Tapton SCHOOL ACADEMY TRUST

Computational Model of Neuroblastoma Shows that the Life Sciences are as Quantitative as Physics

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PRIMAGE
Medical imaging Artificial intelligence Childhood cancer research

The University Of Sheffield.

INSIGNEO
Institute for *in silico* Medicine

Objectives

1. PRIMAGE project.

2. Neuroblastoma.

3. The first multicellular model of neuroblastoma.

4. Model calibration using GPUs.

5. Predictive simulations.

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Explainer

https://vimeo.com/613708072/c59c67572c or https://kywertheim.com/research/PrimageVideo.mp4

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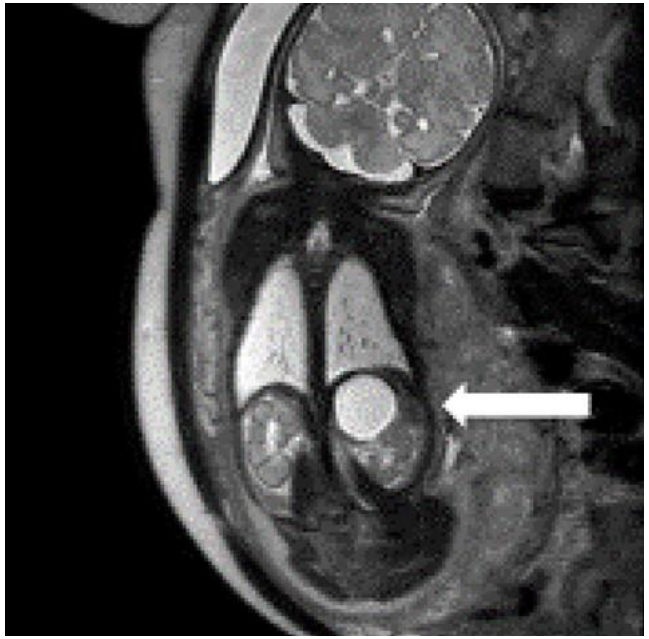

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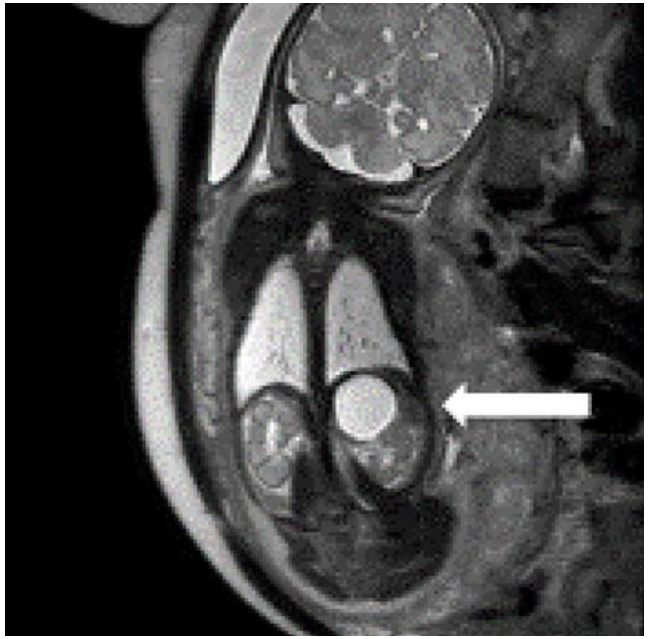
Paediatric cancer:

- Most common extra-cranial solid tumour in children.
- 15 % of cancer-related deaths in children.
- Adrenal medulla is usually the primary site.

Neuroblastoma



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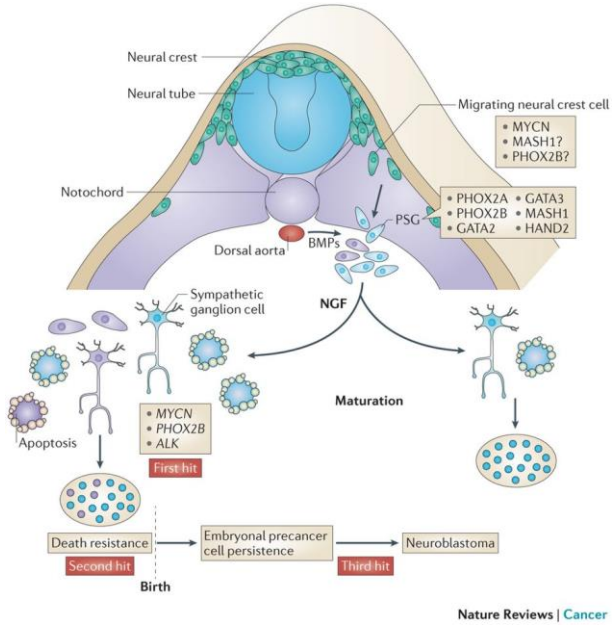

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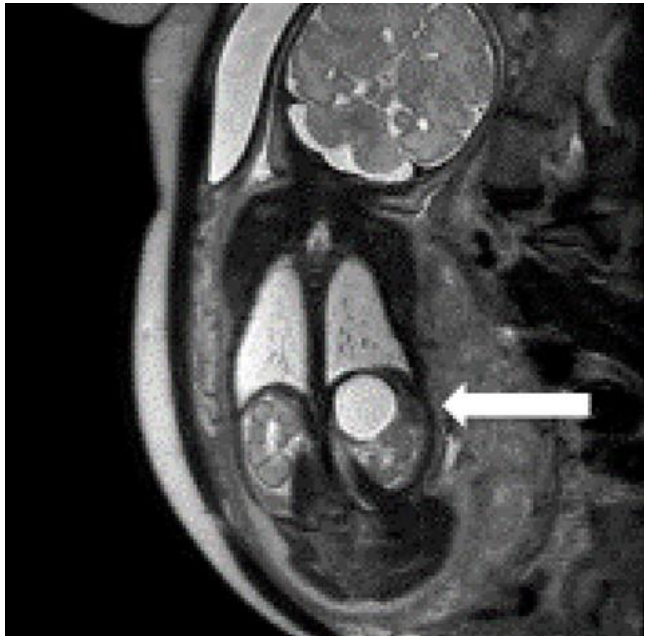
Neural crest:

- Transient structure during embryonic development.
- Migrate and differentiate into different cell types.
- Sympathetic nervous system.

Neuroblastoma



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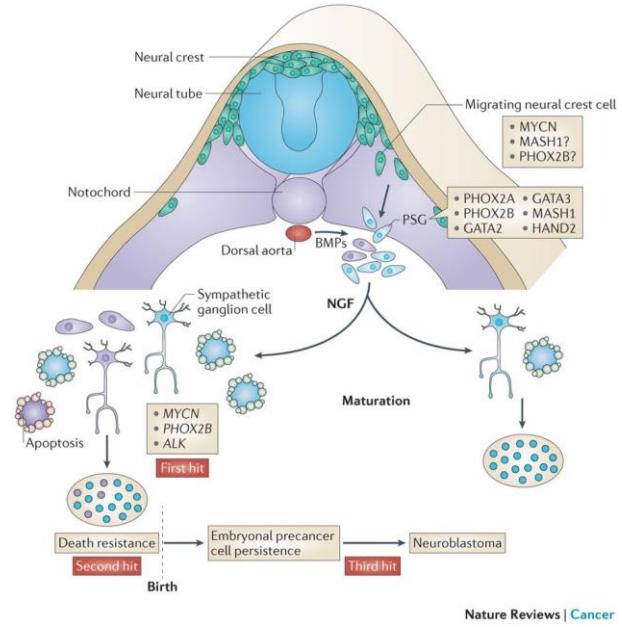

Marshall, Glenn M., et al. "The prenatal origins of cancer." *Nature Reviews Cancer* 14.4 (2014): 277-289.

INRG Stage	Age (months)	Histologic Category	Grade of Tumor Differentiation	*MYCN*	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any, except GN maturing or GNB intermixed		NA			B Very low
				Amp			K High
L2	< 18	Any, except GN maturing or GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	≥ 18	GNB nodular; neuroblastoma	Differentiating	NA	No		E Low
					Yes		H Intermediate
			Poorly differentiated or undifferentiated	NA			
				Amp			N High
M	< 18			NA		Hyperdiploid	F Low
	< 12			NA		Diploid	I Intermediate
	12 to < 18			NA		Diploid	J Intermediate
	< 18			Amp			O High
	≥ 18						P High
MS	< 18			NA	No		C Very low
					Yes		Q High
				Amp			R High

Sokol, Elizabeth, and Ami V. Desai. "The Evolution of Risk Classification for Neuroblastoma." *Children* 6.2 (2019): 27.

Paediatric cancer:

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Heterogeneity:

- Spontaneous regression.
- Drug resistance and metastasis even after multi-modal treatment.
- MYCN amplification.
- < 50 % survival rate in high-risk cases.

Party Conversation Starter 1.

