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Determination of drug sensitivity subgroups in acute myeloid leukemia to increase treatment efficacy by imatinib and its analog imatinib B

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Abstract

While chemotherapeutics without any selective properties were used in cancer treatment in the past, they are now replaced by targeted drugs. Imatinib has common use in the treatment of acute lymphoid leukemia (ALL) and chronic myeloid leukemia (CML) but not Acute myeloid leukemia (AML). It is a highly effective oral drug treatment and provides stable remission in many people treated. Treatment of imatinib mesylate can cause various side effects such as swelling around the eyes, nausea, and vomiting. However, it is likely to act on normal cells, and this effect causes side effects. To create a better design with increased bioactivity with decreased possible side effects of imatinib we calculated the molecular pharmaceutical properties and biological activity and investigated its analogs. Also, we figured out which genetically subclasses of AML can benefit from therapy with imatinib analog, imatinib B. The gene expression data were retrieved from the E-MTAB-783 Cancer Genome Project database. Gene expression data for available genes in cell lines belonging to various types of leukemia were chosen for use in the analysis. Three subgroups were determined using all genome expression profiles and drug sensitivity data of AML cell lines associated with imatinib sensitivity. Genes with statistical significance between groups, network connections, and related pathways to which they belong were introduced. Thirty-two genes and 18 pathways that can predict sensitivity to imatinib in AML cell lines were identified. Also, our results show these genes are closely linked with each other. Today, the use of Imatinib in the treatment of AML is limited. By redesigning the imatinib molecule, it will be possible to effectively treat certain AML subgroups by reducing harmful side effects.

Keywords: Bioactivity, drug-likeness, drug design, boron, biomarker, imatinib

Introduction

TLeukemia is commonly referred to as the growth of blood cells or blood-producing cells in the bone marrow by uncontrolled division [1, 2]. This is caused by damage to their DNA during the development of these blood cells. This damage generally affects white blood cells and leukocytes [3]. As a result of the researches carried out by the National Cancer Institute in 2019, it is estimated that approximately 61.780 people have been diagnosed with leukemia and that leukemia may cause 22.840 deaths in the same year [4].

Acute myeloid leukemia (AML) is one of the most common types of leukemia which prevents the normal functioning of blood cells in the body, can occur at any age. But, in general, this type occurs more often in adults. The most common feature of acute leukemia is to develop very quickly, causing the patient to worsen faster. Treatment options for acute myeloid leukemia cancer are available [5, 6].

Imatinib is specifically a selective tyrosine kinase inhibitor of the C-kit, BCR/ABL, and platelet-derived growth factor - receptor (PDGF-R). C-kit (CD117) expression is observed in patients with more than 80% acute myeloid leukemia. Imatinib drug inhibits C-chitin tyrosine kinase activity, creating a potential treatment method for patients with acute myeloid leukemia [7-10]. Imatinib has common use in the treatment of acute lymphoblastic leukemia (ALL) [1] and chronic myelogenous leukemia (CML) but not the AML because of some observed side effects of imatinib therapy in AML patients. In the present study, we calculated the molecular pharmaceutical properties and biological activity of Imatinib and investigated its analogs to create a better design with increased bioactivity and with decreased possible side effects. For that, we switched the methyl group CH3 with Boron bio isostere B(OH)2 named Imatinib B (shown in Figure 1) and predicted its bioactivity score against the most important drug targets reported in the

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literature [11]. By using the imatinib data reported, we tried to figure out which genetically subclasses of AML can benefit from therapy with imatinib analog, imatinib B with the more promising clinical result.

Various medications, such as imatinib, are used to treat chronic and acute leukemias. However, derivatives of newly approved drugs or existing drugs are needed to control this disease [11].

In addition to the critical information that has been specified, gene biomarkers also play an important role in understanding the molecular basis leading to AML cancer, prognosis, diagnosis, and determining the treatment to be administered [12]. Biomarkers have characteristic biological properties that can be detected and measured in parts such as tissue or blood in the body [13]. Thanks to their characteristic features, they help identify functions, specific changes in pathways, genes, complex organs. In the light of the determined gene profiles, tumor genotypes, and molecular functions, it is possible to find personalized cancer treatment routes and to predict the risk of the disease [14-17].

