

Triagem de drogas *in silico* para Leucemia Linfóide Aguda (LLA)



DataSci4Health – MO413A/MC936A

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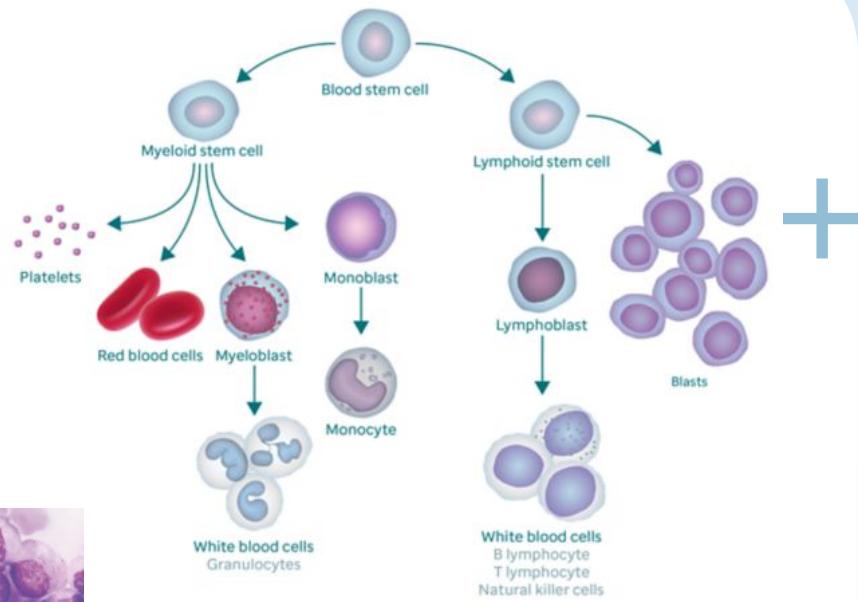
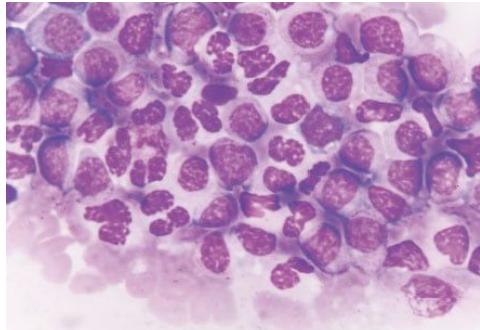


01.

Resumo

Descrição resumida

- Células precursoras de linfócitos B e T;
- Heterogênea;
- Casos de recaída → pior prognóstico;
Medicina de precisão → estratégias terapêuticas individuais.



<https://together.stjude.org/>

Descrição resumida



Redes de interação gênica, com dados de genômica e transcriptômica, para identificar a droga com ação potencial para a rede do paciente.



Adaptada de Davis et al. (2012)



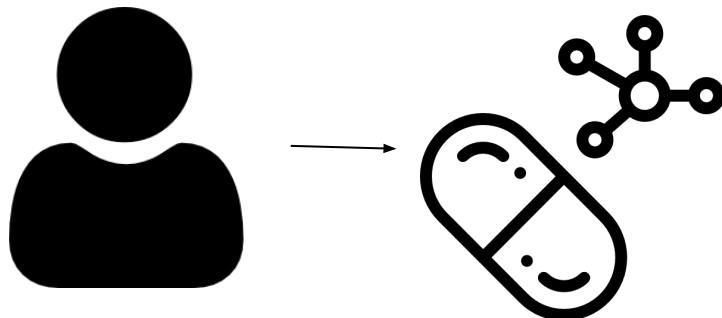
Explorar tratamentos in silico direciona os esforços in vitro e in vivo → diminui custos para o desenvolvimento de alternativas terapêuticas.



02.

Fundamentação

Fundamentação teórica



Dados moleculares de múltiplas ômicas

Letai, A. (2017). Functional precision cancer medicine—moving beyond pure genomics. *Nature Medicine*, 23(9), 1028–1035.
<https://doi.org/10.1038/nm.4389>



Adaptada de Davis et al. (2012)

Aplicações biológicas e clínicas potenciais

Barabási, AL., Gulbahce, N. & Loscalzo, J. Network medicine: a network-based approach to human disease. *Nat Rev Genet* 12, 56–68 (2011).
<https://doi.org/10.1038/nrg2918CV>



03.

Pergunta biológica

Pergunta biológica



As vias moleculares alteradas na LLA se organizam em clusters?