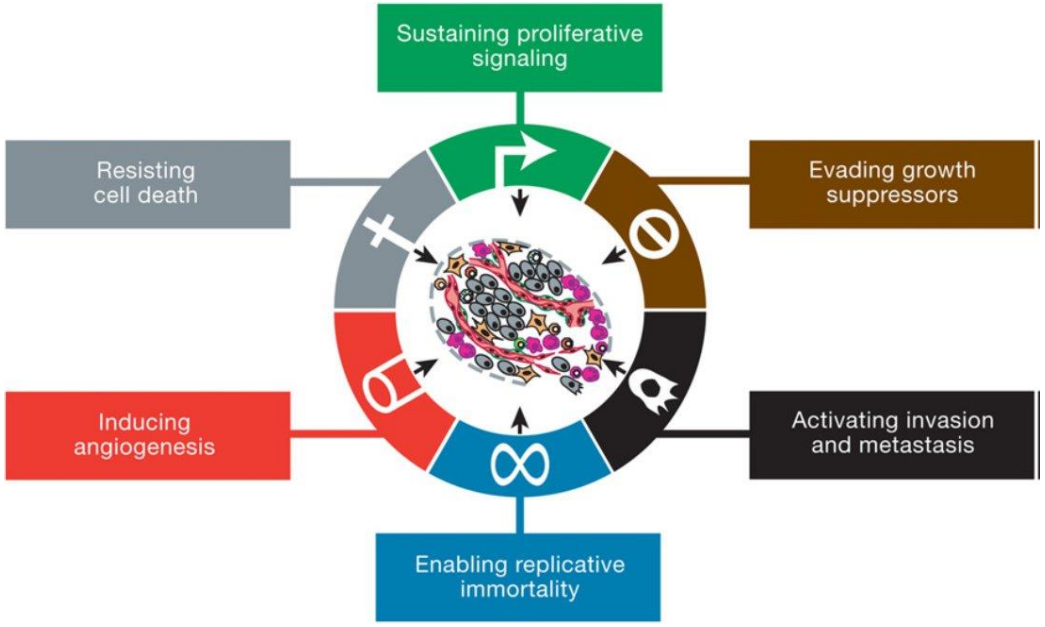
Why do paediatric cancers usually have fewer mutations than adult cancers?

An adult, by definition, has been around for longer than a child. More time, more mutations.

Party Conversation Starter 2.

What are the key hallmarks of cancer?

1. Sustained proliferation.
2. Limitless replicative potential.
3. Resistance to cell death.
4. Immune evasion.
5. Blood supply.
6. Motility.



Hanahan, Douglas, and Robert A. Weinberg. "Hallmarks of cancer: the next generation." *cell* 144.5 (2011): 646-674.

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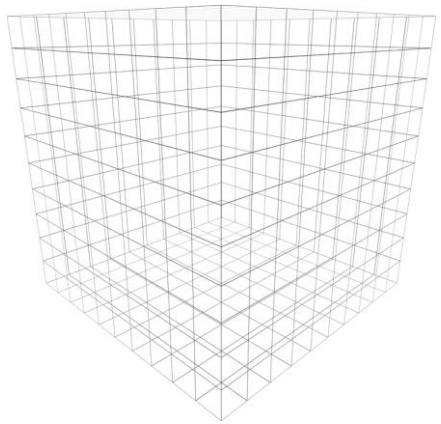
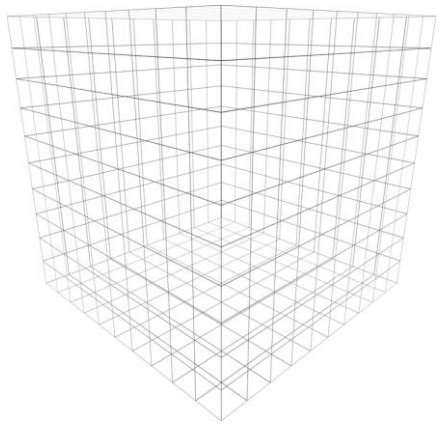
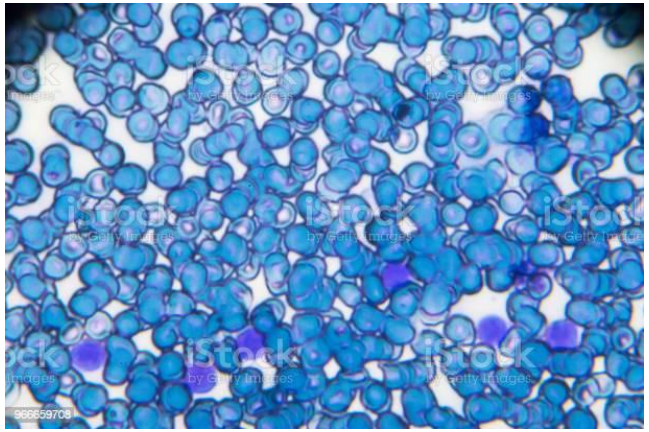


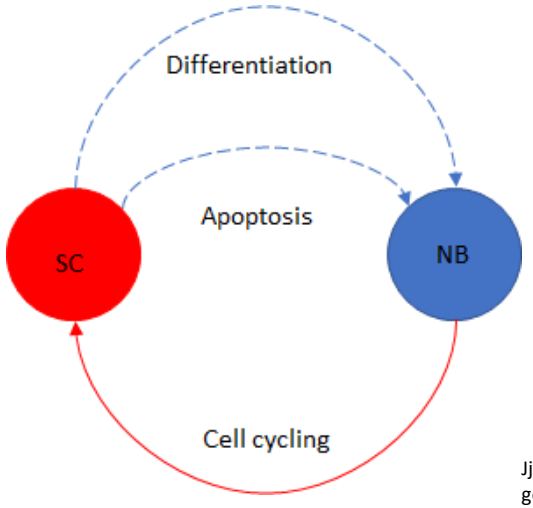
Part 1: continuous automaton.

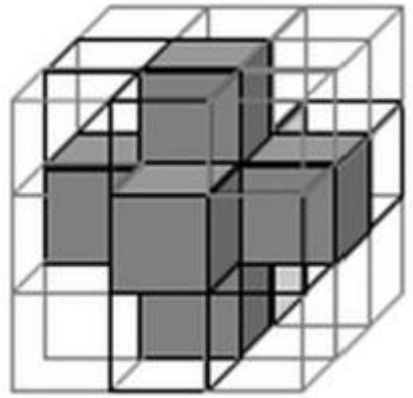
- Voxelate the tumour microenvironment.
- Spatial distributions of cells and matrix.
- Oxygen, nutrients, and chemotherapeutic drugs (uniform).
- Inflammation (uniform).

Model structure









Jjumba, Anthony, and Suzana Dragicevic. "Integrating GIS-based geo-atom theory and voxel automata to simulate the dispersal of airborne pollutants." *Transactions in GIS* 19.4 (2015): 582-603.

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- Neuroblasts and Schwann cells.
- 3D von Neumann neighbourhood in the continuous automaton.

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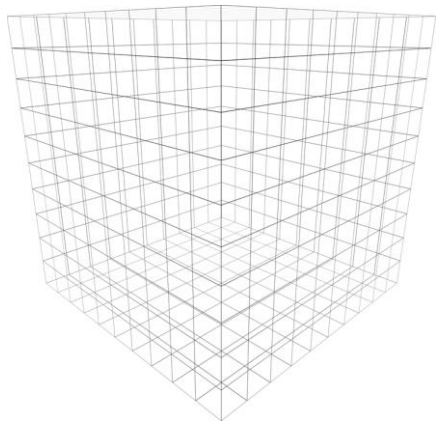

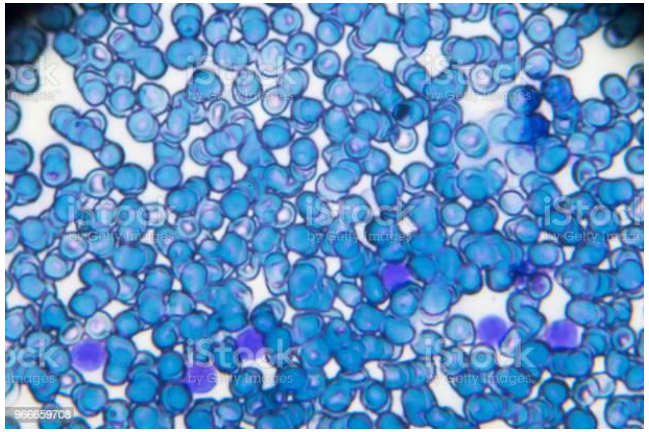

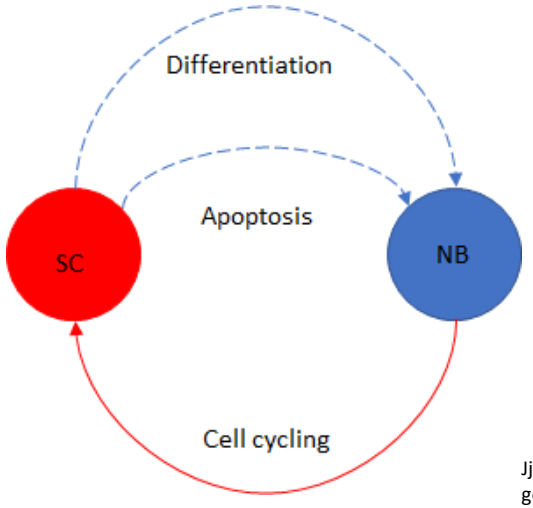

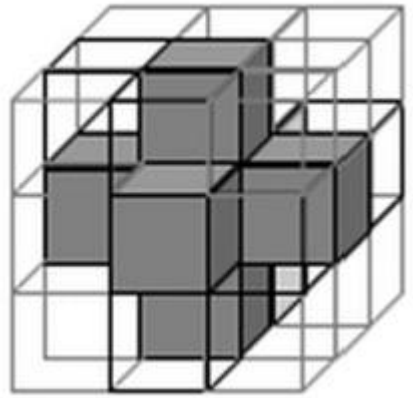

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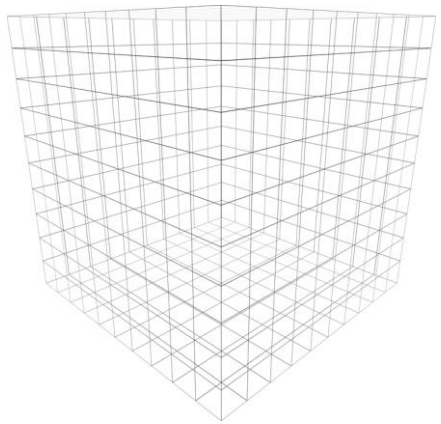