Material and Methods

Drug molecule selection

From the data of our institutional clinics of Hacettepe University, we have resulted in eight chemotherapy drugs as listed: ATRA (all-trans-retinoic acid), Cytarabine, Dasatinib, Doxorubicin, Etoposide, Imatinib, Methotrexate, and Nilotinib. We have investigated their pharmaceutical parameters using their molecular physicochemical properties. From the list, each drug molecule showed one or more violations of drug-likeness criteria commonly used in the drug design community except Cytarabine, Dasatinib, and Imatinib. Dasatinib was excluded because of its final renewal of FDA approval. Dasatinib was found to be stopped its FDA approval in 2016, however, Imatinib approval was renewed in March 2019. So, we continued with Imatinib to search for a better design drug molecule.

Different design compounds were created using bioisosterism concept, molecular editing programs, and calculating their Physico-chemical properties by Molinspiration to investigate drug-likeness parameters of new design compounds.

Physico-chemical properties

The molecular structural properties of Imatinib and its analogs were calculated using the smiles code given in Table 1. Its 2D structure given in Figure 1 was drawn using the free online service of cheminformatics and bioinformatics software Molinspiration, https://www. molinspiration.com/. The results were summarized in Table 2.

Table 1. Canonical SMILES code, the first and the last year of approval, and 2D structure of Imatinib.

	Year of the app.	SMILES
Imatinib	**2001-2019**	CN1CCN(CC2=CC=C(C=C2)C(=O)NC2=C-C(NC3=NC=CC (=N3)C3=CN=CC=C3)=C(C) C=C2)CC1

Table 2. Molecular pharmaceutical parameters of some chemotherapy drugs. MiLog P is the logarithm of partition coefficient, MW molecular weight in the unit of gr/mol, nOH the number of H bond acceptors, nOHNH the number of H bond donors, nvio the number of drug-likeness violation of the drug molecule. The properties were calculated using Molinspiration.

Drug Name	miLog P	TPSA	MW (gr/mol)	nOH	nOHNH	nvio.
ATRA	5.80	37.30	300.4	2	1	1
Cytarabine	-1.93	130.8	243.2	8	5	-
Dasatinib	3.13	106.5	488.0	9	3	-
Doxorubicin	0.57	206.0	543.5	12	7	3
Etoposite	0.70	160.8	588.5	13	3	2
Imatinib	3.89	86.2	493.6	8	2	-
Methotrexate	-1.97	210.5	454.4	13	7	2
Nilotinib	4.99	97.6	529.5	8	2	1
Nilotinib	4.99	97.6	529.5	8	2	1

Biological activity

Computational bioactivity score of imatinib across the most important drug targets G-protein coupled receptor (GPCR), kinase, ion channel modulators (ICM), protease, and enzyme inhibitors was evaluated using Molinspiration, and the results were tabulated in Table 3. The working principle of the program is based on a color code. The scores higher than 0.2 and 0.5 are, respectively, considered as moderate and high biological activity against the drug target. The more negative score the molecule has, the much farther away the molecule is from the bioactive functionality (Table 4).

Table 3. Bioactivity score of Imatinib and its analog Imatinib B on the most important drug targets, GPCR (G-protein coupled receptors,) ICM (ion channel modulator), kinase inhibitors, NRL (nuclear receptor ligands), protease, and enzyme inhibitors.

Drug name	GPCR ligand	ICM	Kinase inhibitor	NRL	Protease inhibitor	Enzyme inhibitor
Imatinib	0.10	-0.09	0.59	-0.40	-0.08	0.07
Imatinib B	0.14	-0.14	0.61	-0.02	0.33	0.54

Table 4. Pathway analysis of differentially expressed genes.