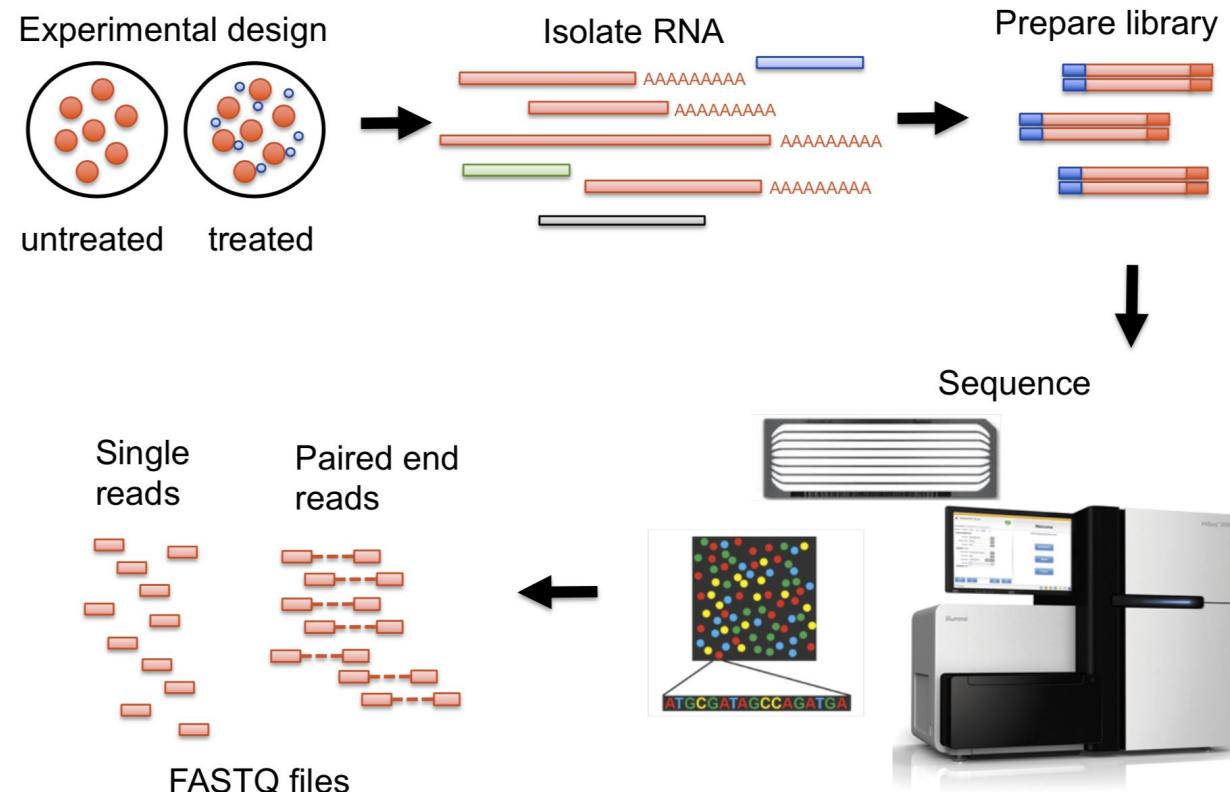


Esses clusters são alvos potenciais para as classes de fármacos usados em LLA que iremos triar in silico?

04.

Dados de RNAseq

Transcriptômica - RNAseq



Transcriptômica - RNAseq

RPKM vs TPM

RPKM

Both TPM
RPKM (and FPKM)
correct for biases in gene
length and sequencing
depth. But....

TPM

Gene Name	Rep1 RPKM	Rep2 RPKM	Rep3 RPKM
A (2kb)	1.43	1.33	1.42
B (4kb)	1.43	1.39	1.42
C (1kb)	1.43	1.78	1.42
D (10kb)	0	0	0.009

Gene Name	Rep1 TPM	Rep2 TPM	Rep3 TPM
A (2kb)	3.33	2.96	3.326
B (4kb)	3.33	3.09	3.326
C (1kb)	3.33	3.95	3.326
D (10kb)	0	0	0.02

Transcriptômica - RNAseq

RPKM vs TPM

Consider 3 pies, each the same size (10).

A 3.33 sized slice is the same in each pie, and is always larger than 3.32.

TPM makes it clear that in Rep1, more of its total reads mapped to gene A than in Rep3.

TPM



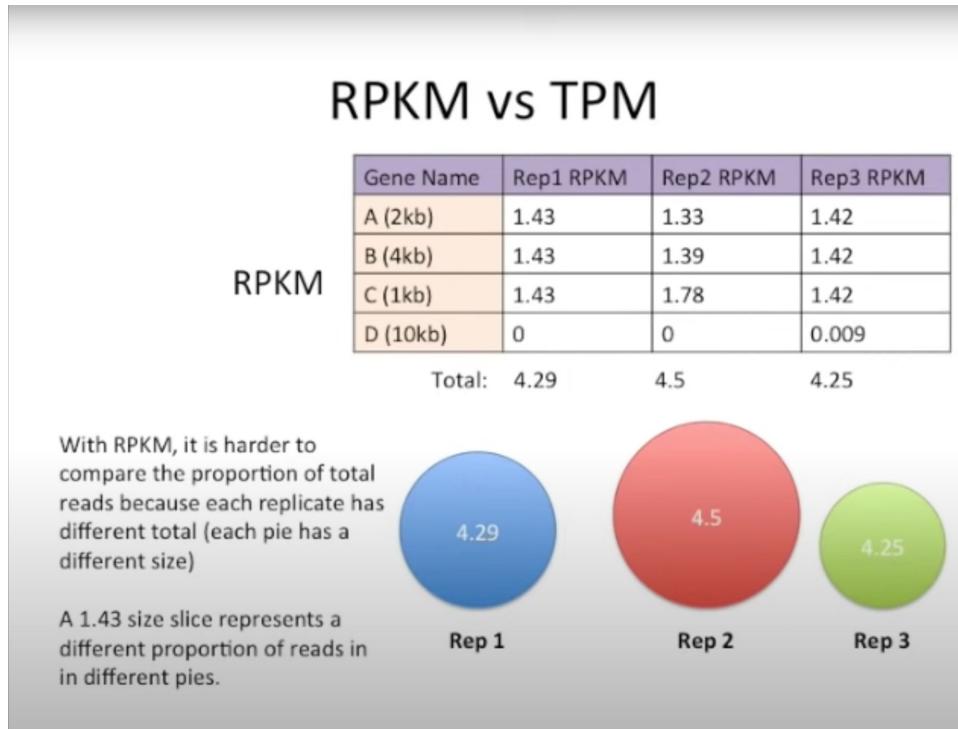
Gene Name	Rep1 TPM	Rep2 TPM	Rep3 TPM
A (2kb)	3.33	2.96	3.326
B (4kb)	3.33	3.09	3.326
C (1kb)	3.33	3.95	3.326
D (10kb)	0	0	0.02

Total: 10

10

10

Transcriptômica - RNAseq



Transcriptômica - RNAseq

Main point: With TPM, everyone gets the same sized pie.

Ha! In all seriousness, folks are using TPM because the numbers can clearly tell you what proportion of reads mapped to what in each sample.

And since RNA-seq is all about comparing relative proportions of reads, this metric seems more appropriate.

