Expression Profiling of mRNA By Next Generation Sequencing and the Development of Algorithm for Predicting Response in Acute Myeloid Leukemia

#1314

Maher Albitar, MD[1], Marina Y Konopleva, MD, PhD[2], Hong Zhang, PhD[3*], Ivan De Dios, BS[4*], Wanlong Ma[4*], Spiraggelos Antzoulatos[4*], Steven M. Kornblau, MD[5], Antonio Cavazos[6*], Sanam Loghavi, MD[7], Koichi Takahashi, MD[8], Hagop M. Kantarjian, MD[9] and Courtney D. DiNardo, MD, MSc[10]

[1]Genomic Testing Cooperative, Valley Center, CA. [2]Department of Leukemia, MD Anderson Cancer Center, Houston, TX. [3]Department of Computer Science, Georgia Southern University, Savannah, Georgia. [4]Genomic Testing Cooperative, Irvine, CA. [5]Department of Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, TX. [6]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX. [7]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX. [8]Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX. [9]M.D. Anderson Cancer Center, Houston, TX. [10]Department of Leukemia, UT MD Anderson Cancer Center, Houston, TX.

Introduction

The principle of precision cancer medicine is to customize therapy based on the genomic profiles of the cancer and the host constitution/response to the cancer. Since RNA expression is influenced by many genetic mechanisms, RNA profiling may provide broader coverage of genomic changes and might be a better predictor of response to therapy. However, incorporating the many biological changes of the host and the cancer in the decision of selecting therapeutic approach is not practical without using computer-aided algorithms. This is particularly relevant when combination therapy is used. We explored the potential of developing algorithms for the prediction of complete response (CR) to novel combination therapy in patients with acute myeloid leukemia (AML) using targeted RNA expression profiling.

Methods

Samples and patients:

Methods: RNA was extracted from the peripheral blood (PB) and bone marrow (BM) samples from patients with AML being treated on two different protocols: FLAG-IDA+venetoclax (F-I-V)(Abou Dalle I et al, ASH 2019; the NCT # is NCT03214562) and ivosidenib+venetoclax (I-V). In the initial study, 22 samples (9 PB and 13 BM) were used as training set. Subsequently 16 PB samples from the F-I-V arm and 4 from the I-V arm were collected and tested blindly as testing set after the development and locking of the algorithm.

	FLAG+IDA+Venetoclax (# 26)	Ivosidenib+Venetoclax (#14)
Age: Median (range)	45 (20-79)	67 (37-83)
Gender: Male	16	7
No prior therapy	9	3
Previously treatment: Median (range)	2 (1-6)	1 (1-4)
WBC: Median (range)(10^9/L)	3.35 (1-20)	1.9 (1-10.5)
Hgb Median (range)(g/dL)	8.55 (7-11)	8.7 (7.7-13.1)
Platelets: Median (range)(10^9/L)	32 (7-178)	56 (6-275)
PB Blasts Median (range)(%)	19.5 (0-78)	9.5 (0-93)
BM blasts Median (range)(%)	45 (2-94)	36.5 (1-72)
Cytogenetics: Good/Intermediate/poor	3/17/6	1/11/2

DNA and RNA Extraction:

The Agencourt FormaPure Total 96-Prep Kit is used for extracted both DNA and RNA from formalin fixed paraffin embedded human tissue. The Agencourt FormaPure Kits allows us to use a split protocol for extracting both RNA and DNA from the same FFPE lysate.

RNA Library Construction and Sequencing

Sample are selectively enriched for 1408 cancer-associated genes using reagents provided in an Illumina® TruSight® RNA Pan-Cancer Panel. Sequencing is performed on Illumina NextSeq 550. Expression levels are measured using Cufflinks FPKM.

