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TRANSCRIPTOMICS-BASED DRUG REPURPOSING OF SP600125 TO TARGET PRONEURAL–MESENCHYMAL TRANSITION IN GLIOBLASTOMA

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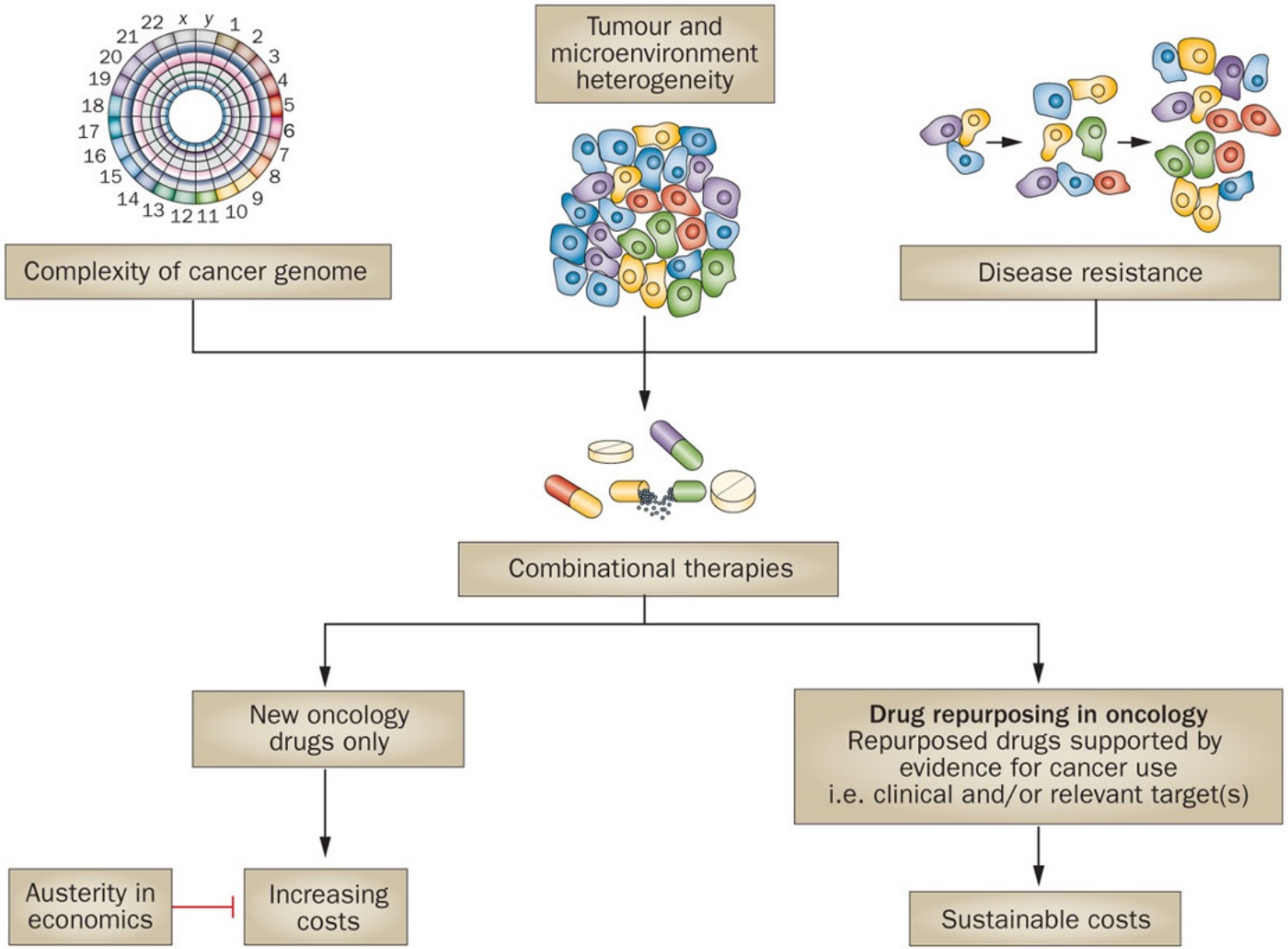
2025

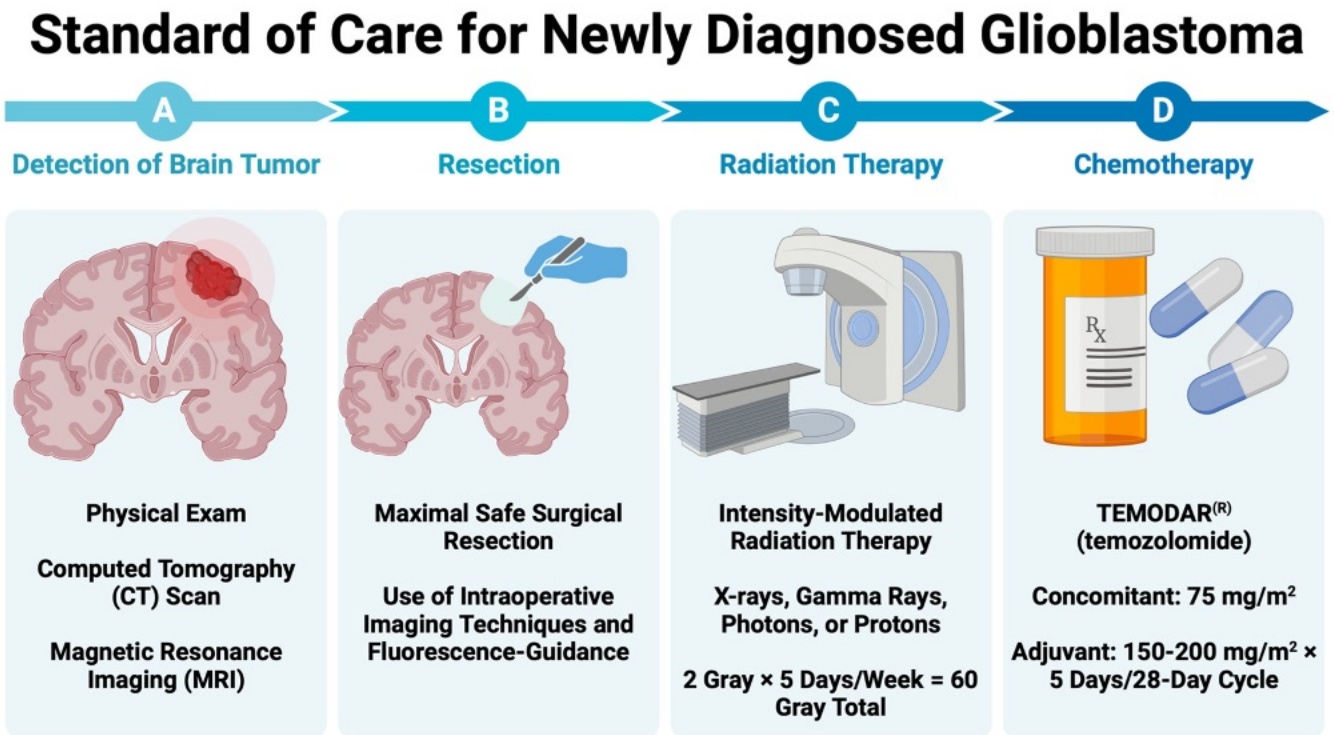
Drug repurposing in oncology



Bertolini, F. et al. (2015). Nat. Rev. Clin. Oncol. doi: 10.1038/nrclinonc.2015.169.