TPM



Gene Name	Rep1 TPM	Rep2 TPM	Rep3 TPM
A (2kb)	3.33	2.96	3.326
B (4kb)	3.33	3.09	3.326
C (1kb)	3.33	3.95	3.326
D (10kb)	0	0	0.02

Total: 10 10 10

Transcriptômica - RNAseq

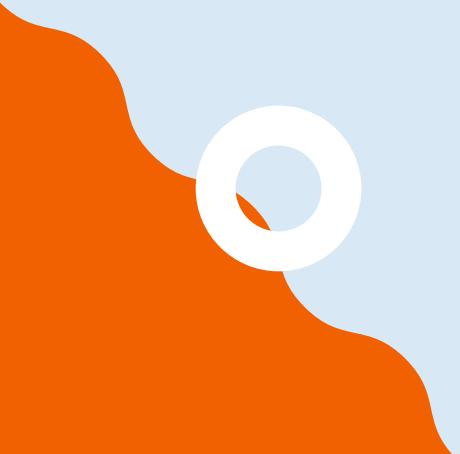
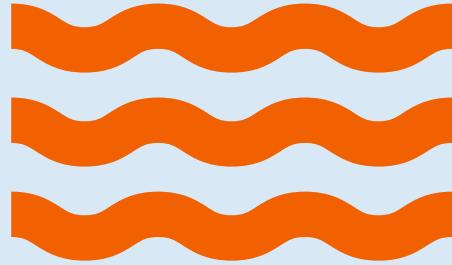
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
	POOR													
gene_id	BCRABL1													
HLA-B	3317.04	4110.27	871.89	1245.51	6138.07	541.5	479.15	3770.78	2486.15	2083.77	3274.07	6053.94	709.53	2904.6
RPL13	2630.97	2275.49	1489.49	534.74	2584	585.97	516.95	2257.45	1168.29	3970.72	1888.04	2293.43	968.46	5339.69
ACTG1	3861.94	1134.99	297.33	242.48	1518.77	173.25	221.86	2821.34	1802.4	1190.84	2012.3	483.96	271.76	2951.09
B2M	5552.06	1508.98	1772.02	1397.63	3662.26	1136.1	1385.94	4119.92	4345.92	2291.1	4893.17	4445.48	1394.75	2797.99
RPLP0	2860.26	2558.15	1249.63	421.11	2685.49	656.32	559.88	2871.33	1225.55	4496.42	1962.54	1804.33	854.48	5199.68
RPL10	2734.47	3697.44	1802.92	634.22	2974.13	1002.2	896.7	2835.95	1630.26	4041.39	2059.51	3343.49	1318.42	4328.41
RPS27	2986	2141.67	3468.58	1288.45	3385.65	2427.61	2143.9	3025.02	1905.74	4608.22	2086.96	2444.6	2664.71	4364.2
RPS11	2881.4	2493.74	1898.91	957.91	3076.46	1363.64	837.77	2965.41	1403.93	4385.74	2411.08	2328.11	1422.94	4774.75
RPS18	2893.08	3258.23	2734	580.81	2315.89	1241.72	979.58	2189.97	1257.6	4566.76	2083.9	1838.32	1931.81	5521.54
HNRNPA1	3227.24	2813.27	474.58	299.11	1976.53	239.41	237.92	2221.1	730.03	3179.9	1577.93	1679.8	411.44	3897.62
RPS3	2653.12	1875.1	1065.89	417.26	2245.76	567.99	424.58	2115.79	1180.29	3949.77	1798.14	1761.09	827.25	4046.96
RPS3A	3274.4	3501.15	1647.93	513.88	2519.61	814.54	852.81	2425.6	1514.28	4228.22	2007.87	2775.13	1139.72	6022.73
RPL13A	2890.37	2170.4	1433.4	585.85	2629.11	1104.26	720.48	3052.71	1587.82	4262.55	1808.39	1969.93	1177.47	4597.41
SNORD97	62.04	9.83	324.67	367.97	82.56	351.07	466.76	64.99	16.06	54.41	31.86	21.89	1696.07	8.47
SCARNA13	0.42	2.29	1653.57	1258.26	0.96	1374.64	1926.95	6.38	0	0.72	0.77	0	2428.48	0.22
RPL7	3124.17	3005.94	974.44	377.96	1876.29	610.36	578.34	2247.68	1505.65	3584.33	1831.38	1741.15	629.78	4783.25
RPL39	2308.05	3374.09	1866.46	673.72	2204.93	764.43	1204.1	2187.51	1259.99	3628.8	1627.54	3122.8	1761.83	3978.3
PTMA	1470.36	934.55	540.19	263.31	2010.25	380.63	553.64	2473.62	1007.17	1627.66	1523.59	1138.3	431.35	1965.85
RACK1	3227.54	1695.2	840.38	396.24	2278.6	449.58	370.68	2477.78	1280.75	3111.07	1392.62	1471.4	539.22	3793.38
RPS9	2111.84	1501.96	648.09	311.34	1612.84	434.93	309.75	1878.85	1195.67	3299.72	1588.11	1213.29	498.05	3606.08
RPS2	2446.6	1759.54	966.68	476.63	1952.41	798.7	393.83	2094.95	850.66	3452.45	1482.61	1389.07	769.06	4432.16
EEF1G	2203.89	2024.8	918.66	353.17	1738.53	480.57	311.12	1714.19	1236.96	3115	1316.32	1646.57	570.83	3741.93
ATP5PD	108.58	35.93	1265.21	1926.01	58.84	2197.01	2302.44	91.16	65.04	52.24	87.76	31.75	1479.25	71.64
HLA-DRA	4954.73	941.11	882.16	918.73	2950.35	832.45	282.24	2300.13	2318.37	1501.02	2447.04	618.01	918.34	1056.79
EEF2	1686.91	1491.18	443.03	207.54	1775.27	237.59	171.4	1528.64	1211.09	2848.59	978.35	1082.26	326.82	3052.84
TMSB4X	2184.19	2091.43	982.07	635.33	2023.21	671.39	1251.83	3522.52	2667.94	1338.72	2300.65	3625.57	662.66	1160.28

*Dados ordenados por MAD

**Mil genes e 280 amostras

***4 subtipos de LLA

05.



Lista de Fármacos



Seleção dos fármacos e seus alvos

- Removidos as drogas cujos os alvos não eram proteínas
- 56 fármacos
- Genes alvos selecionados segundo o DrugBank
- Não foram considerados alvos sem referências

TARGET	ACTIONS	ORGANISM
A Dual specificity mitogen-activated protein kinase kinase 1	inhibitor	Humans
A Dual specificity mitogen-activated protein kinase kinase 2	inhibitor	Humans

