

SUPPLEMENTARY MATERIALS AND METHODS

CONSTRUCTION OF EXPRESSION VECTOR AND TRANSFECTION

IGKV1-5*03/IGKJ3*01 full length sequence (with C region) with 10 mutations found in AML patients were cloned into the pcDNA3.1 myc-His (-) B vector (Invitrogen) using the primer sets BamHI- IGKV1-5*03 (5'-GGATCCATGGTGTTCAGACCCAGGTCTT-3') and IGKJ3*01-HindIII (5'-AAGCTTCCACACTCTCCCCTGTTGAAGCTC T-3'). For overexpression of IGK, the plasmid containing IGKV1-5*03/IGKJ3*01 sequence was transfected into HL-60 and NB4 cell lines by electroporation according to the manufacturer's instructions (Nucleofector 2b Device, Lonza, Switzerland).

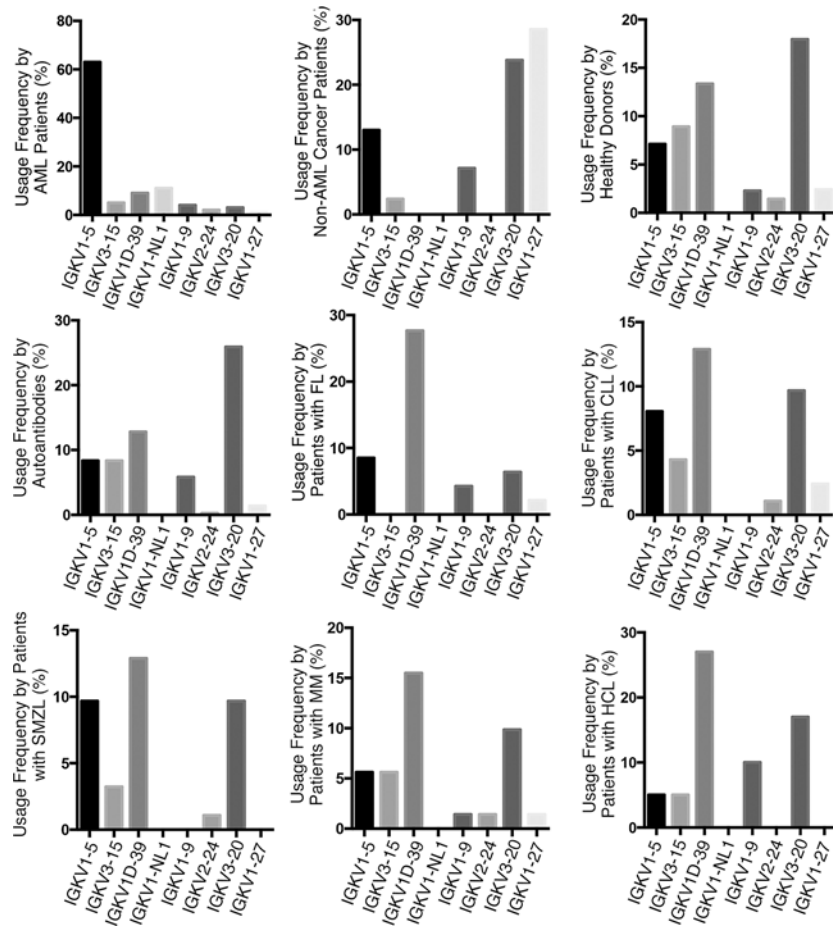
KNOCKDOWN OF IGK EXPRESSION BY SIRNA

siRNAs that target IGK C region sequence and non-specific siRNA (siNC, used as a negative control) were synthesized by Shanghai GenePharma Company (Shanghai, China). The sequences were as follows: siRNA-1, 5'-UCACAGAGCAGGACAGCAA-3'; siRNA-2, 5'-AGGCCAAAGUACAGUGGAA-3'; and siNC, 5'-GUAUGACAACAGCCUCAAGTT-3'. For knockdown of IGK expression, siRNA or siNC was transfected into HL-60 and NB4 cell lines by electroporation according to the manufacturer's instructions (Nucleofector 2b Device).

A IGKV1-5*03/IGKJ3*01
 <---FWR1--><CDR1><-----FWR2-----><C><-----FWR3-----><-CDR3><---JK3--->
 Germline GDRVITICRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPSRFRSGSGSGTEFTLTISLQPDDEFATYYCQQYNSWIFTFGPGTKVDIK
 Patient 1 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 2 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 3 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 4 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 5 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 6 ..K.I.S.....D...L.....M..V.T.Q.....ST.....
 Patient 7 ..K.I.S.....D.....M..V.T.Q.....ST.....
 Patient 8 ..R.I.SR.....D.....M..V.T.Q.....ST.....

B IGKV1-NL1*01/IGKJ5*01
 <---FWR1--><CDR1><-----FWR2-----><C><-----FWR3-----><-CDR3><---JK5--->
 Germline GDRVITICRASQGISNSLAWYQQKPGKAPKLLLYAASRLESGVPSRFRSGSGSGTDYTLTISLQPEDFATYYCQQYYSTPITFGQGRLEIK
 Patient 1D..T.V...HR.....G.....N.....A.....H.....
 Patient 2D..T.V...HR.....G.....N.....A.....H.....
 Patient 3D..T.V...HR.....G.....N.....A.....H.....
 HELD..T.V...HR.....G.....N.....A.....H.....
 NB4D..T.V...HR.....G.....N.....A.....H.....
 KG-1D..T.V...HR.....G.....N.....A.....H.....

Supplementary Figure S1: Alignment of IGK sequences with IGKV1-5*03/IGKJ3*01 and IGKV1-NL1*01/IGKJ5*01 rearrangements from AML patients and AML cell lines and the germline sequence.



Supplementary Figure S2: Comparison of IGKV usage frequency among patients with acute myeloid leukemia (AML), non-hematopoietic neoplasms (non-AML), autoimmune disease, follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), splenic marginal zone lymphoma (SMZL), multiple myeloma (MM), hairy cell leukemia (HCL), and healthy donors.