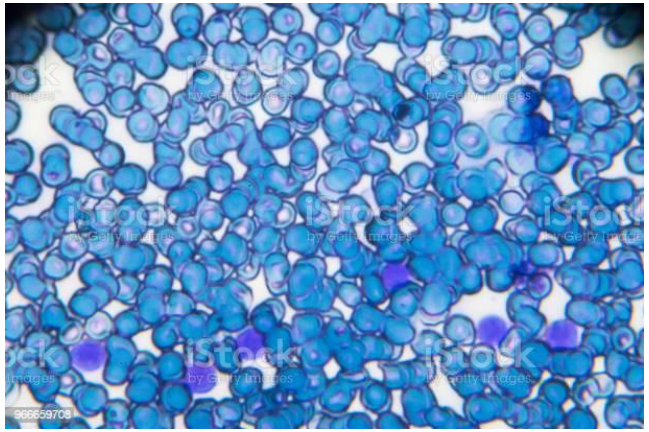

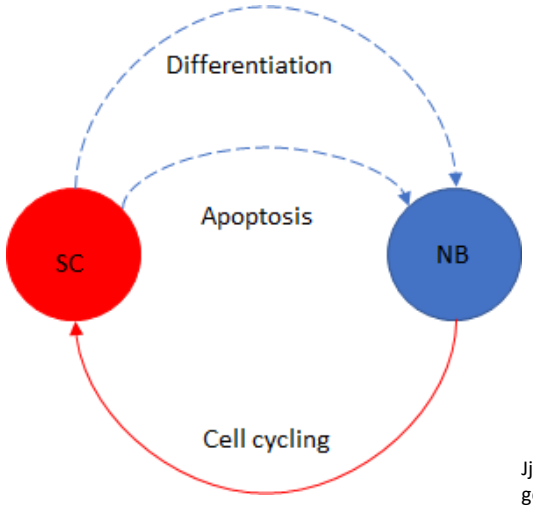

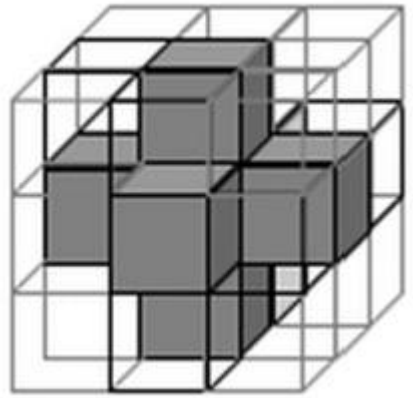

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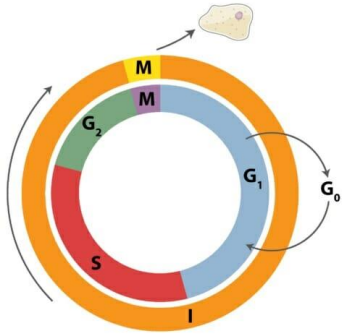
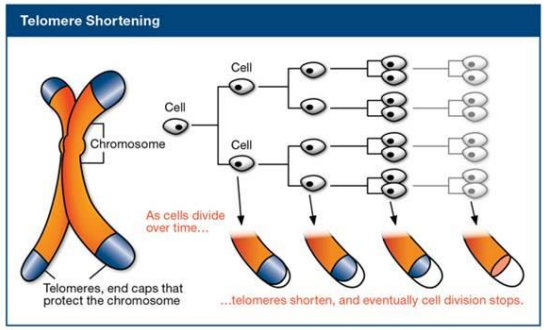
- Mutations, gene expression levels, and DNA status (short telomeres, unreplicated, and generic damage).
- Cell cycling (proliferation and division).
- Cell death (apoptosis and necrosis).



https://biologydictionary.net/cell-cycle/



https://sphweb.bumc.bu.edu/otlt/mph-modules/ph/aging/aging3.html

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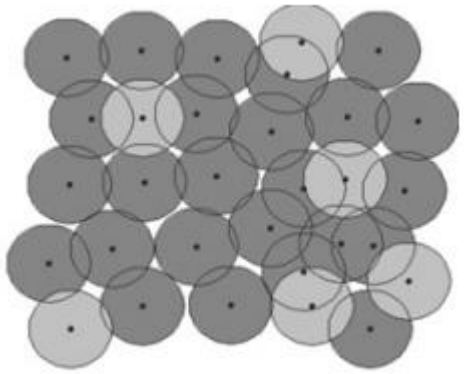
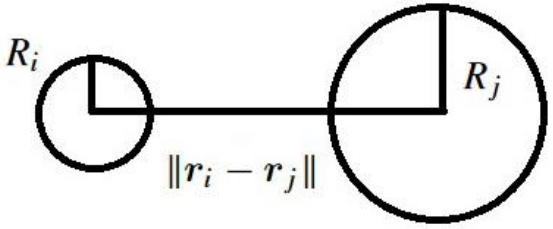


Pathmanathan, P., et al. "A computational study of discrete mechanical tissue models." *Physical biology* 6.3 (2009): 036001.



$$\delta_{ij} = R_i + R_j - \|r_i - r_j\|$$

$$\delta_i = \sum_{j=1}^{N_j} \delta_{i,j} \qquad |\boldsymbol{F_i}| = k_1 \delta_i$$

$$\boldsymbol{F_i} = \mu_{eff} \frac{d\boldsymbol{r_i}}{dt}$$

μ_{eff} scales with the abundance of matrix.

Part 3: centre-based mechanical model.

- Cell gets bigger throughout cell cycle.
- Migration resolves contact inhibition.
- Boundary conditions and matrix abundance.

Algorithm implementing the model.

- Cell-Cell overlap.
- Linear force law.
- Equation of motion.
- Iterate these steps until convergence.

Dynamic simulation (one time step = one hour in the patient's life)

1. Each cell senses its microenvironment, modifies its behaviour, and updates its attributes.

2. Resolve cell-cell overlap using the centre-based mechanical model.

3. The cells collectively modify their microenvironment.

4. Back to step 1 for the next time step.

Python for model development.

CUDA for large-scale simulations.

Party Conversation Starter 3.

When can we not solve an equation with pencil and paper?

Usually, non-linear equations cannot be solved analytically, meaning by a finite number of standard operations.

$$\frac{dx}{dt} = kx$$

$$\frac{dx}{x} = k\,dt$$

$$\int_{x_0}^{x(t)} \frac{dx}{x} = k\int_0^t dt$$

$$\ln\frac{x(t)}{x_0} = kt.$$

Analytic solution.

$$\ln(1+x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \cdots = \sum_{k=1}^{n} \frac{(-1)^{k+1}x^k}{k}$$

Numerical solution. > 90 % of mathematical models in research are non-linear.

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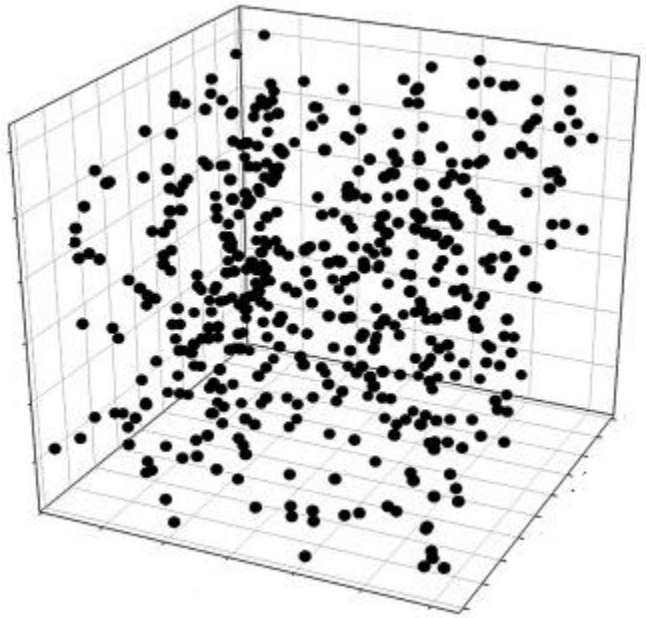
Calibration studies

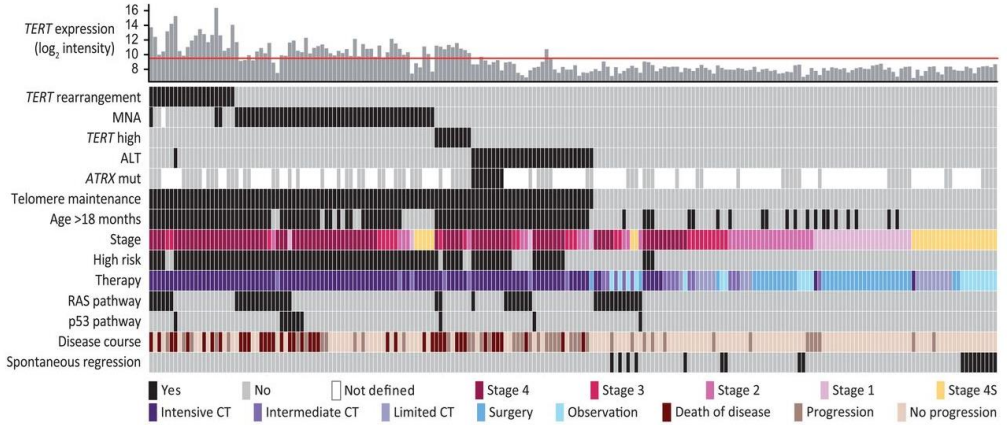


Latin hypercube sampling.