1. Dual specificity mitogen-activated protein kinase kinase 1 [Details]

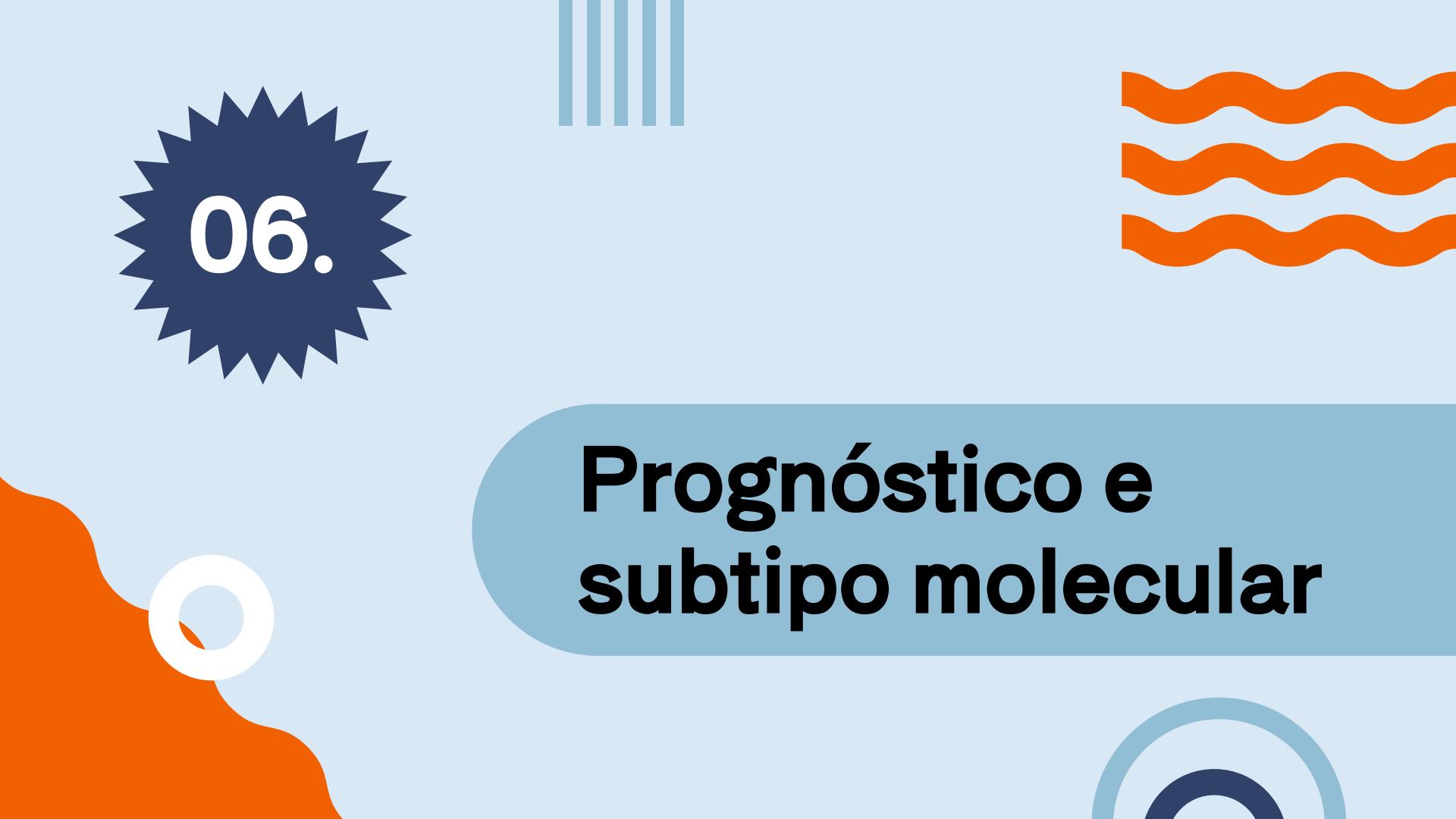
Kind	Protein	General Function	Receptor signaling protein tyrosine phosphatase activity
Organism	Humans	Specific Function	Dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway.
Pharmacological action	Yes		Binding of extracellular ligands such as growth factors, cytokines and hormones t...
Actions	Inhibitor		
Curator comments	The IC50 values for the unphosphorylated and phosphorylated forms of MEK1 are 0.7 nM and 13.2 nM, respectively.	Gene Name	MAP2K1
		Uniprot ID	Q02750
		Uniprot Name	Dual specificity mitogen-activated protein kinase kinase 1
		Molecular Weight	43438.65 Da

References

- 1. Salama AK, Kim KB. Trametinib (GSK1120212) in the treatment of melanoma. *Expert Opin Pharmacother*. 2013 Apr;14(5):619-27. doi: 10.1517/14656566.2013.770475. Epub 2013 Feb 23. [\[Article\]](#)
- 2. Ho MY, Morris MJ, Pirhalla JL, Bauman JW, Pendry CB, Orford KW, Morrison RA, Cox DS. Trametinib, a first-in-class oral MEK inhibitor mass balance study with limited enrollment of two male subjects with advanced cancers. *Xenobiotica*. 2014 Apr;44(4):352-68. doi: 10.3109/00498254.2013.831143. Epub 2013 Aug 23. [\[Article\]](#)
- 3. FDA Approved Drug Products: MEKINIST (trametinib) tablets or solution, for oral use (March 2023) [\[Link\]](#)
- 4. Health Canada Approved Drug Products: MEKINIST (Trametinib) Oral Tablets [\[Link\]](#)

Abreviação	Droga	Gene Alvo
DEXA	Dexametasona	NR3C1
TOPO	Topotecan (hydrochloride)	TOP1
BORT	Bortezomib	PSMB5; PSMB1
DOCE	Docetaxel	TUBB1; MAP2; MAP4; MAPT
PANO	Panobinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
MITO	Mitoxantrone dihydrochloride	TOP2A
IDAR	Idarubicin hydrochloride	TOP2A
TRAM	Trametinib	MAP2K1; MAP2K2
VINC	Vincristine (sulfate)	TUBB
AURO	Auranofin	IKBKB; PRDX5
SELI	Selinexor (KPT-330)	XPO1
BELI	Belinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
CARF	Carfilzomib	PSMB5; PSMB8; PSMB1; PSMB9; PSMB2; PSMB10
VORI	Vorinostat (SAHA)	HDAC1; HDAC2; HDAC3; HDAC6
VENO	Venetoclax	BCL2
GEMC	Gemcitabine hydrochloride	RRM1

06.



Prognóstico e subtipo molecular



Review

Genetic Biomarkers and Their Clinical Implications in B-Cell Acute Lymphoblastic Leukemia in Children

Monika Lejman ^{1,*} , Aleksandra Chałupnik ², Zuzanna Chilimoniuk ²  and Maciej Dobosz ²

¹ Laboratory of Genetic Diagnostics, Medical University of Lublin, 20-093 Lublin, Poland

² Student Scientific Society, Laboratory of Genetic Diagnostics, Medical University of Lublin, 20-093 Lublin, Poland; olachalupnik@op.pl (A.C.); zuzia.chil@gmail.com (Z.C.); macdob.98@gmail.com (M.D.)