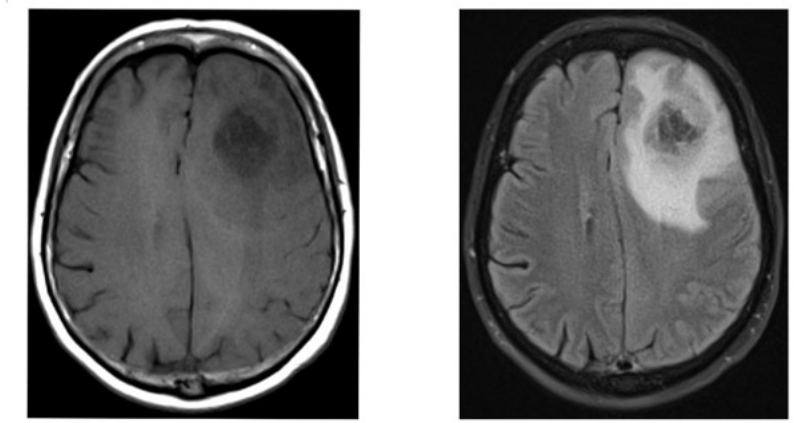
Glioblastoma

- Glioblastoma (GBM) is the most aggressive brain tumor with a median survival of about 14–15 months.
- Standard treatment (Stupp protocol): maximal surgical resection + radiotherapy + temozolomide.
- Major challenge: invasive growth prevents complete tumor removal.



Rodgers et al. (2024). Cancers. doi: 10.3390/cancers16152638.

Magnetic resonance imaging (MRI)

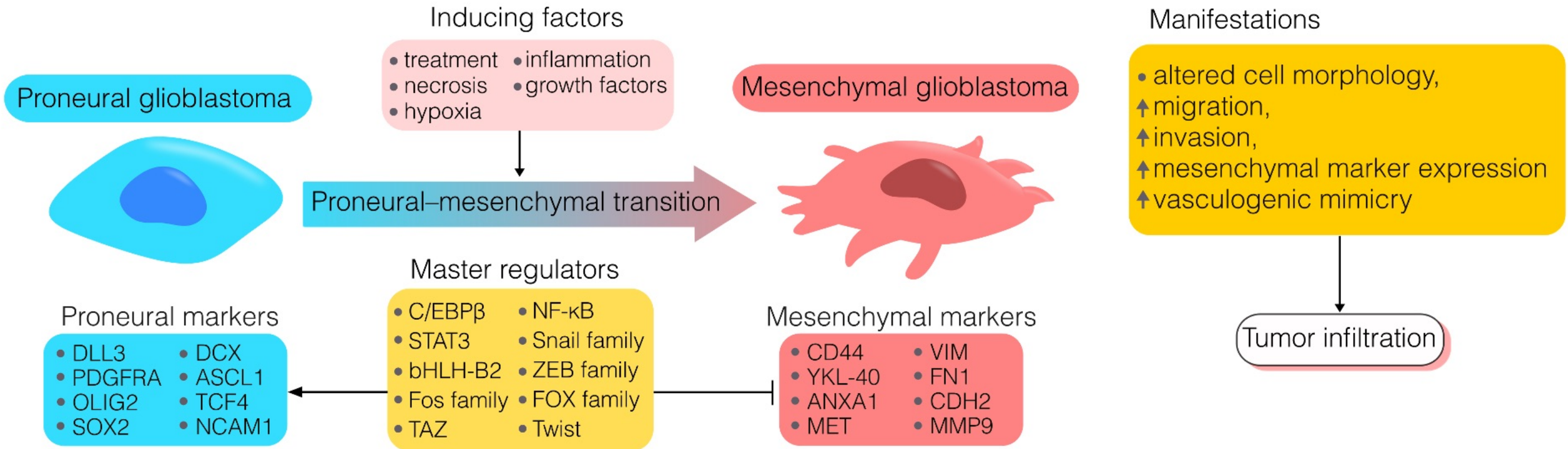


Tumor | Tumor infiltration

Tan et al. (2020). CA Cancer. J. Clin. doi: 10.3322/caac.21613.

Proneural–mesenchymal transition

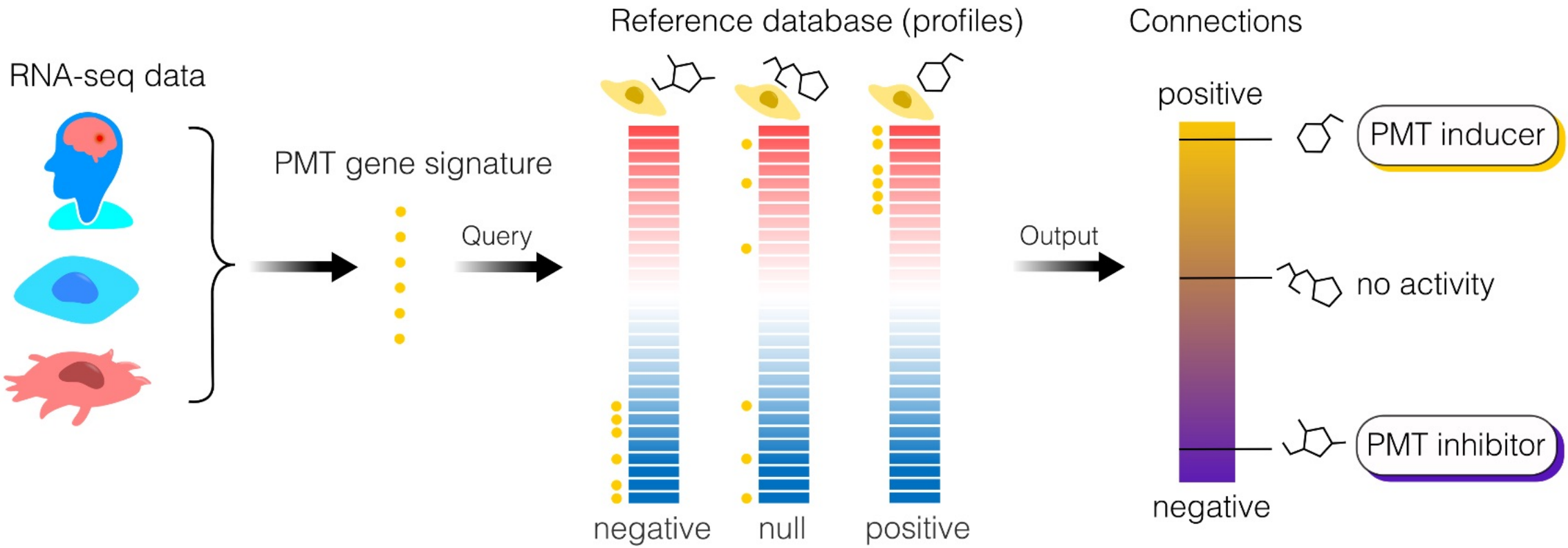
- GBM molecular subtypes: classical, proneural, and mesenchymal.
- Mesenchymal subtype: worst prognosis.
- Proneural–mesenchymal transition (PMT) increases GBM infiltrative growth.



