H61

Detection of Clonal Immunoglobulin and T-cell Receptor Gene Rearrangements in Acute Myeloid Leukemia

Ying Huang Ph.D[1], Zhiyi Xie Ph.D[1], Austin Jacobsen[1], Duy Duong[1], Jeff Panganiban[1], Wenli Huang[1], Bradley Patay M.D.[1], Daniela Hubbard[2], Gillian Pawlowsky[3], Jordan Thornes[2,3], Jeffrey E. Miller Ph.D[1,2,3] and Tim Stenzel M.D., Ph.D[1]

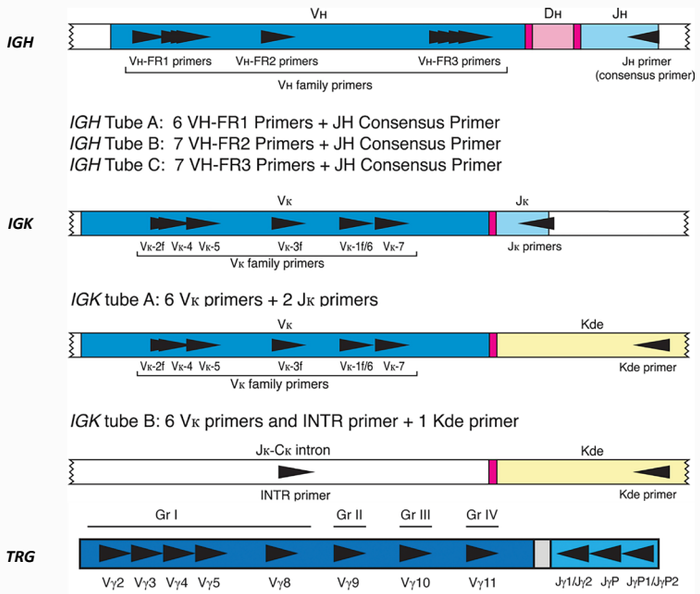
[1]Invivoscribe, Inc., San Diego, USA, [2] LabPMM LLC, San Diego, CA, and [3]LabPMM GmbH, Martinsried, Germany

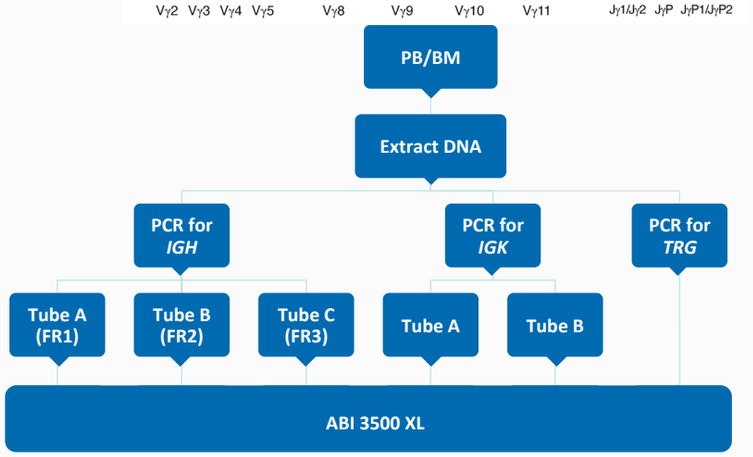
Introduction

Acute myeloid leukemia (AML) carries a high mortality rate and economic burden. Elucidating the heterogeneity of AML will aid in understanding the hematopoietic stem cell (HSC) self-renewal and differentiation. Though AML is classified as a myeloid neoplasm, we were interested in determining the prevalence of clonal rearrangements within the immunoglobulin heavy (*IGH*) and light (*IGK*) chains, as well as the T-cell receptor gamma (*TRG*) loci in AML patient samples.

Materials and Methods

- DNA was extracted from a random sampling of 200 AML anonymized patient residual peripheral blood (PB) or bone marrow (BM) specimens using Qiagen Blood Mini Kit.
- DNA was quantified with NanoDrop and normalized to 10 ng/µL.
- Each DNA sample (50 ng DNA) was tested with 6 different PCR master mixes (MM) from the Invivoscribe Assay kits: IdentiClone® *IGH* Tubes A, B, C, which target the framework (FR) 1, 2, and 3 regions, respectively; IdentiClone® *IGK* Tube A, *IGK* Tube B, and IdentiClone® *TRG* 2.0. Amplicon products were analyzed using the ABI 3500 XL instrument. Based on the florescent signals, clonal (positive) or polyclonal (negative) were assessed.





Results

- The clonal (Pos), polyclonal (Neg) and not testable (N/A) rate detected by different PCR MM for 200 AML samples. Combining multiple PCR MM increased positive detection rate.