- 20 fitting parameters.
- 3000 parametric combinations.

Simulation results against data in literature.

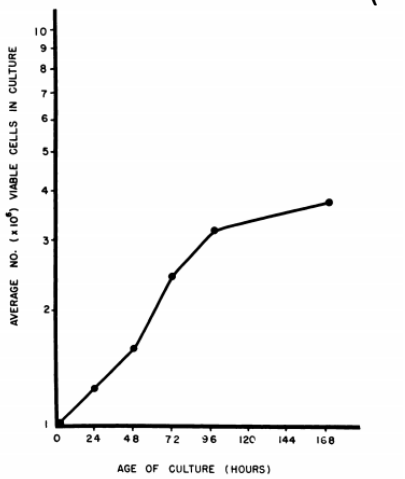
- Clinical outcomes associated with different mutations.
- Cell death triggered by hypoxia. $\mathrm{RSS} = \sum_{i=1}^{n} (y_i - f(x_i))^2$
- Growth kinetics.
- Clinical outcomes and cell behaviours associated with different histology types.



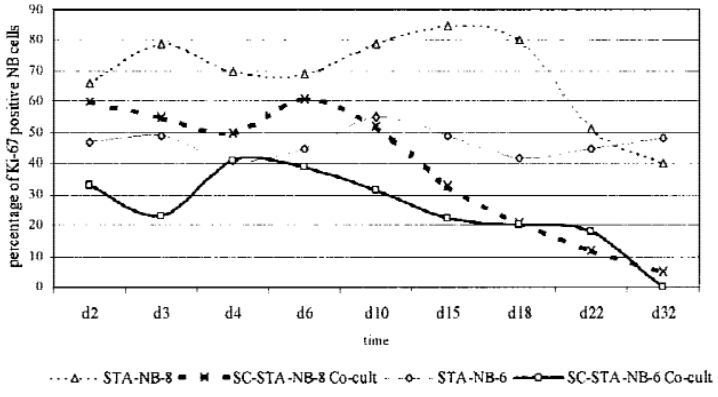
(Ackermann *et al*. 2018)

	Three-stage fit	95% CI	Direct fit	95% CI
Maximum oxygen consumption rate, q_{max} (mmHg · s^{-1})	17.5	15.3–25.1	16.3	15.3–17.9
P_{O_2} for 50% drop in consumption, $P_{50,q}$ (mmHg)	2.7	0.0–12.5	1.6	1.2–2.1
Maximum misonidazole binding rate, $k_{b,0}$ ($\times 10^{-4}$ s^{-1})	4.5	3.9–4.9	4.4	2.5–5.3
P_{O_2} for 50% drop in binding, $P_{50,b}$ (mmHg)	1.4	0.3–2.6	1.4	1.1–2.5
P_{O_2} for 50% necrosis, $P_{50,n}$ (mmHg)	1.2	0.1–4.9	1.0	0.4–1.2

(Warren and Partridge 2016)



(Tumilowicz *et al*. 1970)

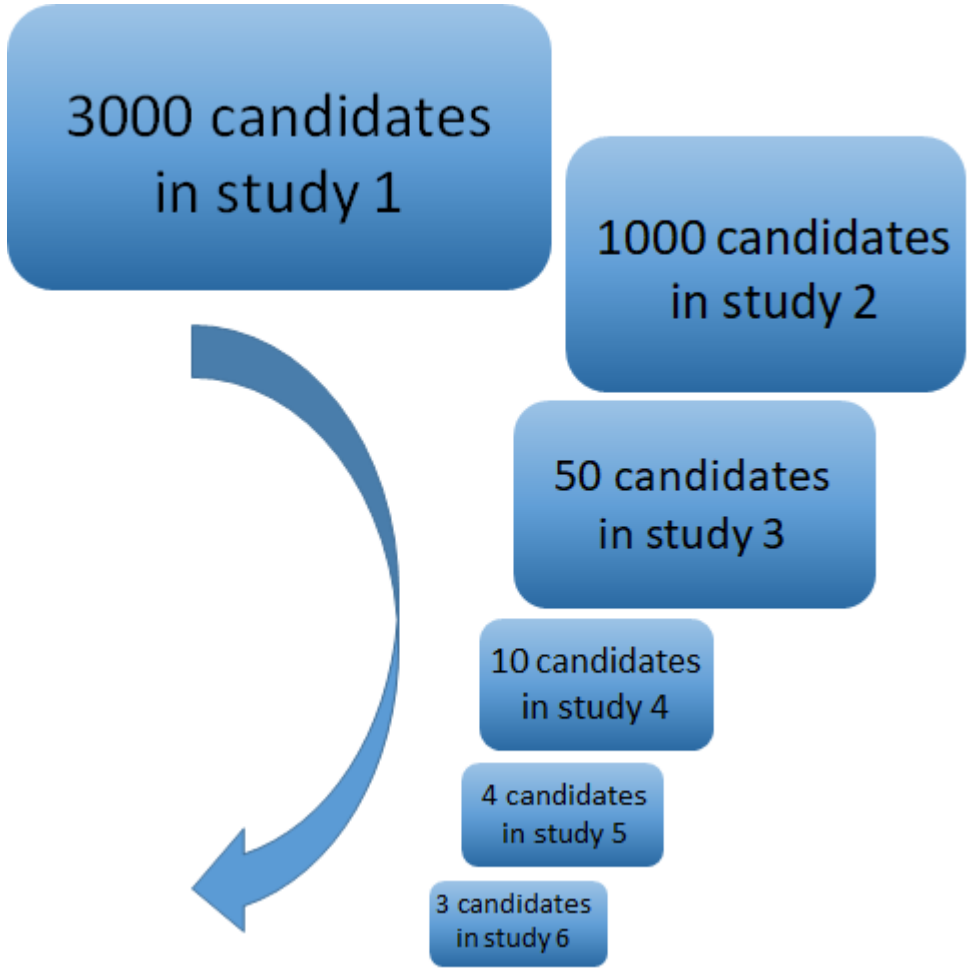


(Ambros *et al*. 2001)

Calibration studies

Squid Game. Created by Hwang Dong-hyuk, Netflix, 2021.

Index	MYCN_fn1	MAPK_RA	MAPK_RA	p53_fn	p73_fn	HIF_fn	P_cycle_s	P_DNA_da	P_DNA_c
677	0.277863	0.296396	0.081294	0.121137	0.168345	0.943243	0.529251	0.222474	0.99045
184	0.484521	0.518488	0.252074	0.676754	0.436464	0.658059	0.519606	0.614104	0.76648
2991	0.301635	0.87196	0.421385	0.797464	0.786514	0.234779	0.385223	0.219635	0.92531
825	0.892225	0.787593	0.215333	0.856983	0.718434	0.925868	0.25681	0.292857	0.98810
564	0.942648	0.377628	0.003161	0.19809	0.141042	0.59177	0.344412	0.772948	0.77149
1540	0.245592	0.997054	0.615927	0.603909	0.193378	0.311584	0.328683	0.659884	0.81446
2193	0.761934	0.675797	0.390508	0.893939	0.19777	0.760859	0.975454	0.337441	0.96006
1556	0.501221	0.879769	0.545846	0.085968	0.131161	0.13793	0.158815	0.126268	0.49899
675	0.69287	0.529858	0.232187	0.806742	0.69036	0.254842	0.541578	0.989668	0.97136
1892	0.547878	0.673346	0.579237	0.132174	0.816287	0.973364	0.553501	0.631952	0.87172
2307	0.832634	0.59399	0.204702	0.913315	0.654981	0.393086	0.472165	0.259098	0.90294
2198	0.485041	0.909258	0.218517	0.203592	0.042106	0.460479	0.623141	0.795116	0.95873
1106	0.815031	0.984735	0.400839	0.28017	0.267183	0.39705	0.947455	0.01568	0.10544



Selection process.

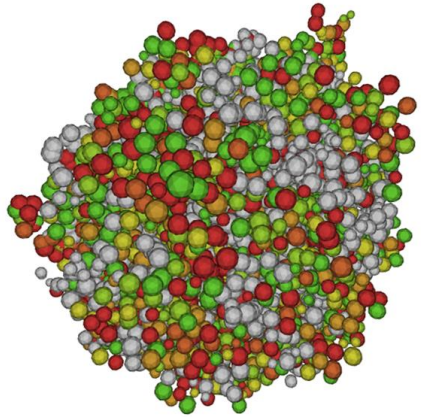
- Eliminated the 3000 candidates gradually in a tournament of 6 studies.
- The set of parameters that describe the experiments and observations best.

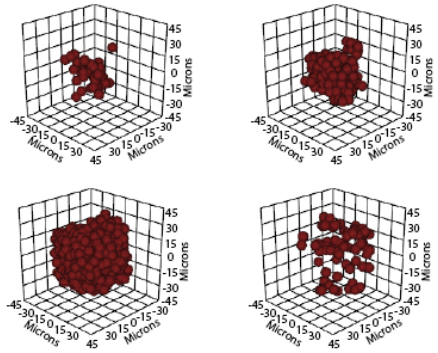
Party Conversation Starter 4.

What is an inverse problem?

Given a set of observations about the effects (data in the literature), infer the causes (parameters) responsible for the observations.