Development of an algorithm for predicting CR

The RNA levels were first normalized to ABL1 mRNA levels. Each gene is normalized by the mean and standard deviation of the gene. in each arm and first evaluated the performance of each of the 1408 genes using receiver operating characteristic (ROC). The top genes in predicting CR are listed in the tables below.) curve

AG120+Venetoclax

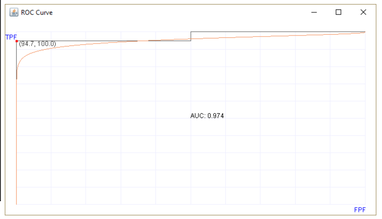
Sensitivity	Specificity	AUC	Gene
0.95	1.00	0.96	**STL**
0.95	1.00	0.98	**TNFRSF10D**
0.89	1.00	0.91	**PTGS2**
0.89	1.00	0.93	**RET**
0.89	1.00	0.95	**TFRC**
0.84	1.00	0.97	**NAV3**
0.84	1.00	0.95	**WSB1**
1.00	0.83	0.91	**GAS1**

FLAG-IDA+Venetoclax

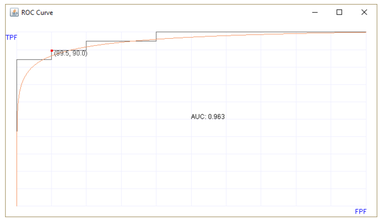
Sensitivity	Specificity	AUC	Gene
0.84	0.90	0.85	**GLI3**
0.89	0.80	0.81	**SETBP1**
0.84	0.80	0.74	**SH3D19**
0.89	0.70	0.82	**ARHGAP20**
0.79	0.80	0.68	**ETS1**
0.79	0.80	0.77	**IKZF2**
0.68	0.90	0.78	**GNG4**
0.68	0.90	0.74	**MAGEE1**

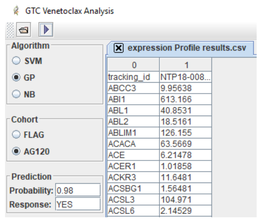
Using top 8 genes, high accuracy in predicting CR is achieved using SVM or Bayesian statistics.

AG120+Venetoclax



FLAG-IDA+Venetoclax





We developed a software for predicting CR with options to use Support Vector machine (SVM), Bayesian modeling with Gaussian Processes (GP), and Naïve Bayesian (NB). The system is used for the prediction of CR in prospective studies of 16 patients. This included 12 patients treated with GALG-IDA+venetoclax and 4 patients treated with ivosidenib+venetoclax. Since the original study showed that peripheral blood was similar to bone marrow in predicting CR, only peripheral blood is used in the prospective study.

Results

Prediction results using the entire 1408 genes are shown in this table.

			Support Vector machine (SVM)		Bayesian modeling with Gaussian Processes (GP)		Naïve Bayesian (NB)	
		Clinical Response	Probability	Predicted Response	Probability	Predicted Response	Probability	Predicted Response
FLAG-IDA + Venetoclox	MDA1	CR	0.81	YES	0.64	NO	1	YES
	MDA2	NR	0.73	NO	0.5	NO	0	NO
	MDA4	CR	0.77	YES	0.57	NO	1	YES
	MDA6	CR	0.89	YES	0.8	YES	1	YES
	MDA7	CR	0.93	YES	0.86	YES	1	YES
	MDA8	CR	0.75	YES	0.54	NO	1	YES
	MDA9	CR	0.89	YES	0.79	YES	1	YES
	MDA11	NR	0.73	NO	0.5	NO	0	NO
	MDA12	NR	0.73	NO	0.5	NO	0	NO
	MDA13	NR	0.73	NO	0.5	NO	0	NO
	MDA14	NR	0.73	NO	0.5	NO	0	NO
	MDA15	CR	0.73	NO	0.5	NO	0.03	NO
	MDA16	CR	0.88	YES	0.77	YES	1	YES
	MDA18	CR	0.81	YES	0.64	NO	1	YES
	MDA19	CR	0.8	YES	0.63	NO	1	YES
	MDA20	CR	0.91	YES	0.85	YES	1	YES
ivosidenib + venetoclax	MDA21	NR	0.99	YES	0.94	YES	0	NO
	MDA23	CR	0.61	NO	0.65	NO	1	YES
	MDA24	CR	1.09	YES	0.95	YES	1	YES
	MDA26	CR	0.55	NO	0.6	NO	1	YES

Conclusion

-Although more data is needed, using RNA expression profiling by targeted RNA sequencing can provide an excellent tool for customizing therapeutic approach, especially combination therapy.
- Nave Bayesian is reliable approach in developing prediction algorithms.