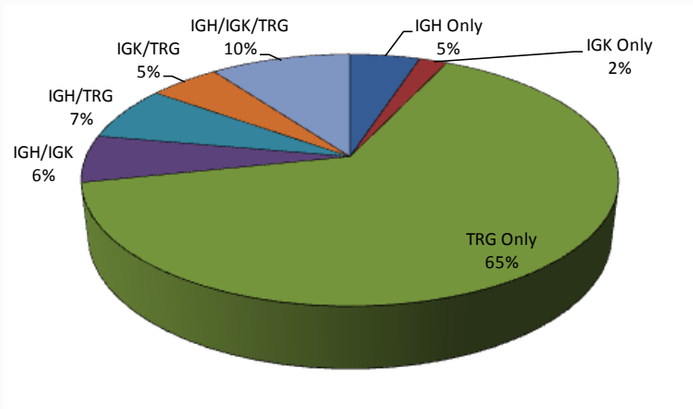
Results	IdentiClone *IGH*				IdentiClone *IGK*					
	Tube A (FR1)	Tube B (FR2)	Tube C (FR3)	*IGH* Overall	Tube A	Tube B	*IGK* Overall	*IGH*+*IGK* Overall	*TRG* 2.0	*IGH*+*IGK* +*TRG* Overall
Pos	23 (12%)	14 (7%)	16 (8%)	28 (14%)	17 (9%)	11 (6%)	23 (12%)	35 (18%)	85 (43%)	99 (50%)
Neg	121 (61%)	81 (41%)	181 (91%)	172 (86%)	176 (88%)	175 (88%)	175 (88%)	165 (83%)	114 (57%)	101 (51%)
*N/A	56 (28%)	105 (53%)	3 (2%)	0 (0%)	7 (4%)	14 (7%)	2 (1%)	0 (0%)	1 (0.5%)	0 (0%)
Total	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)	200 (100%)

* N/A: not amplifiable

- The concurrent positive rates for different PCR MM combinations

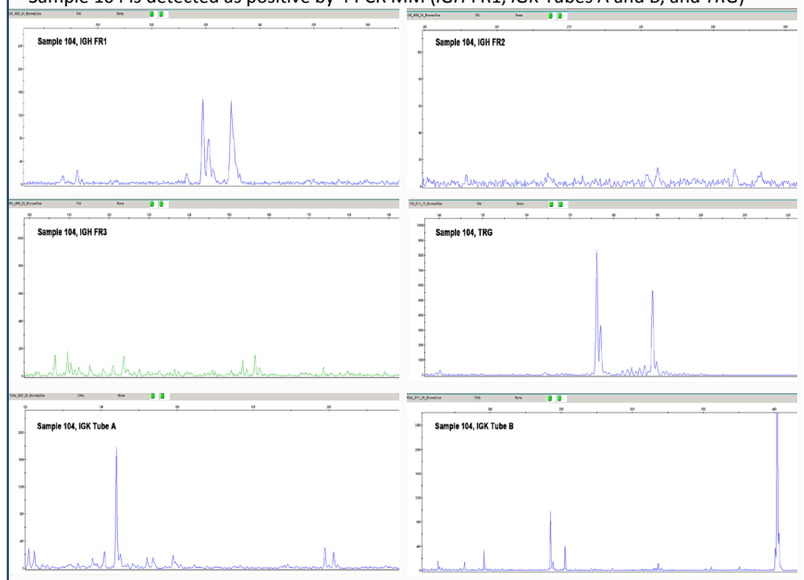
Concurrent Positive Rate			
IGH (FR1+FR2+FR3)	*IGK* (Tube A +Tube B)	*IGH* (FR1+FR2+FR3) + *IGK* (Tube A + Tube B)	*IGH* (FR1+FR2+FR3) + *IGK* (Tube A + Tube B) + *TRG*
8/28 (29%)	5/23 (22%)	1/35 (3%)	1/99 (1%)

- Among the 99 positive (clonal) samples, the exclusive rate by specific target combinations.

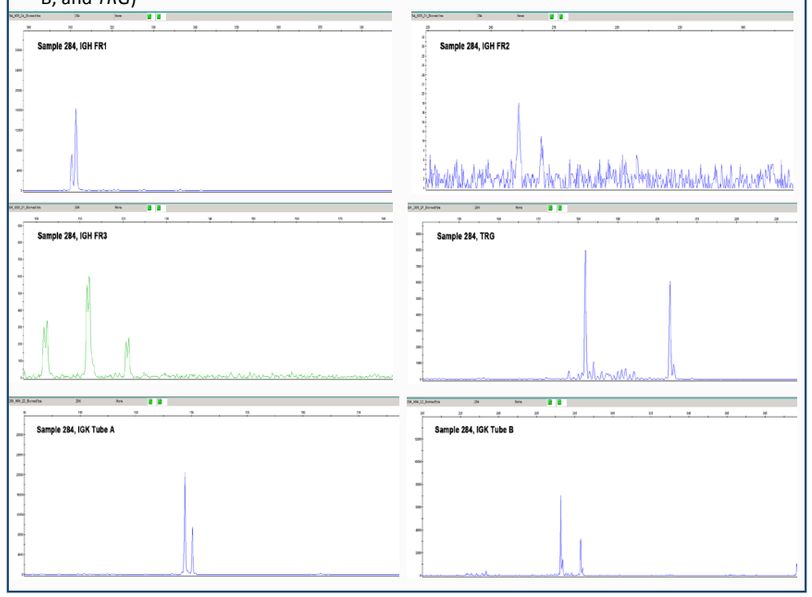


Results

- Sample 104 is detected as positive by 4 PCR MM (*IGH* FR1, *IGK* Tubes A and B, and *TRG*)



- Sample 284 is detected as positive by all 6 PCR MM (*IGH* FR1, FR2 and FR3, *IGK* Tubes A and B, and *TRG*)



Conclusions

- 200 AML samples were tested for clonal rearrangements within the immunoglobulin heavy (*IGH*) and light (IGK) chains, and the chain (*IGK*), T-cell receptor gamma (*TRG*) loci.
- Approximate 50% of AML samples demonstrated at least one clonal *IGH* or *TCR* gene rearrangement.
- While it is unclear if it is the malignant myeloid cells or companion lymphoid cells that harbor these somatic gene rearrangements, the relatively high percentage of clonal rearrangements, and their potential for monitoring in AML makes this an area worthy of further investigation.