MPG	N-methylpurine DNA glycosylase(MPG)
KEGG_PATHWAY	Base excision repair
RAD54L	RAD54-like (S.cerevisiae)(RAD54L)
KEGG_PATHWAY	Homologous recombination
ACSL6	acyl-CoA synthetase long-chain family member 6(ACSL6)
KEGG_PATHWAY	Fatty acid biosynthesis, Fatty acid degradation, Metabolic pathways, Fatty acid metabolism, PPAR signaling pathway, Peroxisome, Adipocytokine signaling pathway
CSF1R	colony stimulating factor 1 receptor(CSF1R)
KEGG_PATHWAY	Ras signaling pathway, Rap1 signaling pathway, Cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, Osteoclast differentiation, Hematopoietic cell lineage, Pathways in cancer, Transcriptional misregulation in cancer
GRID2	glutamate ionotropic receptor delta type subunit 2(GRID2)
KEGG_PATHWAY	Neuroactive ligand-receptor interaction, Long-term depression
MAN1B1	mannosidase alpha class 1B member 1(MAN1B1)
KEGG_PATHWAY	N-Glycan biosynthesis, Metabolic pathways, Protein processing in the endoplasmic reticulum
PNLIPRP2	pancreatic lipase related protein 2(gene/pseudogene)(PNLIPRP2)
KEGG_PATHWAY	Glycerolipid metabolism, Metabolic pathways, Pancreatic secretion, Fat digestion, and absorption
PLN	phospholamban(PLN)
KEGG_PATHWAY	Calcium signaling pathway, cGMP-PKG signaling pathway, cAMP signaling pathway, Adrenergic signaling in cardiomyocytes, Thyroid hormone signaling pathway, Dilated cardiomyopathy
STX6	syntaxin 6(STX6)
KEGG_PATHWAY	SNARE interactions in vesicular transport

Obtaining gene expression data and drug cytotoxicity data

The acute myeloid leukemia cell line expression data were obtained from ArrayExpress (E-MTAB-783) [18]. After obtaining 789 cell lines expression data which consist of many different cancer types, seventeen AML cell lines (KMOE2, MONOMAC6, P31FUJ, OCIAML2, KASUMI1, GDM1, NOMO1, ML2, CESS, QIMRWIL, HEL, CTV1, CMK, NKM1, K052, HL60, THP1) were selected to further analysis. In addition to the cell lines expression data, the selected drug, imatinib half-maximal inhibitory concentration (IC50) data were retrieved from the Cancer genome project (CGP) Published data [18].

Data normalization, Linear Regression, Hierarchical Cluster analysis, and identification of potential gene biomarkers

Data were normalized using Robust multichip average (RMA) normalization by utilizing BRB-Array Tool. Furthermore, linear correlation analysis was performed to the normalized data to understand gene expression profiles correlation with imatinib sensitivity of seventeen cell lines by comparing IC50 values. Whole genes with Pearson product-moment correlation coefficient value (r-value) higher than 0.07 as well as a Pearson's correlation coefficient related absolute p-value below 0.01 taken into consideration as valuable biomarkers. Relying on decided biomarkers and utilizing seventeen AML cell lines that were selected before, a hierarchical cluster was conducted with the Euclidian distance method for arrays, genes as well as complete linkage with the aid of Cluster 3.0 software.

Pathway and Network Analysis

Also, we mapped these genes in biological pathways by using DAVID bioinformatics resources 6.8 [19]. Moreover, network-analysis was carried out using Cytoscape 3.7.2 to be able to find the relevant possible co-expression with this determined gene list [20].

Results

Drug-likeness profile

The Drug-likeness profile of a molecule is commonly evaluated in term of the reported criteria of some molecular structural and physicochemical properties such as lipophilicity which is associated with Log P value, molecular weight, molecular polar surface area, and hydrogen bonding capacity which were discussed in more detail in our previous paper [21-23]. Those properties were shown to be closely related to the pharmaceutical dynamics of the molecule [22-24]. Previous studies performed on the approved drug molecules showed that the pharmaceutical performance of drug molecules strongly depends on a certain range of those molecular parameters listed above. Therefore, to start our investigation with the best structure of minimum side effects, we calculated those molecular descriptors for some chemotherapeutic drugs used in our clinics: ATRA (all-trans-retinoic acid), Cytarabine, Dasatinib, Doxorubicin, Etoposide, Imatinib, Methotrexate, and Nilotinib. The results showed that all drugs violated one or more drug-likeness parameters except Cytarabine, Dasatinib, and Imatinib (Table 2). Cytarabine showed a negative log P value of -1.93 indicating a hydrophilic nature. On the other hand, a previous virtual screening study performed on the approved drugs showed that log P values ranged from 3.5 to 4.5 [1]. which means the approved drugs mostly had a hydrophobic nature, so we excluded cytarabine from our list of lead molecules. When we searched for the approval history, we found that the last FDA approval of Dasatinib stayed in 2016 which could be related to intolerable side effects of the molecule. Imatinib, on the other hand, was found to have a stable approval history of FDA showing duration of successful clinical results. Its approval was renewed in 2019, March. For that, we used the 2D structure of imatinib for further structural and pharmaceutical analysis.

