1946 Clonotype- naive Detection Of Clonality in Patients Suspected of Having Multiple Myeloma or Monoclonal Gammopathy Using Peripheral Blood Cell-Free RNA (cfRNA)

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INTRODUCTION

here is no data on using cell-free RNA (cfRNA) in detecting clonality. Since plasma cells contain unproportional quantities of immunoglobulin RNA for the synthesis and secretion of immunoglobulin, using RNA in the detection of B-cell clonality is particularly relevant. This higher sensitivity in testing is needed when there is no prior knowledge of the specific clonotype expressed in multiple myeloma. Furthermore, using peripheral blood cfRNA in screening for B-cell clonality in patients with multiple myeloma (MM) has the potential of replacing the need for bone marrow biopsies for both the diagnosis and the monitoring of patients with MM.

AIM

We explores the potential of using peripheral blood cfRNA in detecting heavy chain and light chain clonality using next generation sequencing (NGS). We tested patients suspected of having MM, Waldenstrom macroglobulinemia (WM) or monoclonal gammopathy of uncertain significance (MGUS) and compared with normals and patients with xlonal hematopoiesis of indeterminate potential (CHIP)

METHOD

The cfRNA was sequenced using a 1600-gene targeted RNA next generation sequencing (NGS) panel that included all immunoglobulin heavy chain (IgH), Kappa (IgK), and Lambda (IgL) genes, and all T-cell receptors alpha (TRA), beta (TRB), and gamma (TRG) genes. Sequencing was performed using the Illumina NovaSeq 6000 instrument.

RESULTS

Establishing a cut-off point for B-cell and T-cell clonality

Most dominant : second most dominant = 10

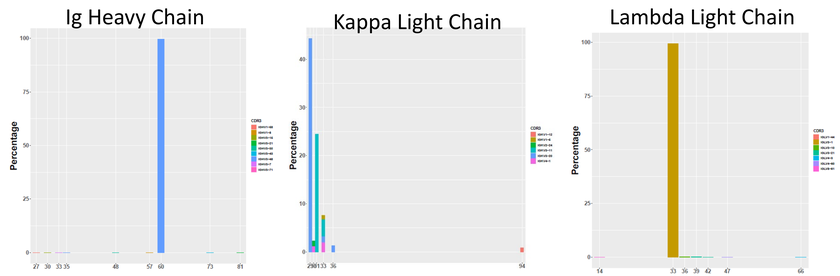
Analyzing expression data of various Immunoglobulin and T-cell receptors in cfRNA of 430 normal individual showed relative ration of the most dominantly expressed clonotype to the second most dominantly expressed clonotype of 10 to be reliable ration of separating normals from patients with known lymphoid neoplasms. Using this criteria showed that 100% of these normal individuals were negative for clonality.

Clonality Using cfRNA Expression of Heavy and Light chain

Clonality was detected in

- 44 (9%) of the 502 patients with CHIP
- 35 (44%) of 80 tested patients suspected of MM, MGUS or WM.
- 6 of the 25 showed clonality in light chain only
- 7 (78%) of 9 WM
- 23 (43%) of 53 MM
- 5 (28%) of 18 MGUS

Example of Clonality using cfRNA with multiple myeloma



CDR3	Counts
IGHV3-48	**256653**
IGHV1-58	65
IGHV3-21	33
IGHV3-71	29
IGHV3-7	27
IGHV3-15	20
IGHV3-30	19
IGHV3-48	19
IGHV1-8	18
IGHV3-43	15

CDR3	Counts
IGKV3-20	471
IGKV3-11	260
IGKV3-11	38
IGKV4-1	21
IGKV3-20	14
IGKV3-20	13
IGKV4-1	13
IGKV2-24	11
IGKV1-12	9
IGKV1-5	9

CDR3	Counts
IGLV3-1	**90463**
IGLV3-10	50
IGLV3-21	49
IGLV8-61	26
IGLV4-3	18
IGLV3-21	14
IGLV4-60	13
IGLV1-44	11
IGLV3-21	10
IGLV3-21	9

Clonality by Mutation profile

Mutations in plasma cells/B-cell-specific genes (MYD88, KRAS, and NRAS…) were detected in :

- 64 (80%) of 80 tested patients suspected of MM, MGUS or WM.
- 9 of 9 (100%) WM
- 44 (83%) of 53 MM
- 11 (61%) of 18 MGUS

Clonality using both Immunoglobulin and Mutations:

Clonality was detected in 78 (98%) of 80 patients suspected of having MM, MGUS or WM.

Levels of top 10 cfRNA biomarkers distinguishing between various diseases:

	Normal (TPM)	MM (TPM)	Log P-Value
HIPK1	6.54	5.82	-5.18
MDM4	4.39	3.68	-4.27
FANCL	5.32	3.91	-4.11
USP7	5.67	5.11	-4.09
CD247	4.24	3.27	-4.07
NSD3	5.81	5.31	-4.05
EML4	5.22	4.60	-3.98
PCM1	5.71	5.13	-3.87
SETD2	6.13	5.75	-3.82

	Normal TPM)	Lymphoid NOS (TPM)	Log P-Value
IGLL5	5.01	3.57	-12.89
DPP4	3.45	2.64	-10.40
HGF	3.35	2.66	-10.35
FANCI	4.06	3.59	-10.31
FLNA	7.58	8.67	-10.29
EGF	4.87	5.80	-10.12
FLT3	3.44	2.85	-9.78
TRAF2	2.64	1.68	-9.78
HDAC5	5.07	5.30	-9.73

CONCLUSIONS

- In the absence of prior tissue-based information, liquid biopsy and cfRNA expression levels of heavy and light chain immunoglobulin are reliable in confirming clonality.
- Evaluating immunoglobulin clonality should be combined with mutation profile to obtain optimal clonality evaluation.
- Tumor-informed testing should be used, whenever possible, to maximize the sensitivity of cfRNA/cfDNA testing.

CONTACT INFORMATION

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