* Correspondence: lejmanm@poczta.onet.pl

Abstract: Acute lymphoblastic leukemia (ALL) is a heterogeneous group of hematologic malignancies characterized by abnormal proliferation of immature lymphoid cells. It is the most commonly diagnosed childhood cancer with an almost 80% cure rate. Despite favorable survival rates in the pediatric population, a significant number of patients develop resistance to therapy, resulting in poor prognosis. ALL is a heterogeneous disease at the genetic level, but the intensive development of sequencing in the last decade has made it possible to broaden the study of genomic changes. New technologies allow us to detect molecular changes such as point mutations or to characterize epigenetic or proteomic profiles. This process made it possible to identify new subtypes of this disease

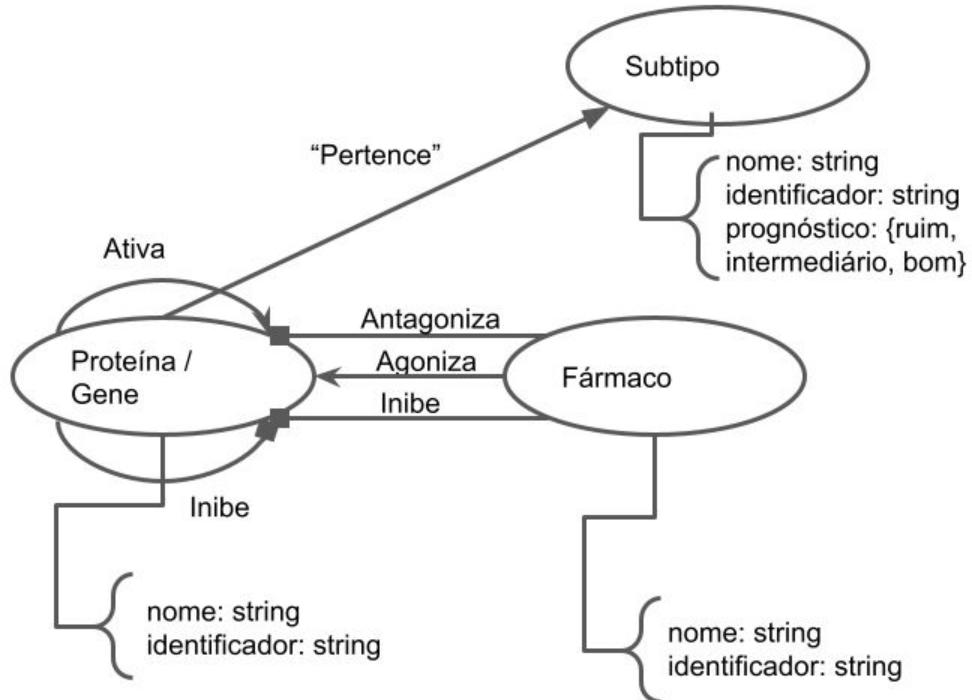
Subtipo molecular	Alteração genética	Prognóstico
HeH	Aneuploidia (51-65)	Bom
LeH	Aneuploidia (47-50)	Ruim
NeH	Aneuploidia (25-31)	Ruim
LeH	Aneuploidia (32-39)	Ruim
HeH	Aneuploidia (40-44)	Ruim
iAMP21	Amplificação	Intermediário
BCL::ABL1	Translocação	Ruim
Ph-like	Fusão gênica	Ruim
TCF::PBX1	Translocação	Intermediário
TCF3::HLF	Translocação	Intermediário
IKZF1	Fusão gênica/mutação pontual/deleção	Ruim
CRLF2	Fusão gênica/mutação pontual	Ruim
MEF2D	Fusão gênica	Ruim
CDKN2A	Deleção/hipermetilação	Ruim
ETV6::RUNX1	Translocação	Bom
ETV6::RUNX1-like	Translocação	Ruim
KMT2A	Translocação/inversão	Ruim
DUX4	Fusão gênica	Bom
PAX5-alt	Fusão gênica/deleção/amplificação	Intermediário
PAX5-P80R	Hotspot mutation	Intermediário
ZNF384	Fusão gênica	Intermediário
NUTM1	Fusão gênica	Bom

Subtipo molecular	Alteração genética	Prognóstico
HeH	Aneuploidia (51-65)	Bom
LeH	Aneuploidia (47-50)	Ruim
NeH	Aneuploidia (25-31)	Ruim
LeH	Aneuploidia (32-39)	Ruim
HeH	Aneuploidia (40-44)	Ruim
iAMP21	Amplificação	Intermediário
BCL::ABL1	Translocação	Ruim
Ph-like	Fusão gênica	Ruim
TCF::PBX1	Translocação	Intermediário
TCF3::HLF	Translocação	Intermediário
IKZF1	Fusão gênica/mutação pontual/deleção	Ruim
CRLF2	Fusão gênica/mutação pontual	Ruim
MEF2D	Fusão gênica	Ruim
CDKN2A	Deleção/hipermetilação	Ruim
ETV6::RUNX1	Translocação	Bom
ETV6::RUNX1-like	Translocação	Ruim
KMT2A	Translocação/inversão	Ruim
DUX4	Fusão gênica	Bom
PAX5-alt	Fusão gênica/deleção/amplificação	Intermediário
PAX5-P80R	Hotspot mutation	Intermediário
ZNF384	Fusão gênica	Intermediário
NUTM1	Fusão gênica	Bom

07.

Modelo lógico

Modelo lógico



08.

Metodología

Metodologia



Exploraremos os conceitos de **centralidade**, detecção de **comunidade**, e **link prediction** para avaliar as **interações** e possíveis **escapes** em interações de drogas conhecidas com determinadas proteínas que fazem parte do problema estudado.



09.

Ferramentas

Ferramentas



Neo4j

organizar os dados brutos que vieram dos bancos de dados em gráficos de redes.



Cytoscape

analisar o gráfico em rede gerado de acordo com os conceitos de centralidade, comunidade e link prediction.



String

verificar a quais vias protéicas os genes diferencialmente expressos estão inseridos.



10.