Evaluation of bioactivity

By using the concept of bioisosterism, we investigated bioisosteric analogs of imatinib. To predict the computational bioactivity score of imatinib and its analogs, we used the virtual screening engine of Molinspiration working with a training set of bioactive molecules reported in the literature. Previous studies classified the most important drug targets as G-protein coupled receptors (GPCR), kinase inhibitors, ion channel modulators, nuclear receptors, protease, and enzyme inhibitors [11]. Our trials resulted in a promising structure. The scores were shown that a boron incorporated analog of imatinib, imatinib B, shown in Figure 1 exhibited a better bioactivity score than even the approved molecule imatinib. Although Imatinib showed a good activity only on kinase receptor, Imatinib B resulted in a good activity on not only kinase but also on enzyme inhibitor and a moderate activity on protease inhibitor. That shows a considerable meaning serving a popular approach of cancer treatment, known as the multi-targeted approach. The resulting multi-target bioactivity of our compound imatinib B makes it significant to further investigation. From the molecular point of view, we expect from our analog Imatinib B to show a similar, maybe better pharmaceutical behavior than Imatinib. Therefore, we could construct our bioinformatics analysis on the imatinib data to develop a genetic biomarker.

Imatinib B was generated by replacing a methyl group (CH3) with a Boronic acid (B(OH)2) as shown in Figure 1. Investigation of the latest FDA-approved drugs showed that Boron-based molecules gathered increasing interest in the drug development community. For instance, bortezomib has approved by FDA in 2003 first and still in use for the treatment of relapsed multiple myeloma. Tavaborole is another boron-based approved anti-fungal medication. Therefore, our design compound might become another boron-based therapeutic agent.

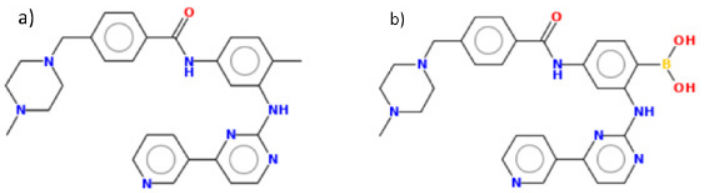

Figure 1. The 2D structure of a) Imatinib and b) boron incorporated analog, Imatinib B

Biomarker gene discovery

To identify individual genes in AML cell lines whose expression profiles matched that of the one generated by cytotoxicity experiments for imatinib, we used a linear regression-based approach, where we searched for statistically significant

correlations between gene expression values and IC50 data. The intersections of the genes were identified in 17 AML cell lines and used for further analysis. Our linear regression model identified 35 probests belong to 32 different genes.

When all 35 gene probesets were used in a hierarchical cluster analysis, three major clusters of cells representing relatively sensitive and resistant cells could be identified, as seen in Figure 2.

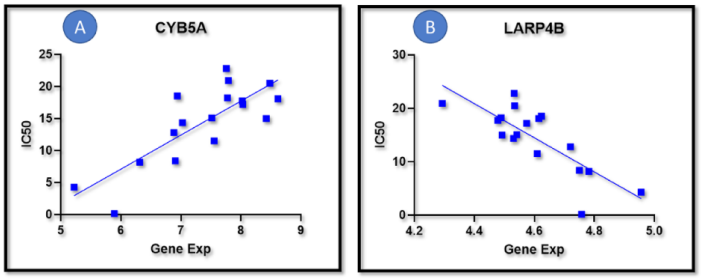

Figure 2. Examples to concordance between gene expression and imatinib IC50 values. (A) positively and (B) negatively correlated genes with imatinib resistance profile in AML cell lines.

