraC treatment; bottom figure with corresponding leukemic burden and survival upon AC220 treatment







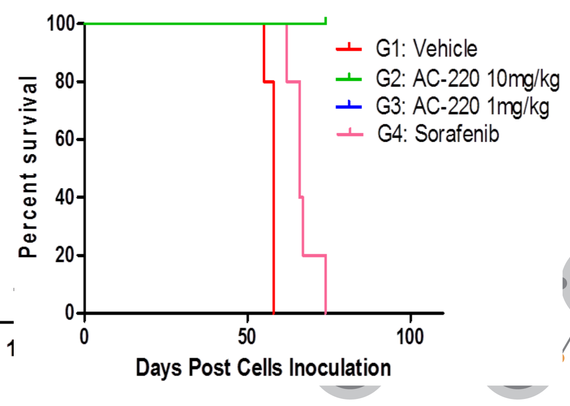


Table 2. Summary of AM8096patient information and model information.

	Patient	Model
	Male, 21-yrs old, M2	
Chromosome	Normal karyotype	
Blood-Rt.	WBC 20X10e9/L, blast cells 70%	
Mutations	wt: TP53; flt3; NPM1; CEBPA-exon1: insertion 570-587, 3GCACCC>4GCACCC	wt: TP53; flt3; NPM1; CEBPA-exon1: insertion 570-587, 3GCACCC>4GCACCC
Immunological phenotypes		Express:CD13,CD33; Not express:CD19
Treatment & response	Alleviative Treatment initial complete response; recurrent	Complete responses to AraC, sorafenib & AC220. No relapse for AC220 after drug withdrawal

Figure 1. A. Leukemic burden in peripheral blood of AM8096-mice (below left) and survival (below right) upon AraC and AC-220 treatment.

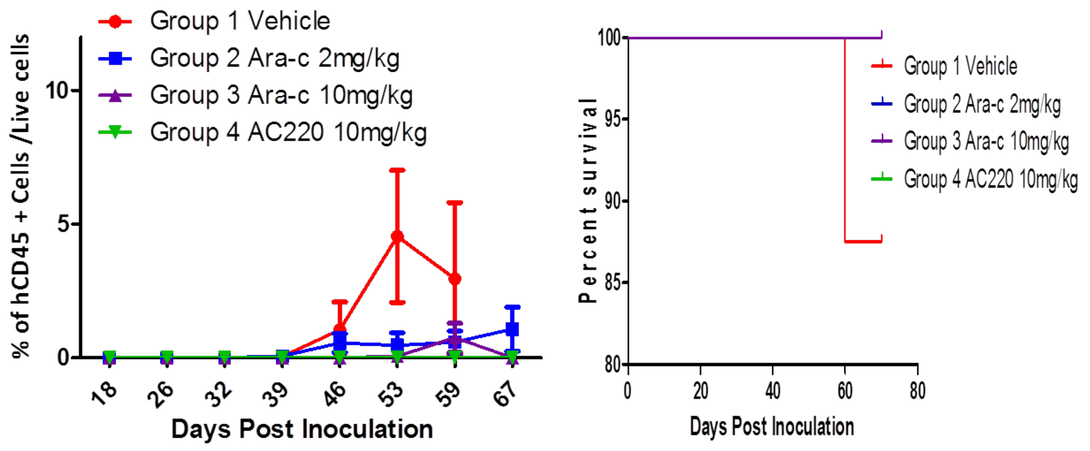
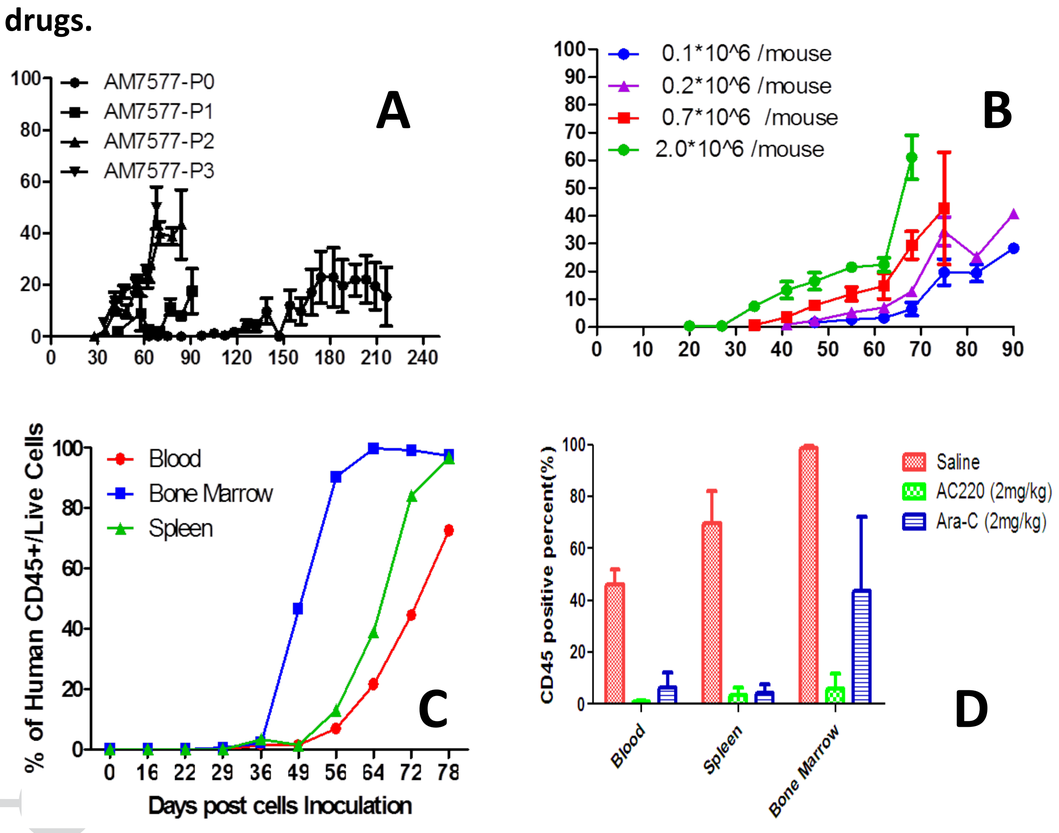


Figure 3. AM7577: leukemia growth of different passages; B: leukemia growth impacted by the number of cells engrafted; C: leukemia growth in differnet organs; D: disease inhibitions in different organs and by different drugs.



We have also established additional two AML models, AM8070 and AM9999, at P0 passages, and currently are being expanded and characterized similarly.

Conclusions

1. 4 AML PDX models derived from patient bone marrow have been successfully engrafted in NOD/SCID mice;
2. They have different subtypes, genotypes and growth phenotypes;
3. Two of them have been pharmacologically tested for standard of cares, along with investigational targeted therapy;
4. Pharmacology responses are different among different disease organs and different drugs.