Calibration studies implemented on GPUs



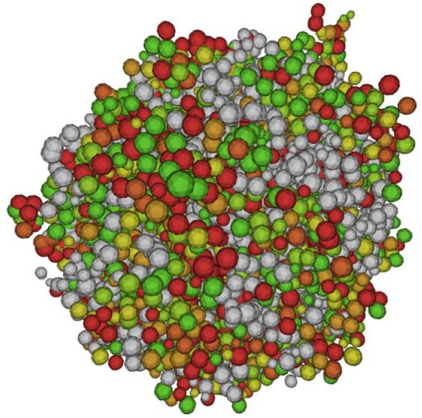


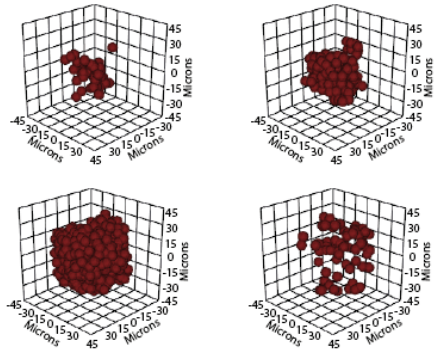
FLAME GPU

High computational costs.

- Millions of cells.
- Four months in a patient’s life.
- Stochastic simulations.

Calibration studies implemented on GPUs





FLAME GPU

High computational costs.

- Millions of cells.
- Four months in a patient's life.
- Stochastic simulations.

High-Performance computing.

- GPUs are not just for gaming.
- CUDA is used to program GPUs.
- FLAMEGPU maps model descriptions to optimised CUDA code.

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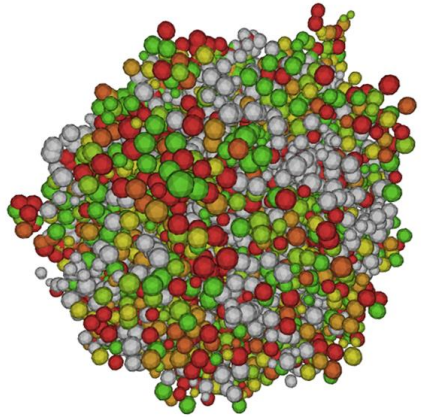

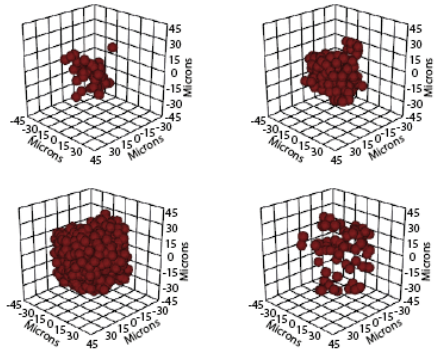

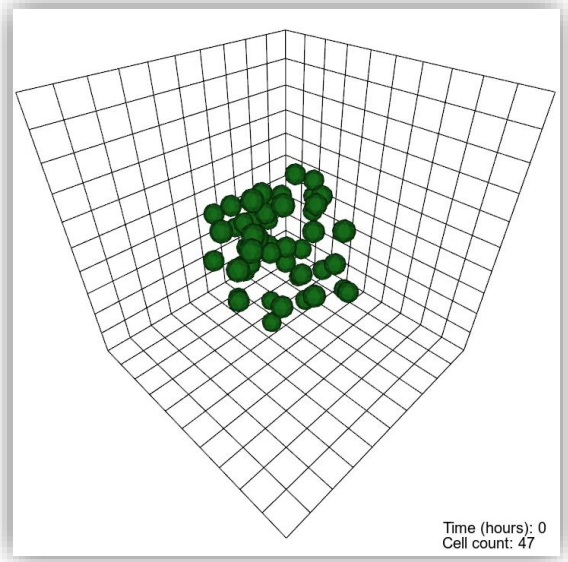

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Computational time.

- 2 TITAN V GPUs, 1 TITAN XP GPU, and 1 TITAN RTX GPU.
- 3000 time steps (3000 hours) took 5 to 10 minutes to simulate.
- **All 6 studies took around 40 days.**
- Impractical without GPUs.

Party Conversation Starter 5.

How can one speed up the computation of a Taylor series?

$$\ln(1+x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \cdots = \sum_{k=1}^{n} \frac{(-1)^{k+1} x^k}{k}$$

We want the first 1000 terms.

Method 1.
Calculate the 1000 terms one by one and add them together on one computer.

Method 2.
1. Compute and sum the first 500 terms on one computer.
2. Do the same for the next 500 terms on another computer.
3. Add the two sums for the final result.

Objectives

1. PRIMAGE project.

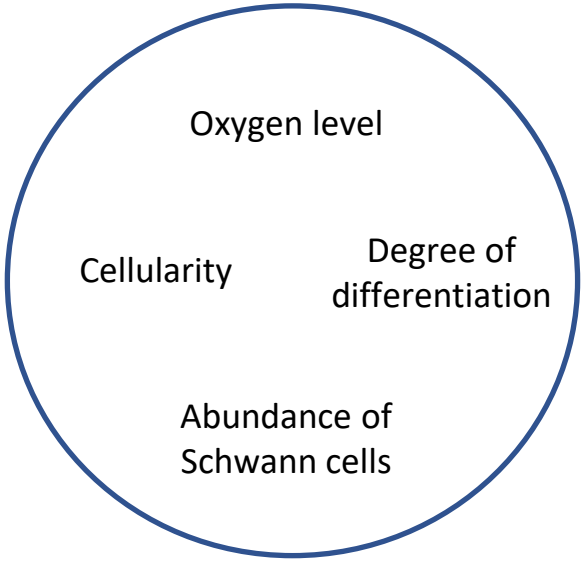
2. Neuroblastoma.

3. The first multicellular model of neuroblastoma.

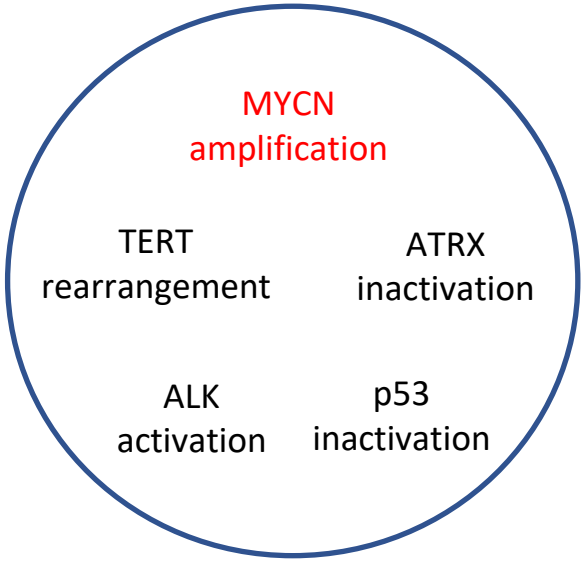
4. Model calibration using GPUs.

5. Predictive simulations.

Are macroscopic or microscopic features more important for neuroblastoma?



Macroscopic features.



24 clones with different combinations of these mutations.

Microscopic features = clonal composition.

Are macroscopic or microscopic features more important for neuroblastoma?

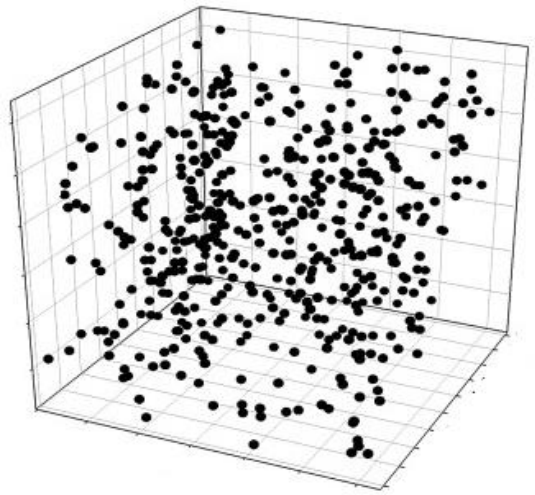
Oxygen level

Cellularity

Degree of differentiation

Abundance of Schwann cells

Macroscopic features.



1200 random combinations of these 28 variables.

1200 virtual tumours.

MYCN amplification

TERT rearrangement

ATRX inactivation

ALK activation

p53 inactivation

24 clones with different combinations of these mutations.

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Cellularity

Degree of differentiation

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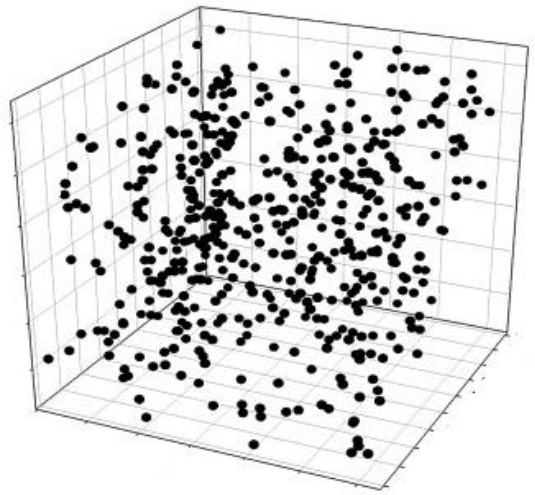

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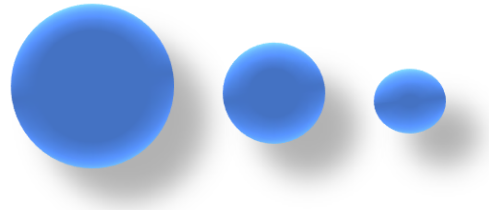
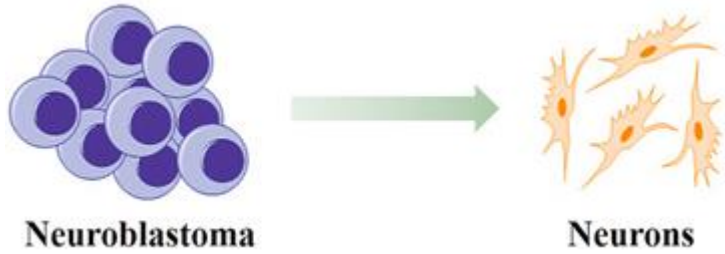


Regression.