Heatmap representing the clustering of 17 AML cell lines based on 35 gene probesets which show a significant association with imatinib chemosensitivity. The average imatinib IC50 values are significantly different between these three groups (Figure 3).

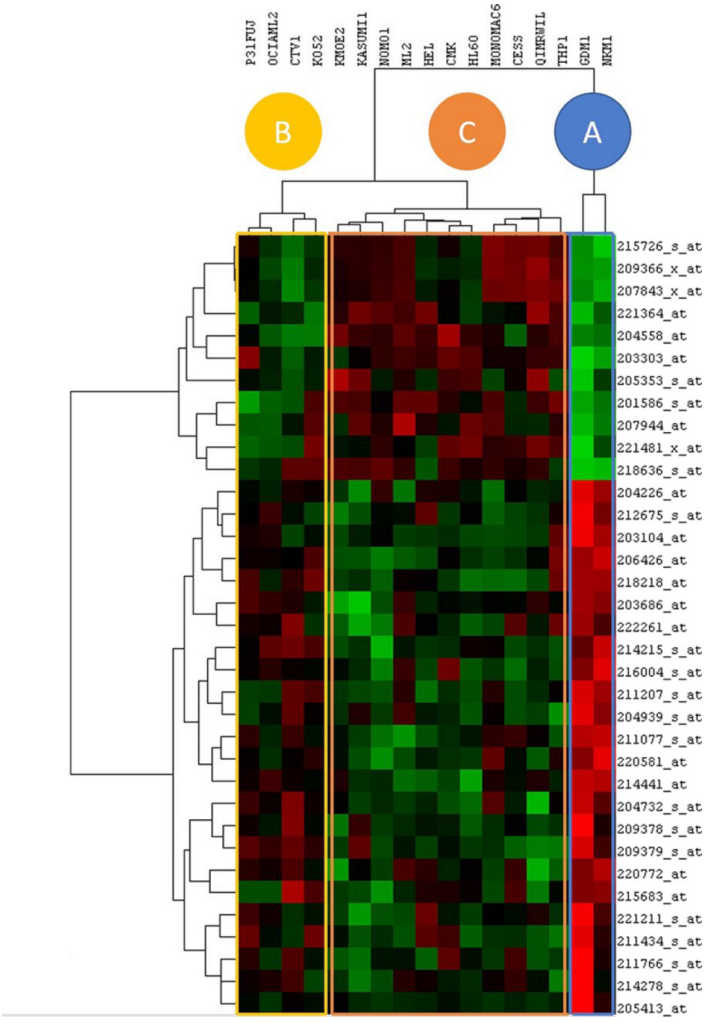

Figure 3. Hierarchical clustering of AML cell lines based on differentially expressed genes

Pathway and network analysis of significantly differentially expressed genes

Co-expression network analysis of all significantly differentially expressed genes is determined. Many of these 32 genes are highly connected in terms of expression.

Table 4 presents the pathways in which these genes are involved, suggesting that other members of these pathway families might have an effect on and responsibility for imatinib in AML.

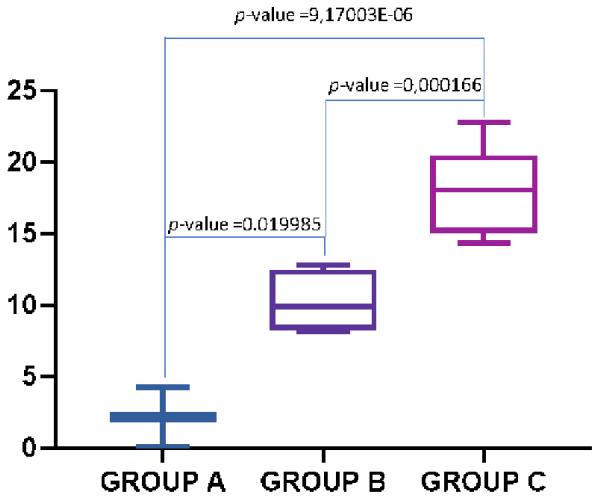

Figure 4. Comparison of average IC50 values between three major clusters of cells representing relatively sensitive and resistant cells.

