Report: Culturing patient leukemic cells

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Rationale:

Leukemic stem cells (LSCs) are cells with the capacity to self-renew and give rise to progenitor cells. The LSCs are thought to be the cells that are responsible for relapse in AML. To be able to screen chemical probes, we would like to be able to maintain CD34 expression, a marker of LSCs (CD34+CD38- are often markers of LSCs). The epigenetic probes that we are using often require long term exposure, so we wanted to determine if we could maintain CD34 expression long term to be able to treat these cells with probes.

Methods and Results:

The patient cells were thawed and their initial immunophenotype was profiled using flow cytometry with the following antibodies: CD45-FITC (clone: HI30), CD34-APC (clone: 581), CD38-PE-Cy7 (clone: HB7) (all from BD Biosciences) and 7-AAD (Life Technologies). We then cultured these cells in 6 different media with varying cytokine and serum/serum-free conditions as listed in table 1 in co-culture with OP9 stroma.

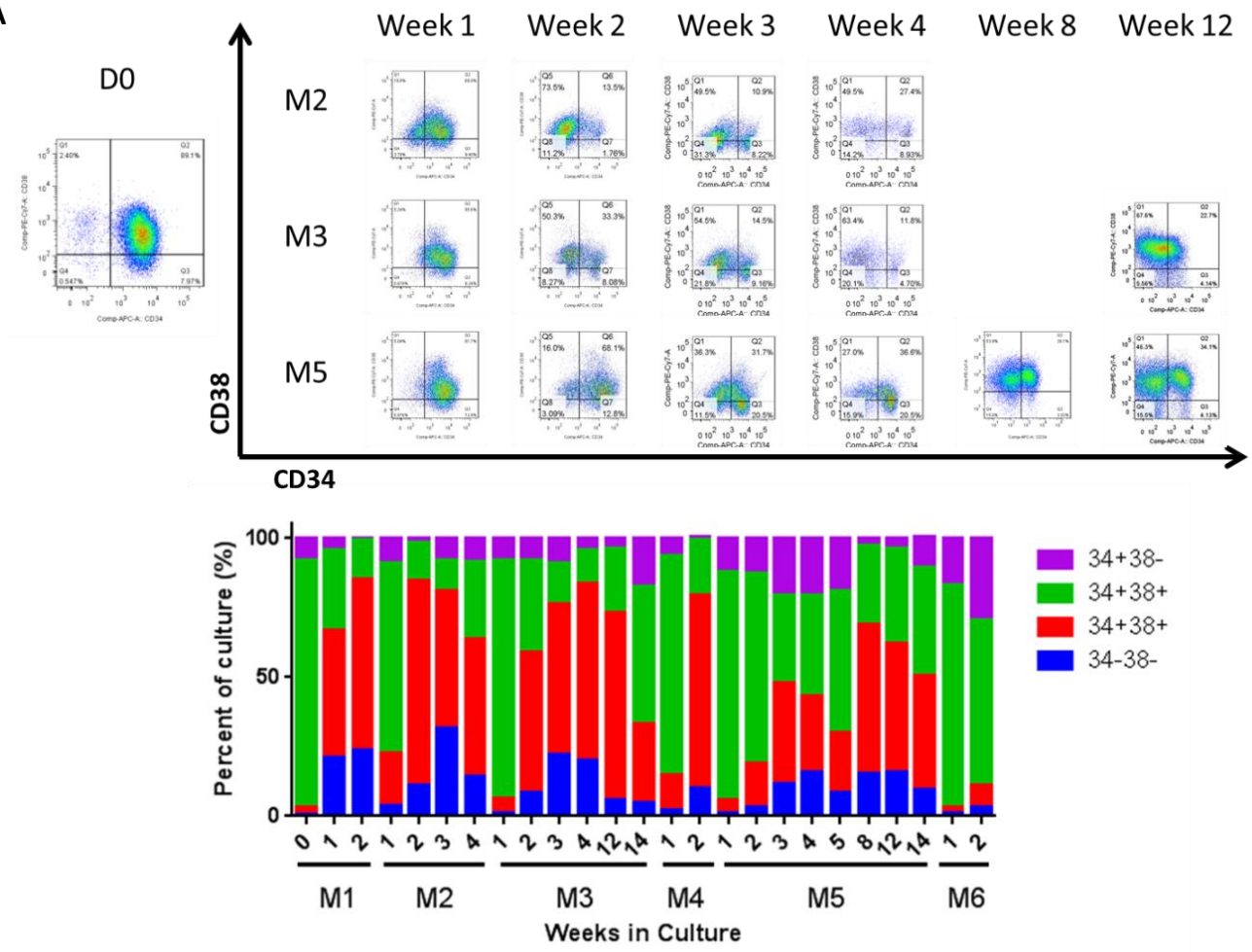
Table 1: Summary of media ingredients used to grow patient cells