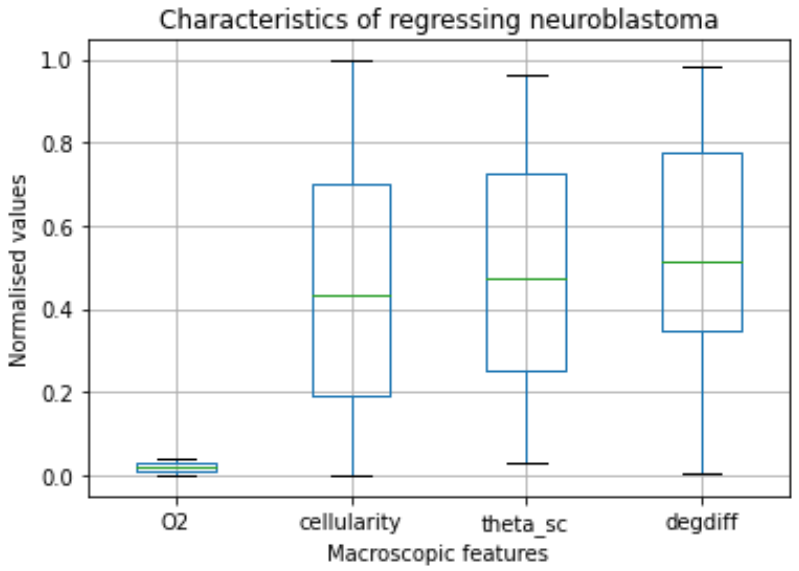


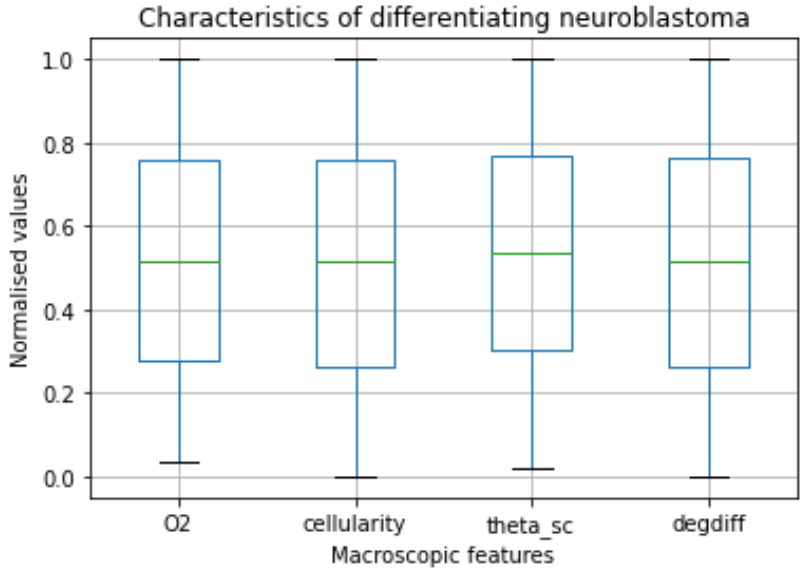
Jin, Zegao, et al. "Development of differentiation modulators and targeted agents for treating neuroblastoma." *European Journal of Medicinal Chemistry* (2020): 112818.

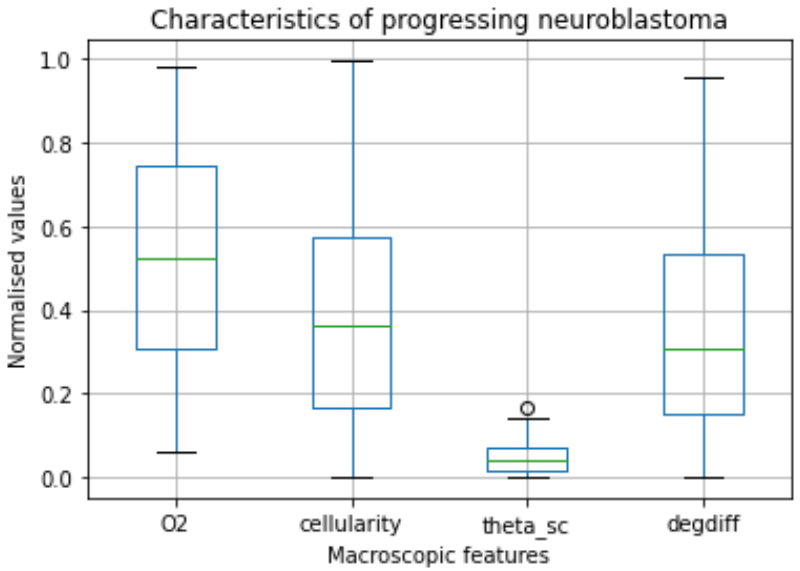
Differentiation into heathy neurons.

Progression.

Driving mechanisms of regression and progression



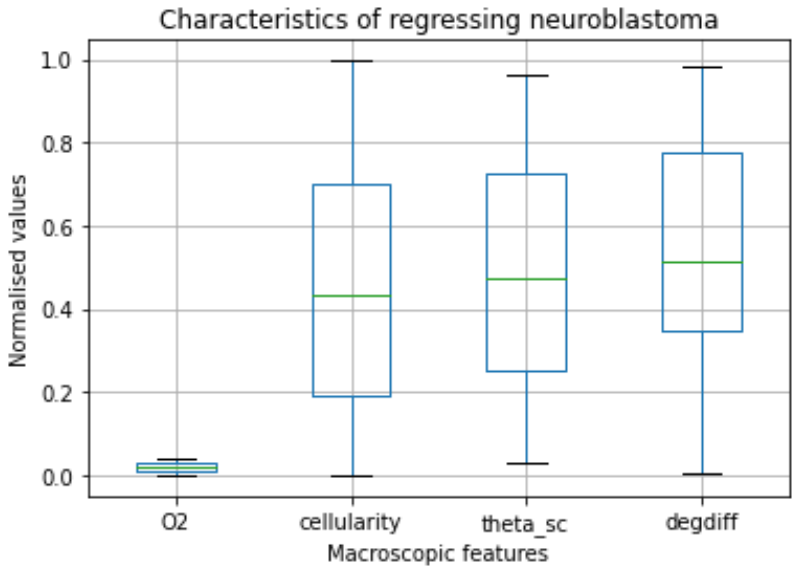


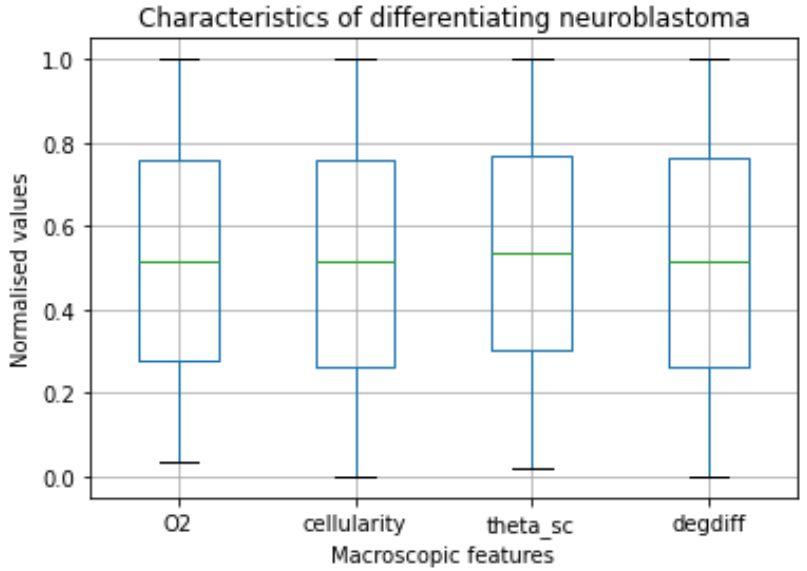


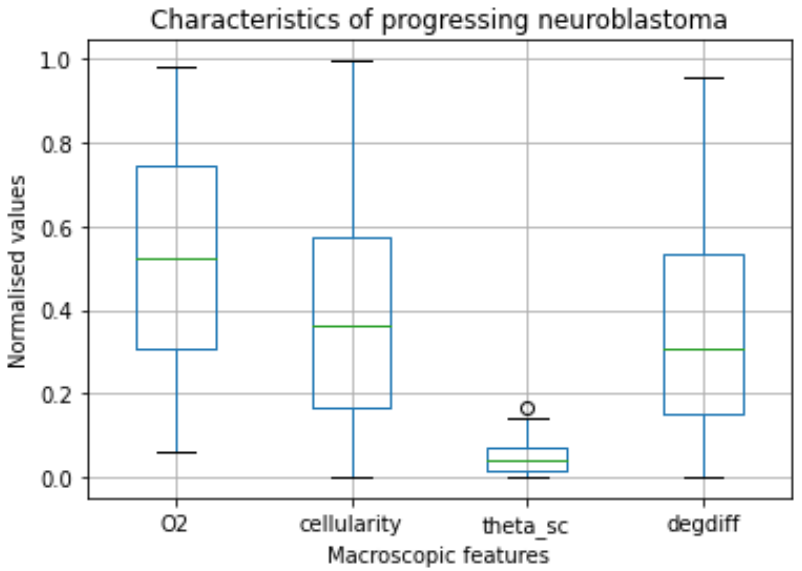
A lack of oxygen results in regression.

Even with sufficient oxygen, neuroblastoma can only progress without too many Schwann cells.