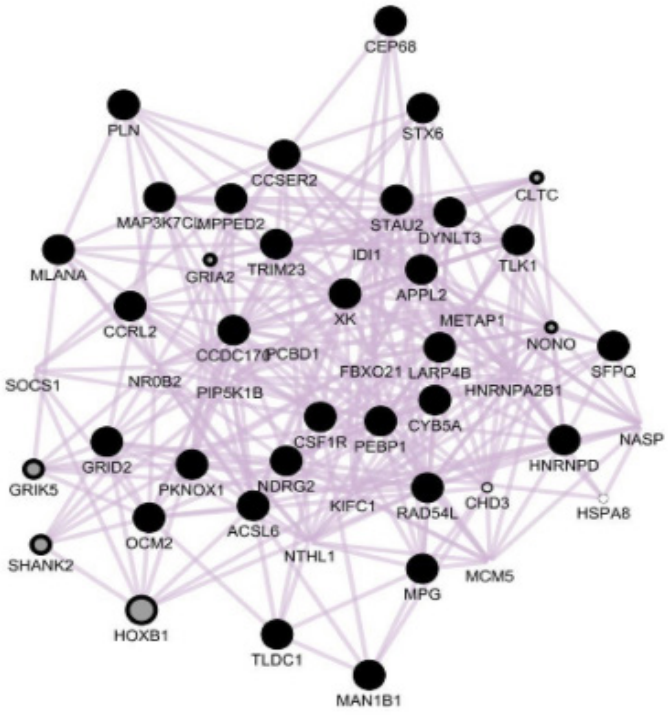

Figure 5. Co-expression network analysis of differentially expressed genes.

Conclusion

Elucidation of the gene expression profiles (GEP) of the protein-tyrosine kinase inhibitors in the pharmaco-biological basis of

AML is extremely important for the clinical activity of anti-AML drugs with regards to effectivity, safety, tolerability, toxicity, and pharmaco-economy. The use of predictive simulation technology seems to be vital in designing therapeutics for targeting novel biological mechanisms using existing or novel chemistry.

AML is a very heterogeneous disease. Genetic changes could play a major role in the prognosis of AML. Molecular analyses using GEP dissected the genetic basis of AML. Although various GEP-based signatures have been reported to identify high-risk leukemia disease and predict prognosis, the inability of GEP to predict clinical response in AML is also evident.

In this study, the hierarchical clustering of AML lines according to imatinib chemosensitivity biomarker genes has been described. The heat map represented the clustering of 3 AML line groups based on 32 genes disclosing either imatinib resistance or less sensitive AML cells. Likewise, the concordances between gene expression and IC50 values of 17 AML cell lines are shown for 32 genes. Furthermore, the relevant biological pathway analyses of the genes whose expressions are concordant with imatinib cytotoxicity were explored via the molecular functional analyses of differentially expressed genes. The genes involved in specific pathways regarding the Bcr-Abl tyrosine-kinase inhibitors, and particularly imatinib, are related to the critical pathological events of acute myeloid leukemia such as the Rap1 signaling pathway as well as RAS signaling. Those pathways are essentially important in the progression of AML such as the peroxisome proliferator-activated receptor signaling pathway. Peroxisome proliferator-activated receptor gamma (PPARγ) a member of this signaling pathway is a multifunctional transcription factor with important regulatory roles in inflammation, cellular growth, differentiation, and apoptosis.

On the other hand, our network analysis showed that 29 genes whose expression could be used to predict chemosensitivity in AML for imatinib have a strong connection in terms of co-expression.

The findings of our present in silico study could be important not only for the understanding of the genomics of AML but also for the better arrangement of targeted anti-leukemia therapies, such as imatinib. Improvement in the understanding of AML pathogenesis will refine the molecular dissection of the disease, especially in the context of novel anti-myeloma drugs affecting the disease course. Genomics, proteomics, transcriptomics, and metabolomics studies should be integrated to understand their significance in the management of AML, as well as to offer better therapeutics and treatment strategies to patients with AML.

As a result of our analysis, we determined genes associated with imatinib sensitivity in AML cell lines. Further in vivo and clinical validation experiments are needed for the clinical application of identified potential biomarker genes.

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Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
The local ethical committee approved our study protocol numbered 104, dated October 2, 2020.

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