	1	2	3	4	5	6
Base media	IMDM	IMDM	IMDM	Stem span	αMEM	αMEM
BIT or FBS	10% BIT-9500	15% BIT	10% FBS		12.5%, 12.5% HS	12.5%, 12.5% HS
L-glutamine	2mM	2mM	2mM		2mM	2mM
low density lipoprotein	5mg/mL					
SCF	100ng/mL	100ng/mL	100ng/mL	100ng/mL		
FLT3L	100ng/mL	50ng/mL	50ng/mL	100ng/mL		
IL6	20ng/mL			20ng/mL		
IL3	20ng/mL	20ng/mL	20ng/mL		20ng/mL	20ng/mL
G-CSF	20ng/mL	20ng/mL			20ng/mL	
GM-CSF	20ng/mL		20ng/mL			
TPO	20ng/mL		40ng/mL	20ng/mL	20ng/mL	
β-mercaptoethanol	55µM	0.1mM	55µM		55µM	
Hydrocortisone					1µM	1µM
Monothioglycerol						1µM

1. From (McDermott, Eppert et al. 2012)
2. From (Pabst, Krosl et al. 2014)
3. Combination of previous publications
4. From (Lechman, Gentner et al. 2012)
5. From (Rozenveld-Geugien, Baas et al. 2007)
6. From (Hartwell, Miller et al. 2013)

We profiled the cells over a series of weeks to determine CD34 and CD38 expression in viable leukemic cells and determined which media best suited the growth of a particular patient sample. It should be noted that the serum free media 1 and 4 were not well tolerated by the OP9 stroma and have been excluded from later testing (OCI-AML-20). I have included a summary of the immunophenotype profile for two patients, 150333 (Figure 1A) and another that we are calling OCI-AML-20 (Figure 1B).

A



B

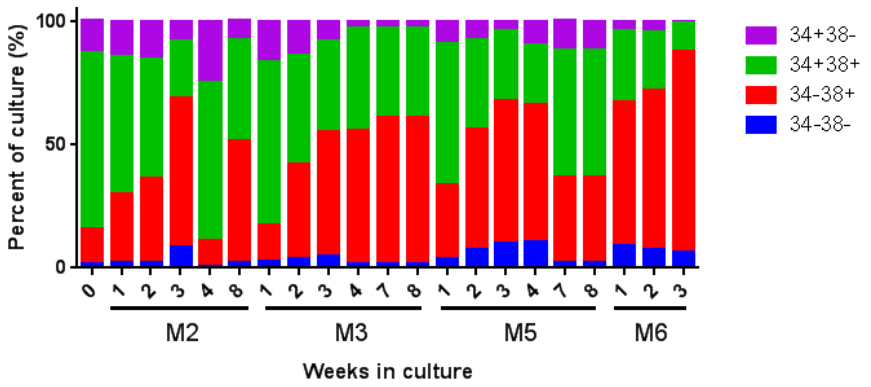


Figure 1: Summary of the profile of CD34 and CD38 expression in (A) 150333 and (B) OCI-AML-20. M refers to media. Media 5 and 3 maintained CD34 expression and allowed cells to proliferate best for the samples, respectively.

For these cells, media 5 for 150333 and media 3 for OCI-AML-20 were best for maintaining CD34 expression and allowing for substantial proliferation of the cells.

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