Resultados

String - aquisição das vias de sinalização citadas no artigo

JAK-STAT

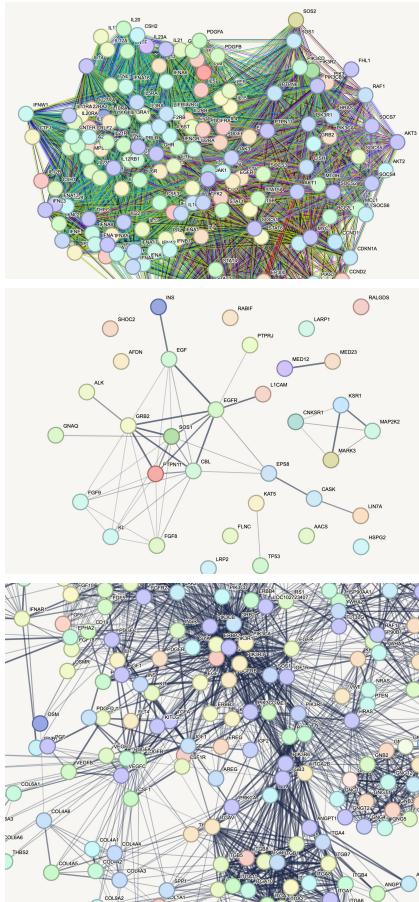
divisão celular,
morte celular e
formação de
tumor.

RTK-RAS

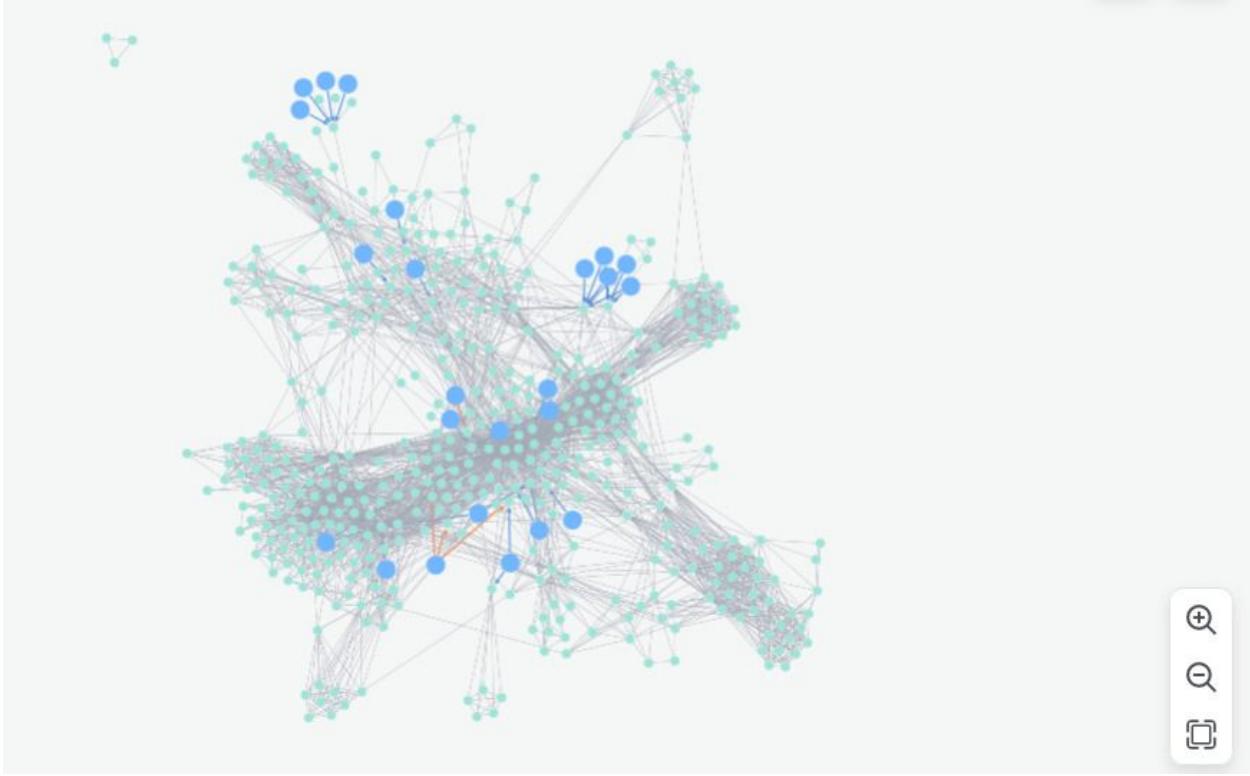
crescimento
celular,
diferenciação e
sobrevivência.

PI3K

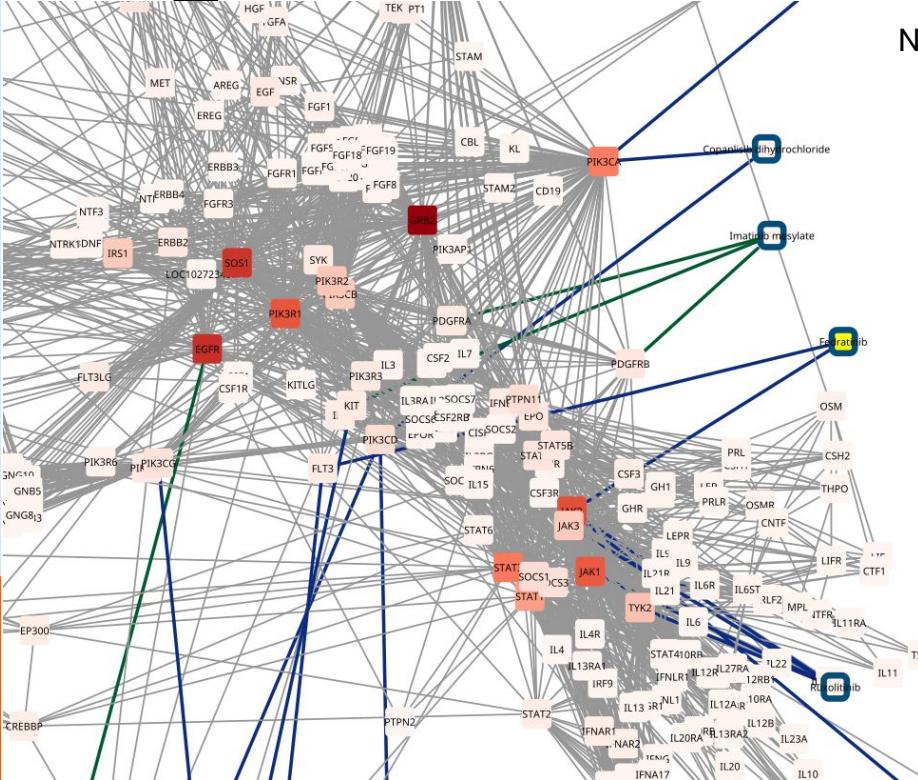
crescimento celular,
sobrevivência,
motilidade e
angiogênese.