Markov et al. (2025). Biomedicines. doi: 10.3390/biomedicines13010133.

Connectivity Map

- Reference dataset: expression profiles of tumor cells treated with ~3000 small molecules (CLUE platform).
- Connectivity map: the similarity between the PMT gene signature and small molecule profiles was determined using the Kolmogorov-Smirnov enrichment statistic.
- Potential PMT inhibitors were identified based on negative connectivity scores.

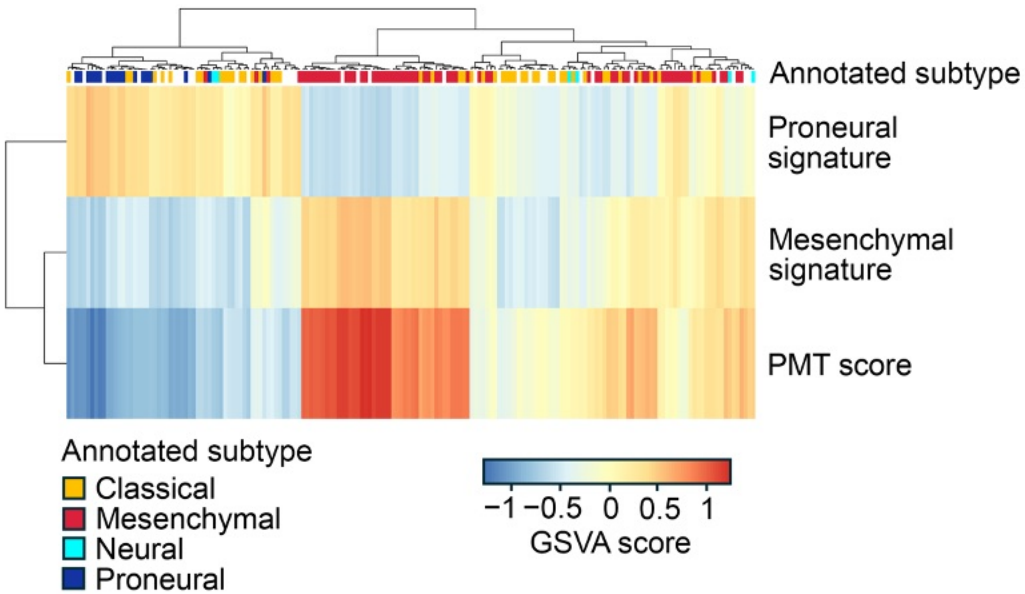


The image is adapted from Trapotsi et al. (2021). RSC Chem. Biol. doi: 10.1039/d1cb00069a.

Construction of PMT signature 1

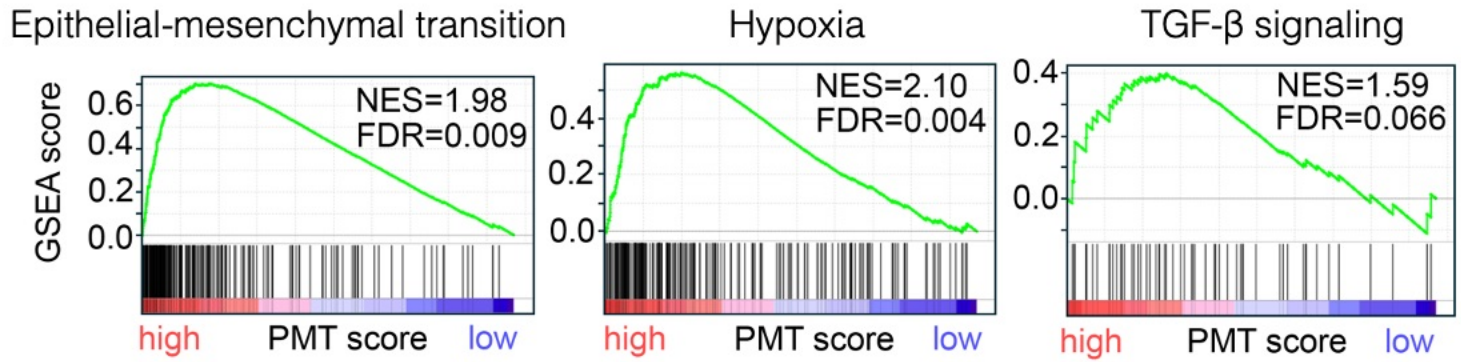
The Cancer Genome Atlas
168 GBM samples

Gene set variation analysis (GSVA)

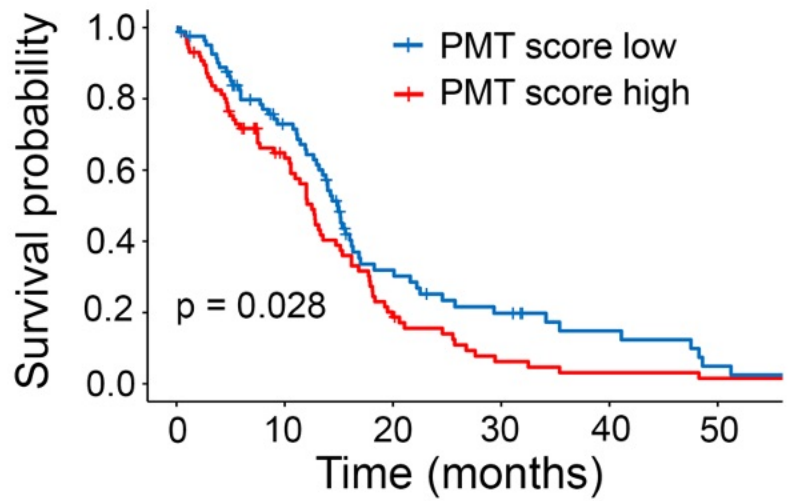


PMT score = ES(MES) – ES(PN)

Gene set enrichment analysis (GSEA)