Driving mechanisms of regression and progression





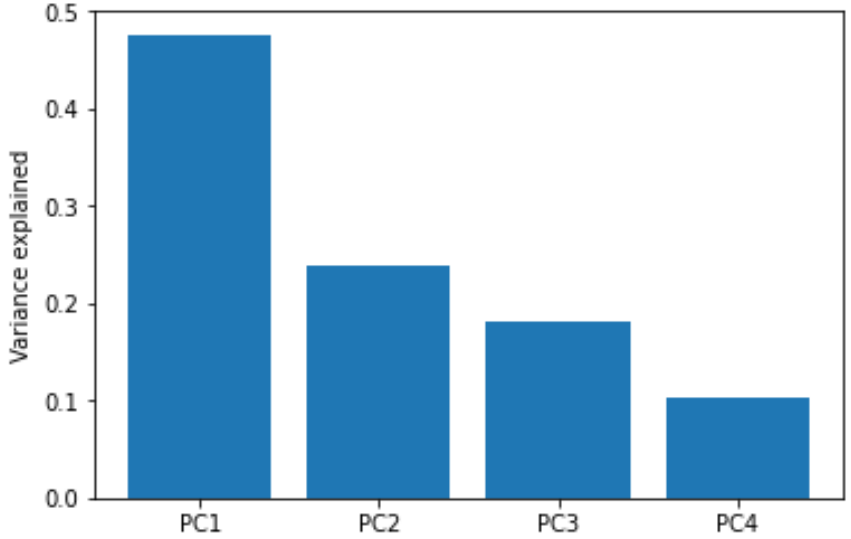


Low oxygen.
Many Schwann cells.

High oxygen.
Few Schwann cells.

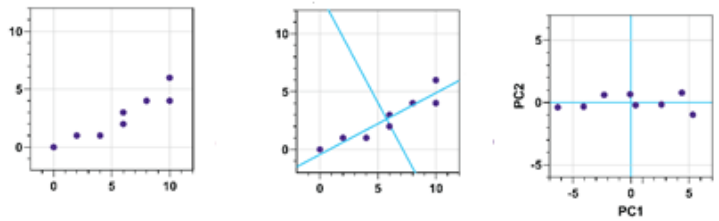
Driving mechanisms of regression and progression

Potential latent features distinguishing regression from progression

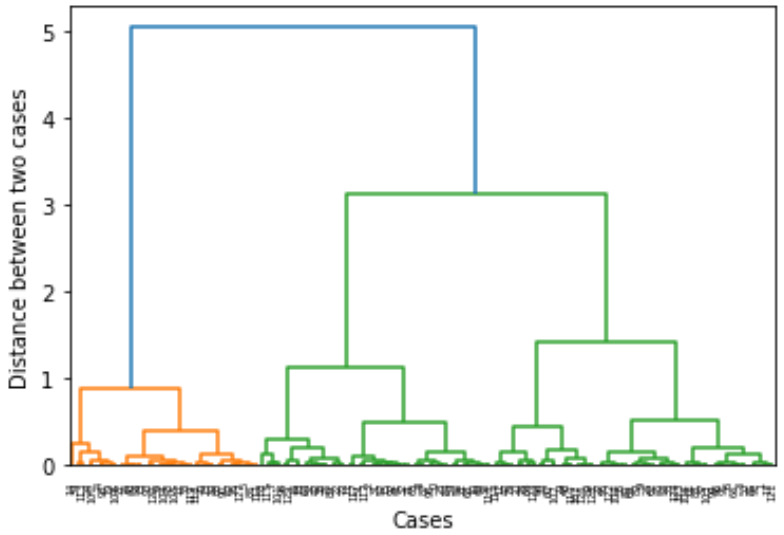


Combined the regression and progression datasets.

Principal component analysis (PCA) on the four macroscopic features.



Clustering regressing and progressing cases, using one principal component



Projected the macroscopic features on the first principal component (PC1) and produced two clusters there.

Hierarchical clustering and DBSCAN.

Tried to reproduce the two original datasets without knowing the label of each case (regression or progression).

Adjusted rand index: 0.748.

Party Conversation Starter 6.

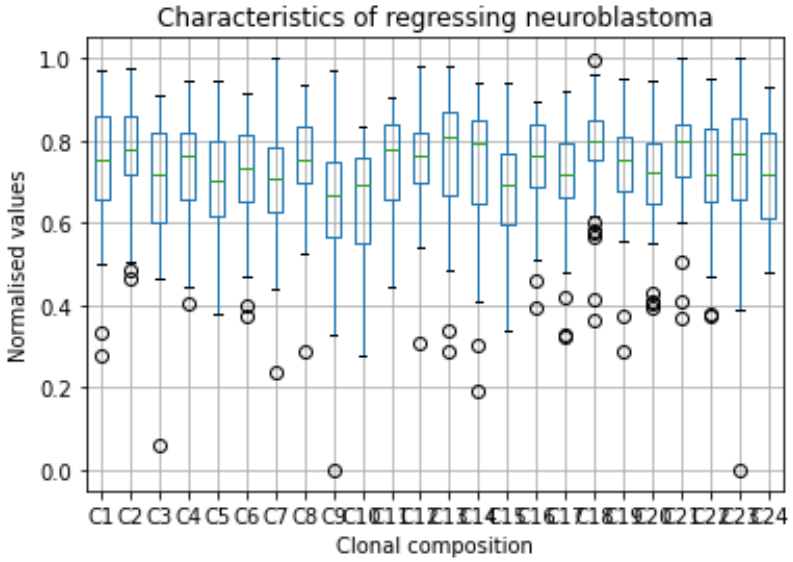
What is unsupervised machine learning?

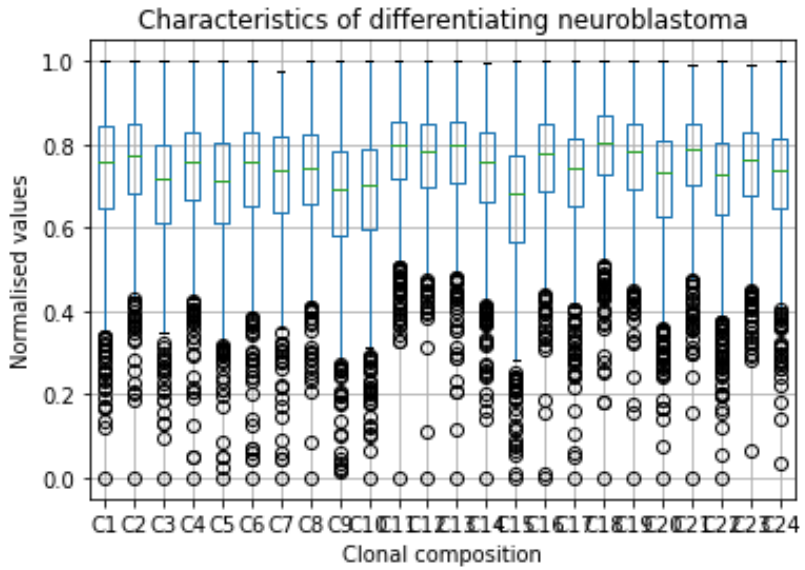
It aims to find the structure of an unlabelled dataset.

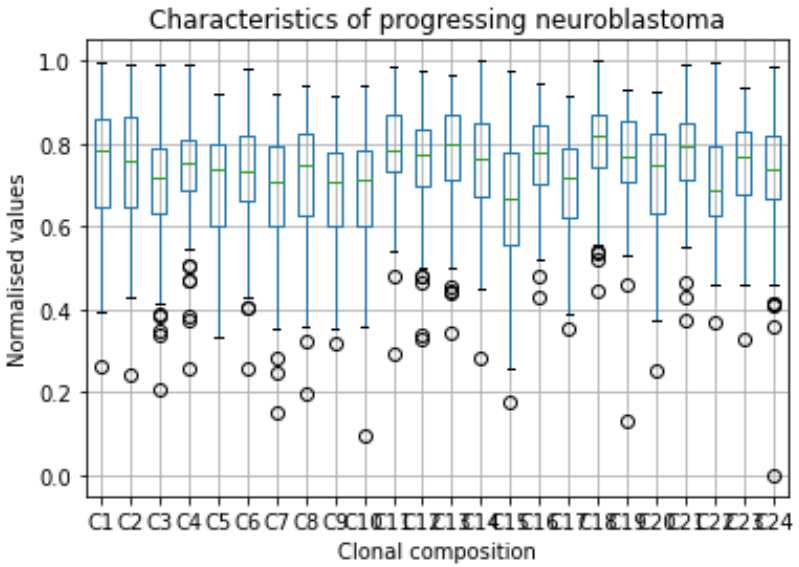
Dimensionality reduction (PCA): Represent heights and weights of teenagers as one latent feature, their ages.

Clustering: Create grade boundaries in a set of test scores.

Driving mechanisms of regression and progression







Clonal composition does not affect the disease outcome!

Consistent with clinical observations.