Rede das principais vias da literatura integradas com os fármacos



Rede das principais vias da literatura integradas com os fármacos



Nota: foram utilizadas as configurações

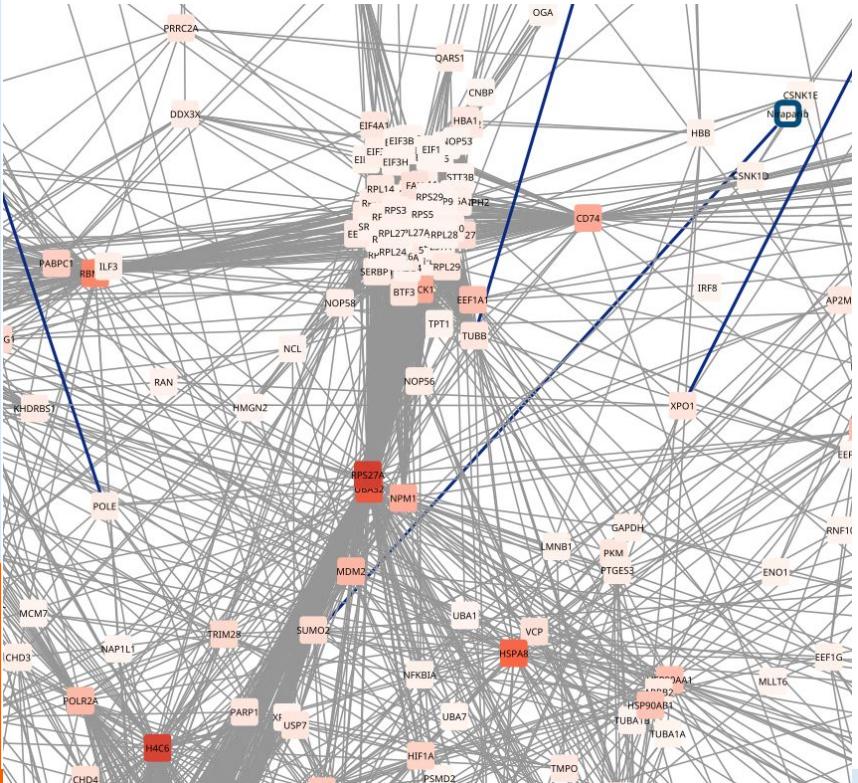
- **Network type:** physical subnetwork
 - **active interaction sources:** experiments, databases



24 drogas que atuam nas principais vias

Abreviação	Droga	Gene Alvo
PANO	Panobinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
AURO	Auranofin	IKBKB; PRDX5
BELI	Belinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
VORI	Vorinostat (SAHA)	HDAC1; HDAC2; HDAC3; HDAC6
VENO	Venetoclax	BCL2
FEDR	Fedratinib	JAK2; FLT3
COPA	Fedratinib	PIK3CA; PIK3CD
ROMI	Romidepsin	HDAC1; HDAC2
COBI	Cobimetinib	MAP2K1
BOSU	Bosutinib	ABL1; LYN; SRC; MAP2K1; MAP2K2; MAP3K2; CAMK2G; FGR; HCK; TEC; SLK
GILT	Gilteritinib	FLT3; AXL; ALK
BINI	Binimetinib	MAP2K2; MAP2K1
SELU	Selumetinib	MAP2K1; MAP2K2
IDEI	Idelalisib	PIK3CD
XL22	XL228	ABL; AURKA; IGF1R; SRC; LYN
DASI (DASA)	Dasatinib	ABL1; SRC; EPHA2; LCK; YES1; KIT; PDGFRB, FYN; BCR
RUXO	Ruxolitinib	JAK2; JAK1; JAK3; TYK2
DUVE	Duvelisib	PIK3CG; PIK3CD
ERLO	Erlotinib hydrochloride	NR1I2 (agonista); EGFR (antagonista)
IMAT	Imatinib mesylate	BCR; KIT (antagonista); RET; PDGFRA (antagonista); ABL1; PDGFRB (antagonista)
EVER	Everolimus	MTOR
AT92	AT9283	JAK2

Genes mais heterogêneos (MAD) integrados com os fármacos



Nota: foram utilizadas as configurações

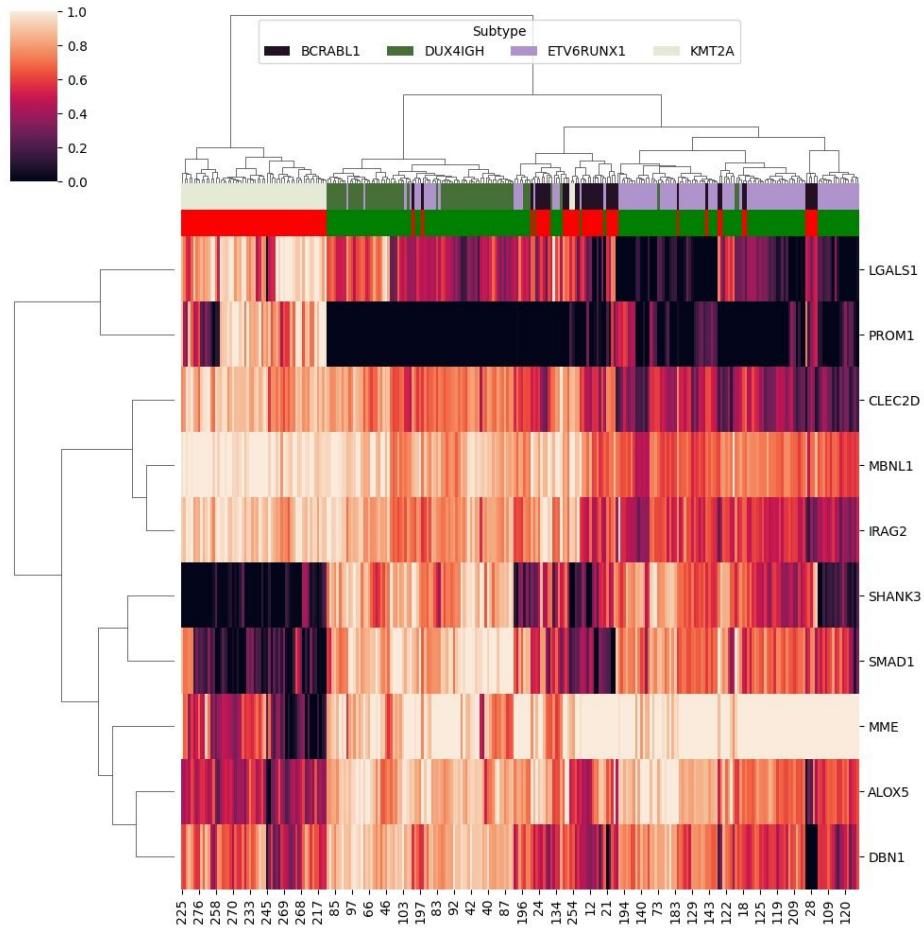
- **Network type:** physical subnetwork
 - **active interaction sources:** experiments, databases