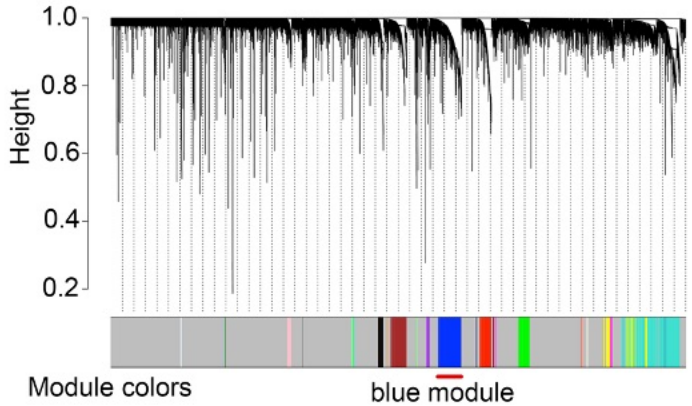
Kaplan–Meier survival curves



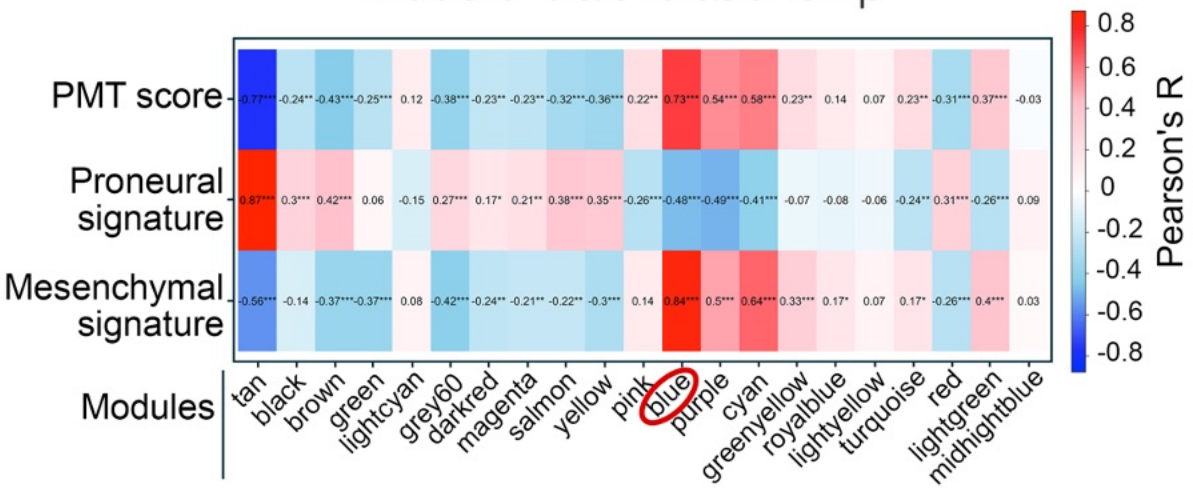
Construction of PMT signature 2

TCGA-GBM

Weighted correlation network analysis (WGCNA)

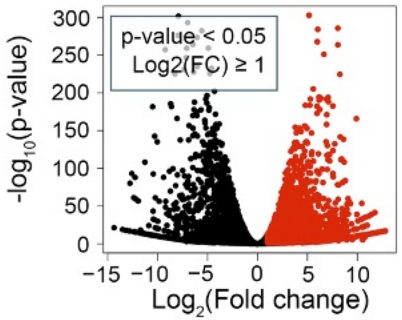


Module–trait relationship

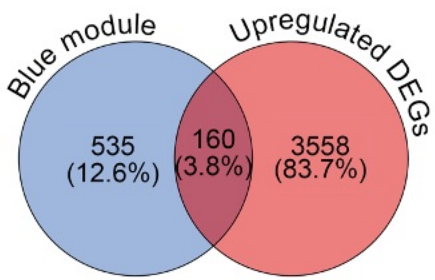


GSE192710: MES GBM cells vs PN GBM cells

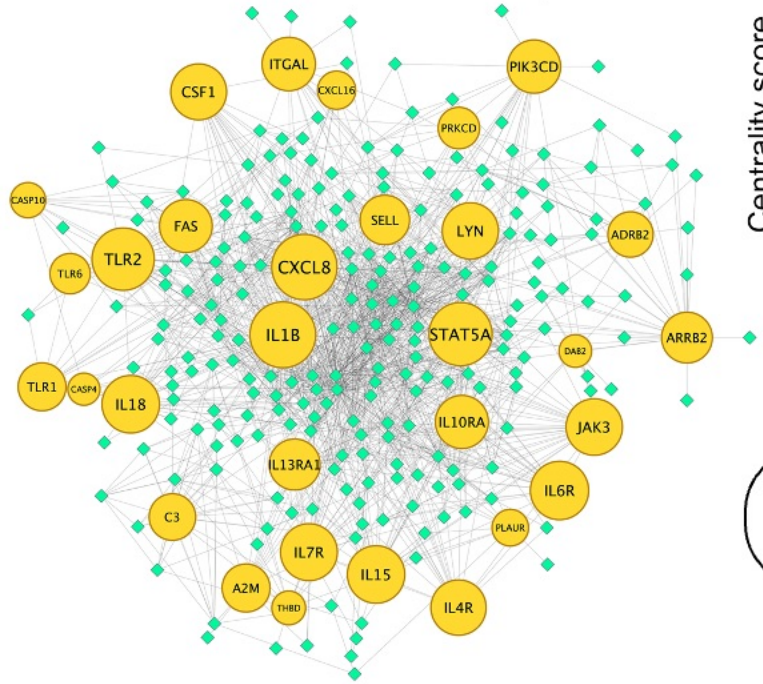
Upregulated DEGs

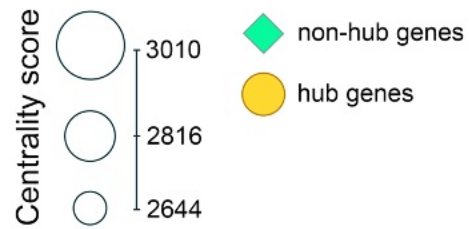


Intersection



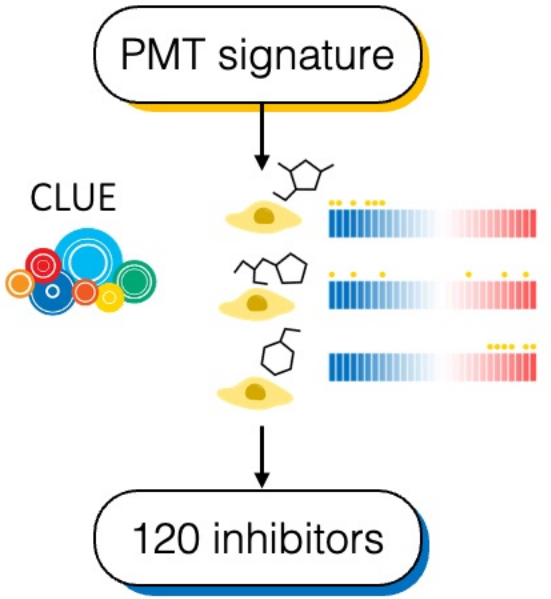
Gene network analysis



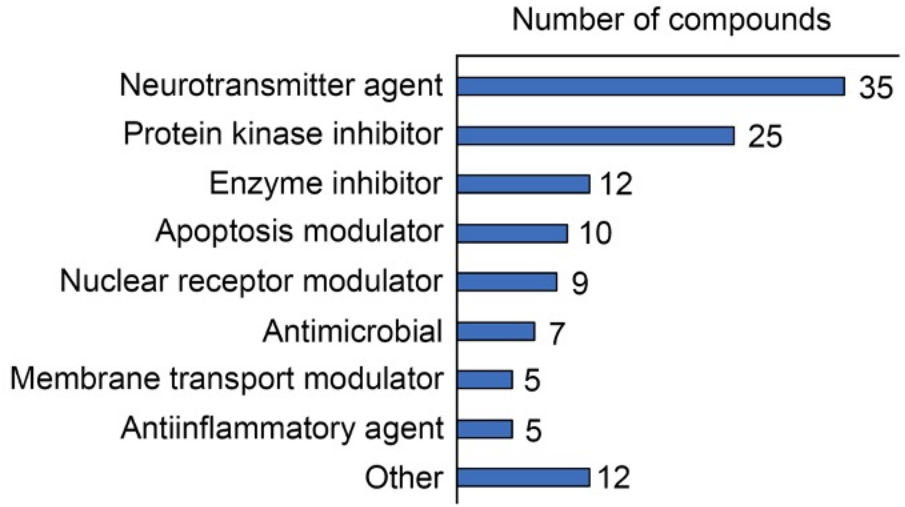


PMT signature
31 hub genes

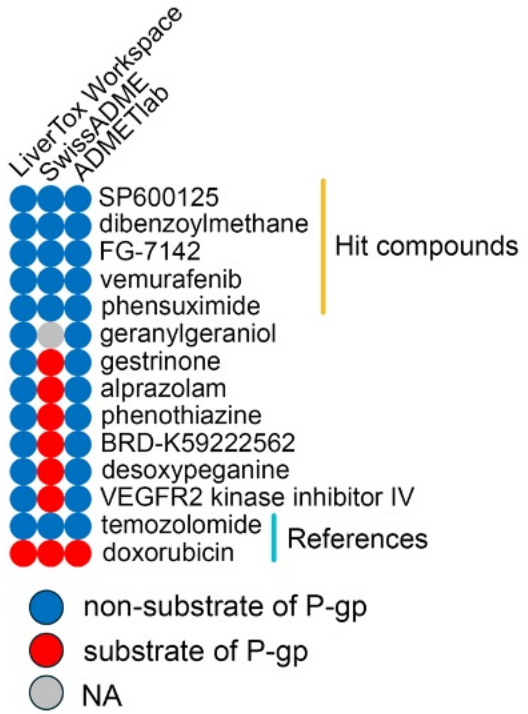
Connectivity Map analysis



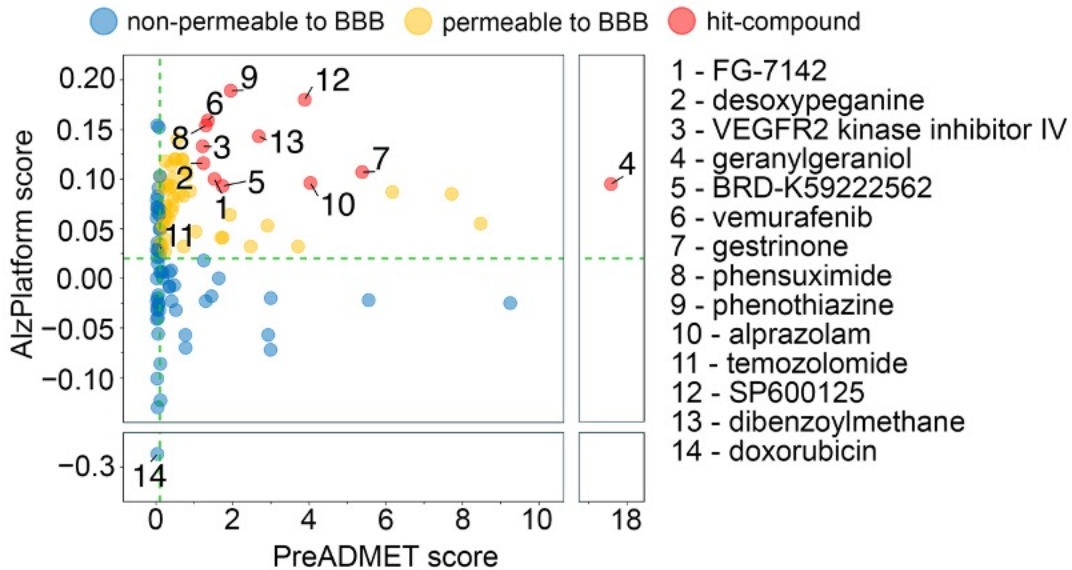
Mechanism of action

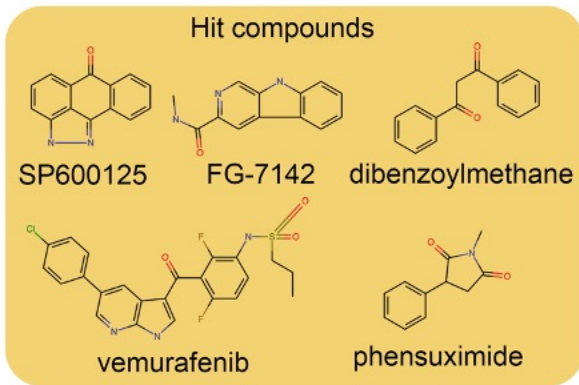


P-glycoprotein substrate specificity



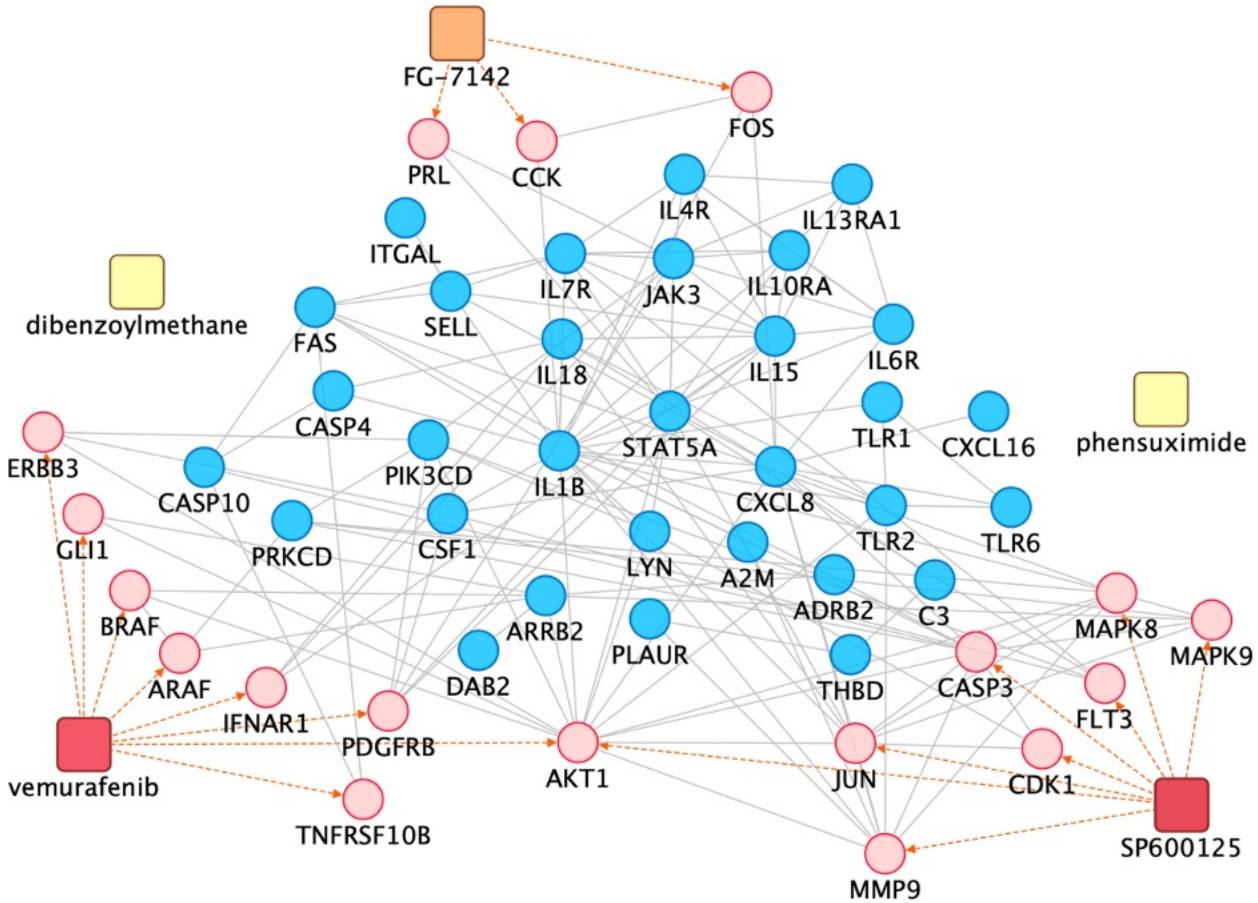
Blood–brain barrier permeability

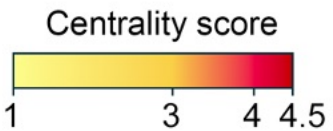




Network pharmacology

Drug–gene network analysis