Foram pesquisadas relações entre os 1000 genes mais heterogêneos, segundo valor de Mean Absolute Deviation

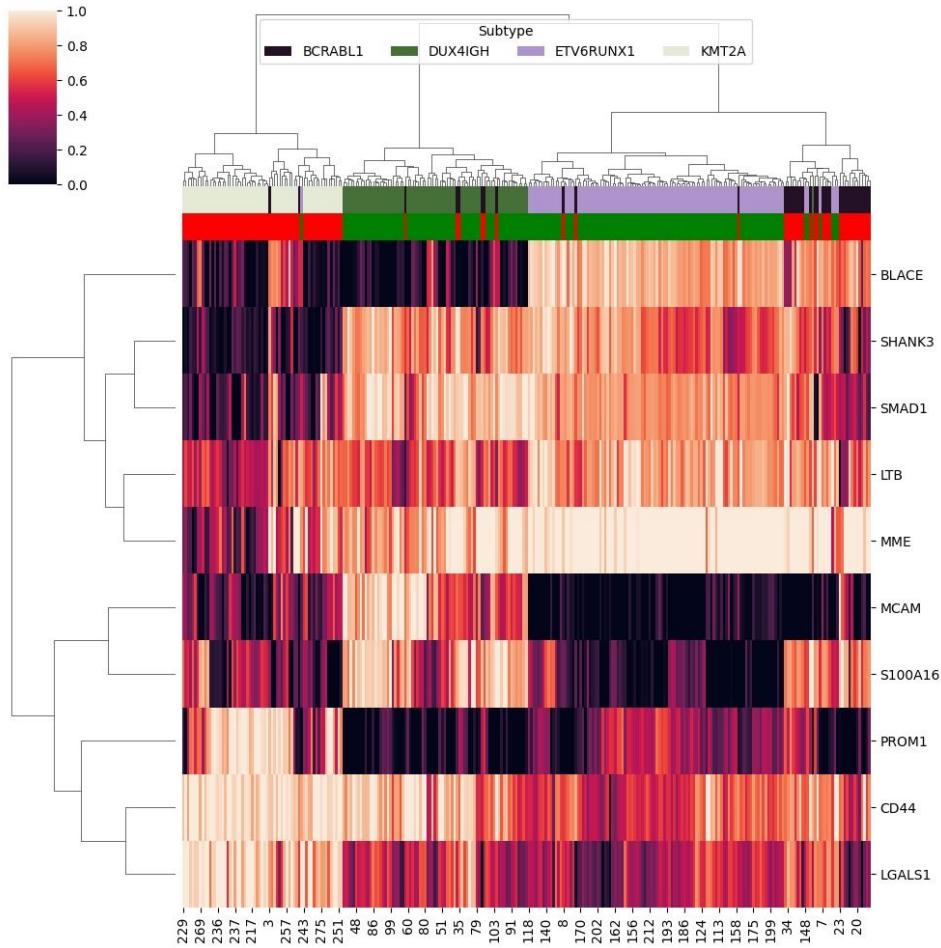
17 drogas que atuam nos genes com maior MAD

Abreviação	Droga	Alvo
AZAX	5-Azacytidine	DNMT1
BELI	Belinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
CLAD	Cladribine	RRM1; RRM2; RRM2B; POLA1; POLE; POLE2; POLE3; POLE4; PNP
COPA	Copanlisib dihydrochloride	PIK3CA; PIK3CD
DASI (DASA)	Dasatinib	ABL1; SRC; EPHA2; LCK; YES1; KIT; PDGFRB, FYN; BCR
DUVE	Duvelisib	PIK3CG; PIK3CD
FEDR	Fedratinib	JAK2; FLT3
GILT	Gilteritinib	FLT3; AXL; ALK
IDEL	Idelalisib	PIK3CD
IMAT	Imatinib mesylate	BCR; KIT (anga); RET; PDGFRA (anga); ABL1; PDGFRB (anga)
NIRA	Niraparib	PARP1; PARP2
PANO	Panobinostat	HDAC1; HDAC2; HDAC3; HDAC8; HDAC4; HDAC5; HDAC6; HDAC7; HDAC9; HDAC10; HDAC11
PONA	Ponatinib	ABL1; BCR
ROMI	Romidepsin	HDAC1; HDAC2
SELI	Selinexor (KPT-330)	XPO1
VINC	Vincristine (sulfate)	TUBB
VORI	Vorinostat (SAHA)	HDAC1; HDAC2; HDAC3; HDAC6

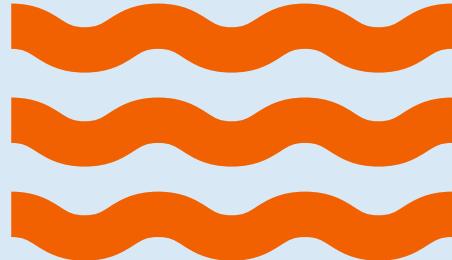
Cluster dos 10 genes mais significativos para o prognóstico



Cluster dos 10 genes mais significativos para os subtipos



11.



Referências



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