GMT inhibitors
primary protein targets
hub genes
protein-protein interaction
compound-protein interaction

Centrality metrics

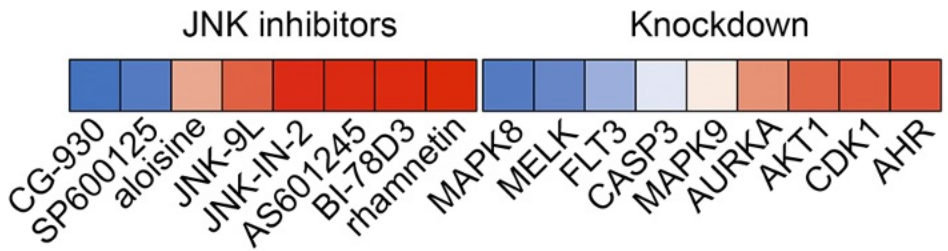
	Degree rank	MCC rank	MNC rank	EPC rank	Closeness rank	Radiality rank	Centrality score
SP600125	4	5	5	4	5	3	4.3
vemurafenib	4	4	4	5	4	4	4.2
FG-7142	3	3	3	3	3	5	3.3
dibenzoylmethane	1	1	1	1	1	1	1.0
phensuximide	1	1	1	1	1	1	1.0

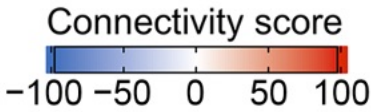
SP600125 targets:

STITCH:
MAPK8 (JNK1)
MAPK9 (JNK2)
AKT1
CASP3
CDK1
FLT3

Literature:
AHR
AURKA
MELK

Connectivity Map analysis

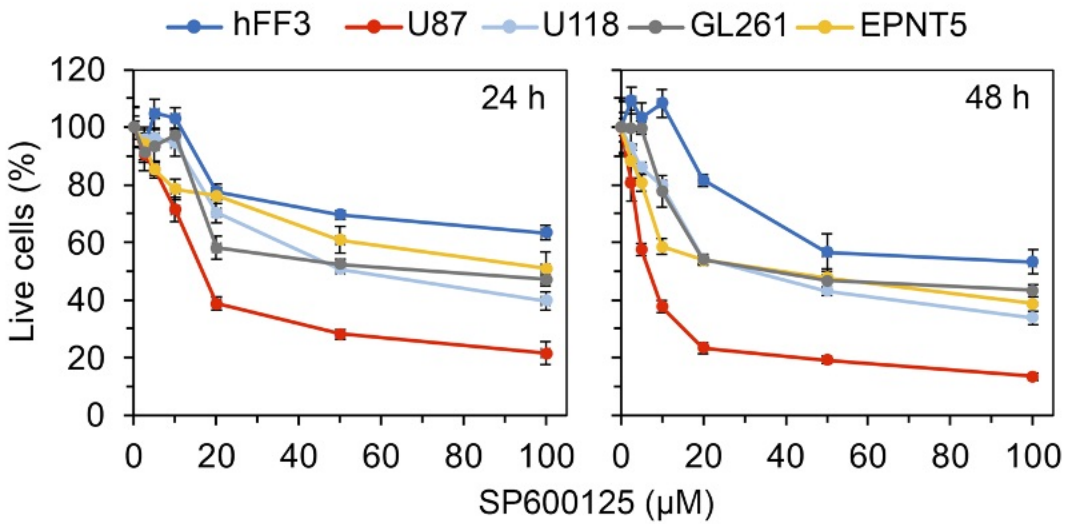




SP600125 is a promising PMT inhibitor

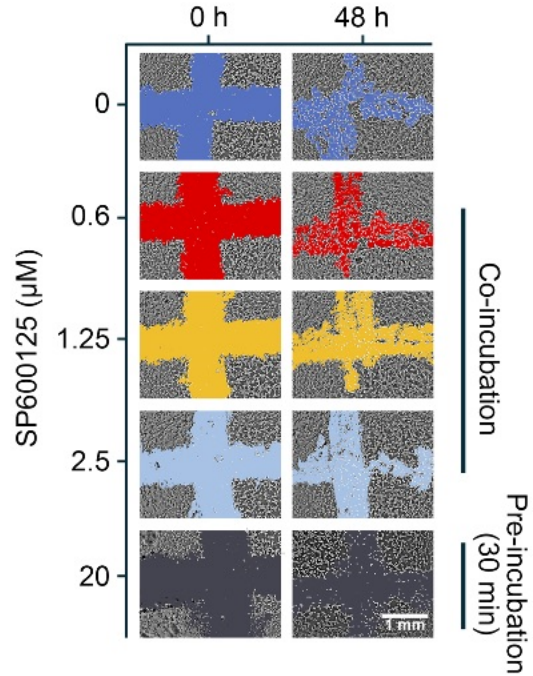
In vitro verification 1

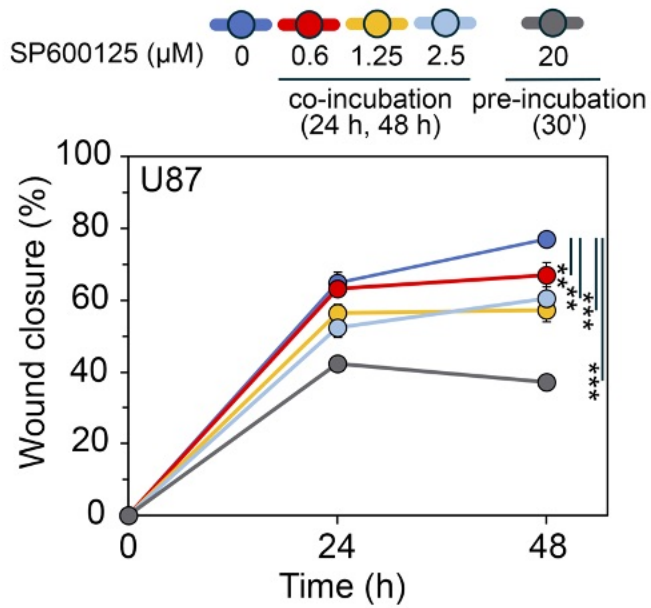
Cytotoxicity assessment (MTT assay)

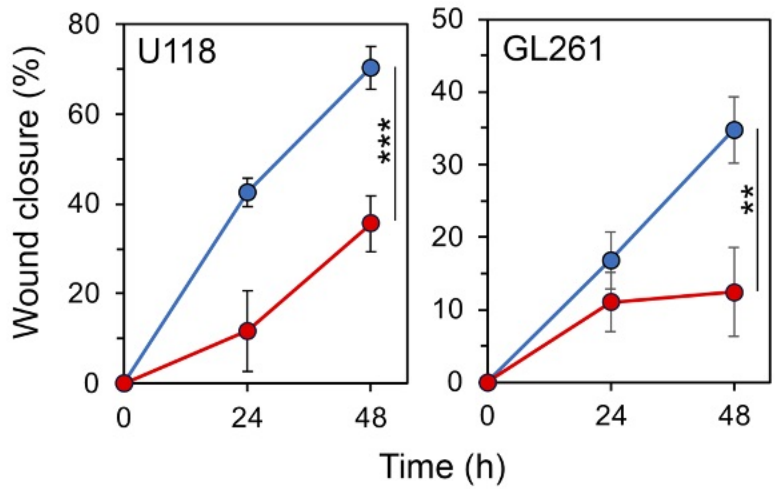


	IC_{50} ± SD (μM)		
	24 h	48 h	
hFF3	>100	87.1 ± 22.7	human fibroblast
U87	19.6 ± 2.5	7.3 ± 0.9	human GBM cells
U118	58 ± 6.8	36.8 ± 4.2	human GBM cells
GL261	65.2 ± 18.5	47.3 ± 13.2	mouse GBM cells
EPNT-5	104.7 ± 29.2	35.9 ± 9.1	mouse GBM cells

Effect on cell migration (scratch assay)







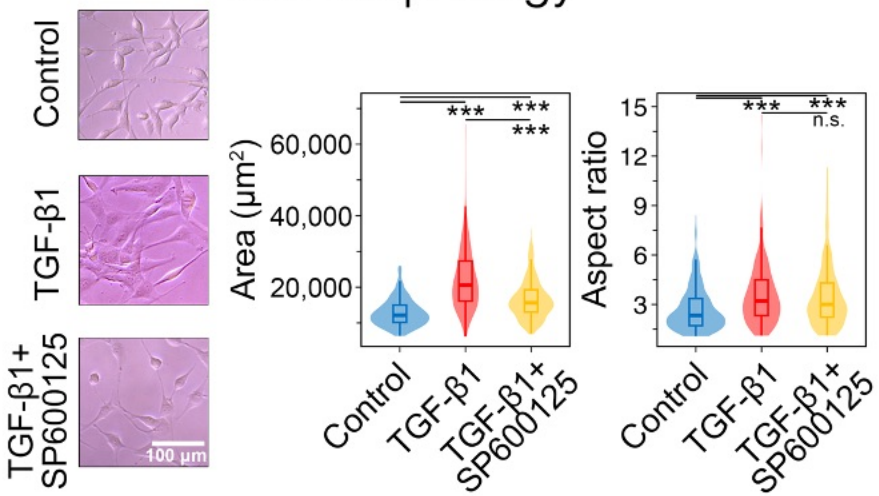
Control
SP600125

T-test:
**p < 0.01
***p < 0.001

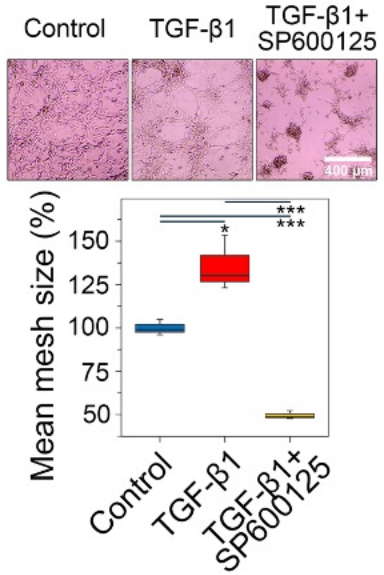
In vitro verification 2

Transforming growth factor beta 1 (TGF-β1) is known PMT inducer.

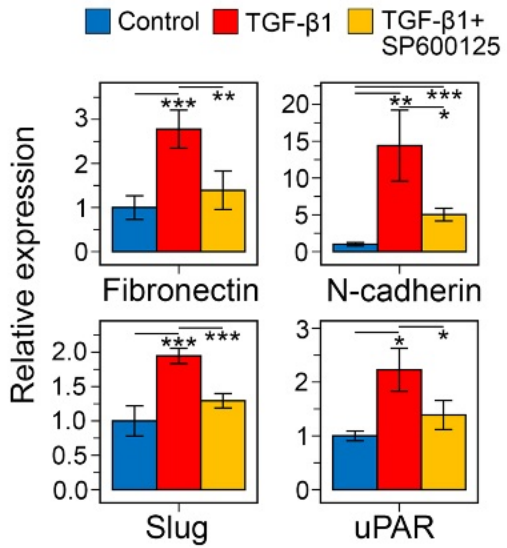
Cell morphology

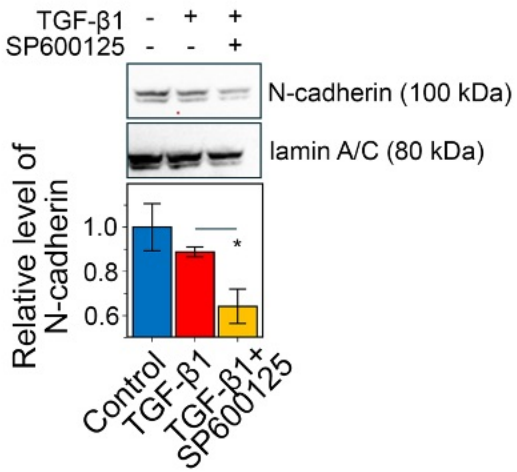


Vasculogenic mimicry

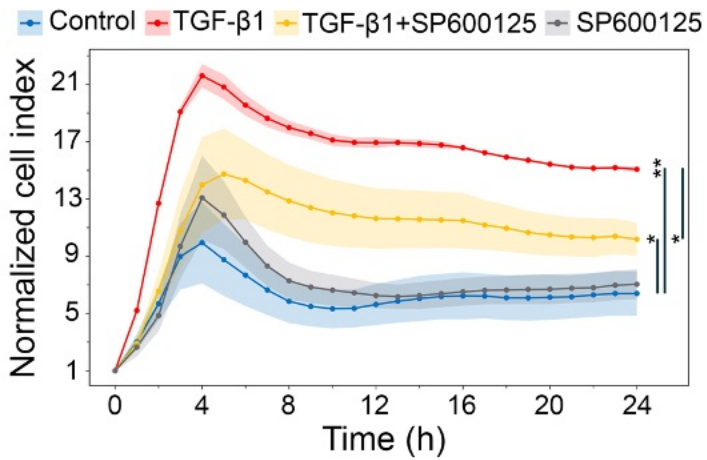


MES markers (RT-qPCR, Western blot)

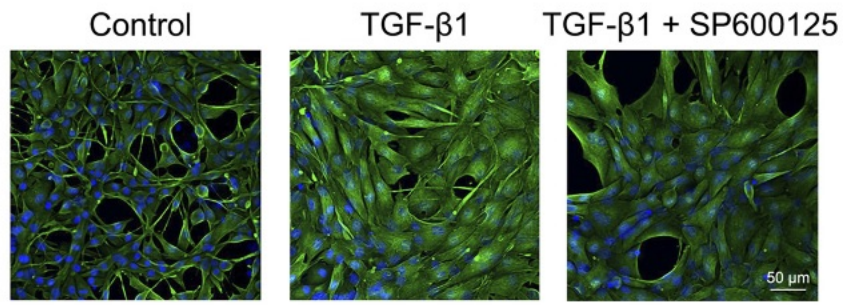




Transwell migration (xCELLigence)



Immunofluorescence of vimentin



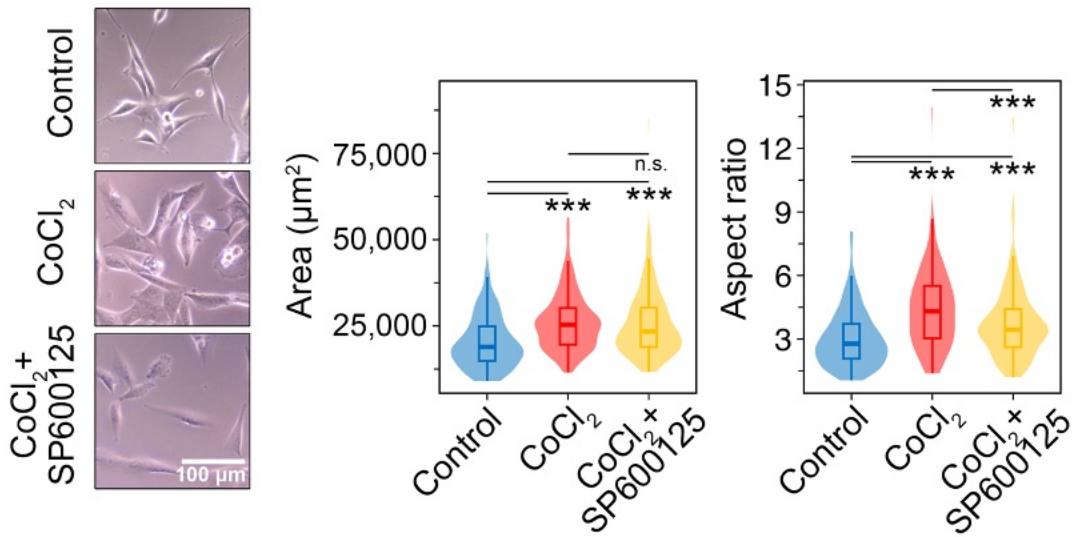
Vimentin
DAPI

T-test:
*p<0.05
**p < 0.01
***p < 0.001

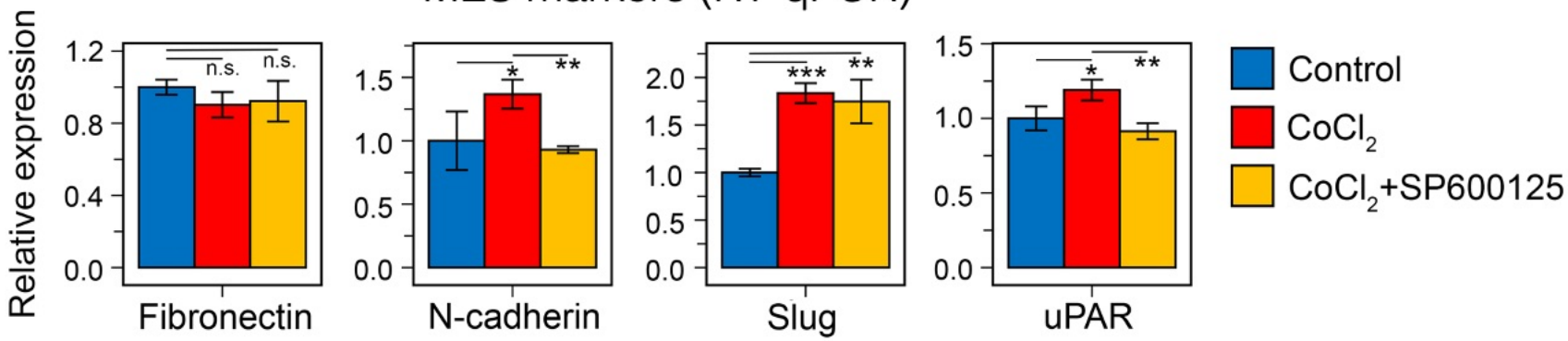
In vitro verification 3

Treatment with cobalt chloride ($CoCl_2$) mimics hypoxia.

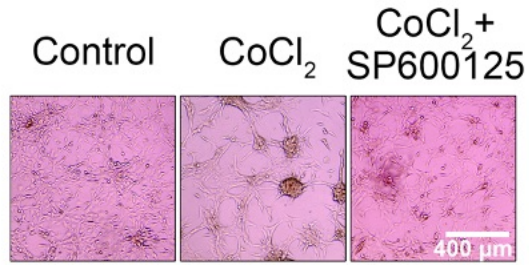
Cell morphology

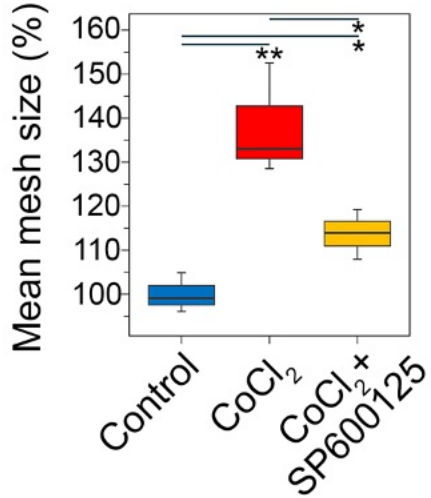


MES markers (RT-qPCR)



Vasculogenic mimicry





T-test:
*p<0.05
**p < 0.01
***p < 0.001

Conclusion

- Connectivity Map analysis repurposed 120 small molecules as potential PMT inhibitors, primarily neurotransmitter agents and protein kinase inhibitors.
- Five hit-compounds with favorable blood–brain permeability were identified: SP600125, dibenzoylmethane, FG-7142, vemurafenib, and phensuximide .
- SP600125 demonstrated the ability to inhibit PMT in GBM cells induced by TGF-β1 or hypoxia, likely by targeting the protein kinases JNK1 and MELK.

Acknowledgements

- The Russian Science Foundation, for financial support (grant number 23-14-00374)
- The Institute of Chemical Biology and Fundamental Medicine SB RAS
- Laboratory of Biochemistry of Nucleic Acids
- But most importantly:

  Dr. Oleg V. Markov, for help with confocal microscopy

  Albina V. Vladimirova, for help with cell culture maintenance

RSF | Russian Science Foundation

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Recently published: Odarenko et al. (2025). Int. J. Mol. Sci. doi: 10.3390/ijms26199772.