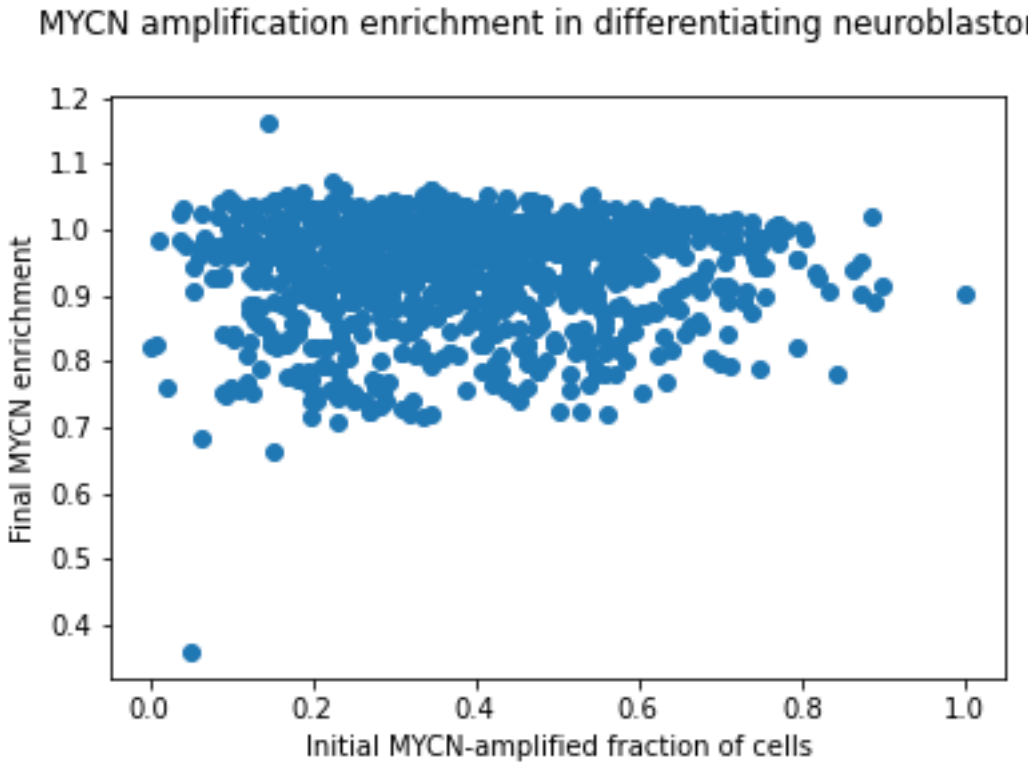
Heterogeneous *MYCN* amplification in neuroblastoma: a SIOP Europe Neuroblastoma Study

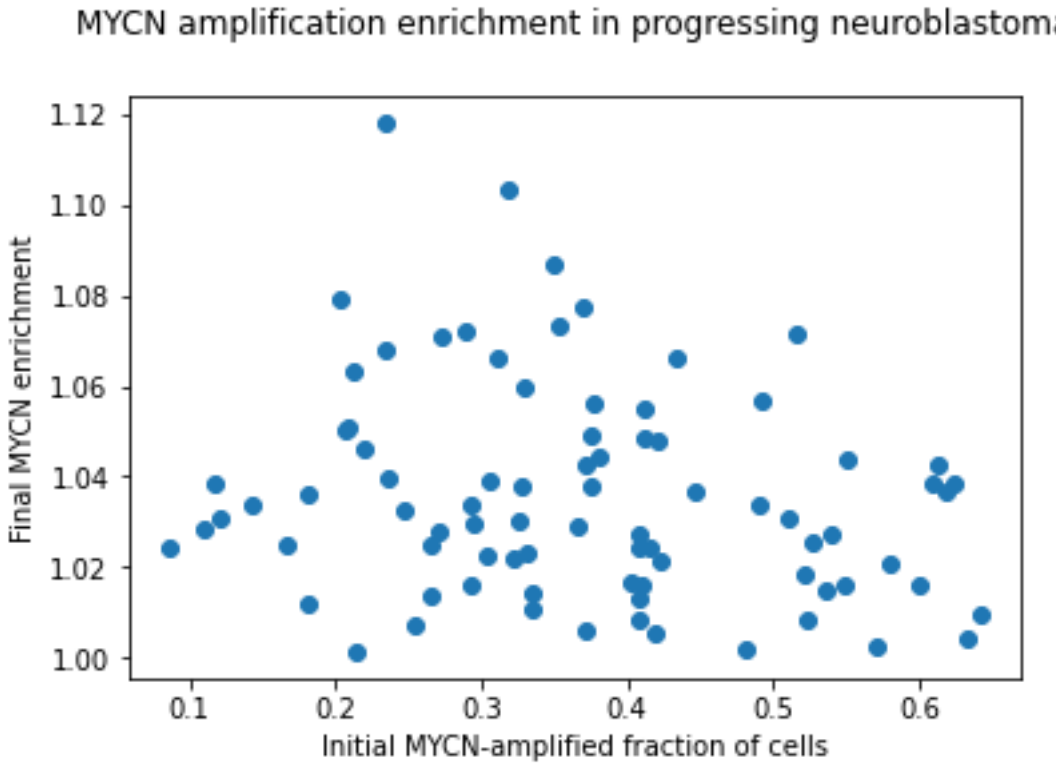
Ana P. Berbegall[1,2], Dominik Bogen[3], Ulrike Pötschger[4], Klaus Beiske[5], Nick Bown[6], Valérie Combaret[7], Raffaella Defferrari[8], Marta Jeison[9], Katia Mazzocco[8], Luigi Varesio[10], Ales Vicha[11], Shifra Ash[12], Victoria Castel[13], Carole Coze[14], Ruth Ladenstein[4,15], Cormac Owens[16], Vassilios Papadakis[17], Ellen Ruud[18], Gabriele Amann[19], Angela R. Sementa[8], Samuel Navarro[1,2], Peter F. Ambros[3,20], Rosa Noguera[1,2] and Inge M. Ambros[3]

BACKGROUND: In neuroblastoma (NB), the most powerful prognostic marker, the *MYCN* amplification (MNA), occasionally shows intratumoural heterogeneity (ITH), i.e. coexistence of *MYCN*-amplified and non-*MYCN*-amplified tumour cell clones, called heterogeneous MNA (hetMNA). Prognostication and therapy allocation are still unsolved issues.
METHODS: The SIOPEN Biology group analysed 99 hetMNA NBs focussing on the prognostic significance of *MYCN* ITH.
RESULTS: Patients <18 months (18 m) showed a better outcome in all stages as compared to older patients (5-year OS in localised stages: <18 m: 0.95 ± 0.04, >18 m: 0.67 ± 0.14, $p = 0.011$; metastatic: <18 m: 0.76 ± 0.15, >18 m: 0.28 ± 0.09, $p = 0.084$). The genomic 'background', but not MNA clone sizes, correlated significantly with relapse frequency and OS. No relapses occurred in cases of only numerical chromosomal aberrations. Infiltrated bone marrows and relapse tumour cells mostly displayed no MNA. However, one stage 4s tumour with segmental chromosomal aberrations showed a homogeneous MNA in the relapse.
CONCLUSIONS: This study provides a rationale for the necessary distinction between heterogeneous and homogeneous MNA. HetMNA tumours have to be evaluated individually, taking age, stage and, most importantly, genomic background into account to avoid unnecessary upgrading of risk/overtreatment, especially in infants, as well as in order to identify tumours prone to developing homogeneous MNA.

British Journal of Cancer (2018) 118:1502–1512; https://doi.org/10.1038/s41416-018-0098-6

Is having a large initial population a competitive advantage for the MYCN-amplified clone?





The spread at each value on the x-axis suggests it is not an advantage.

x-axis: initial clone size.
y-axis: final clone size/initial clone size.
A regressing tumour has no living neuroblasts at the end; dataset not shown.

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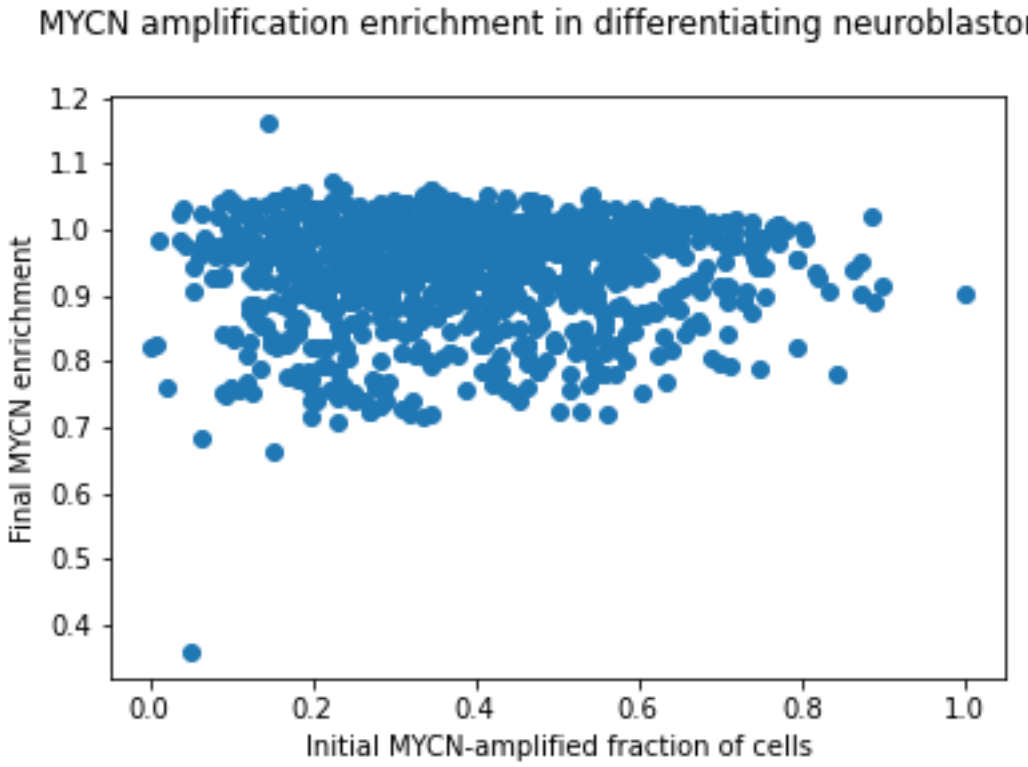
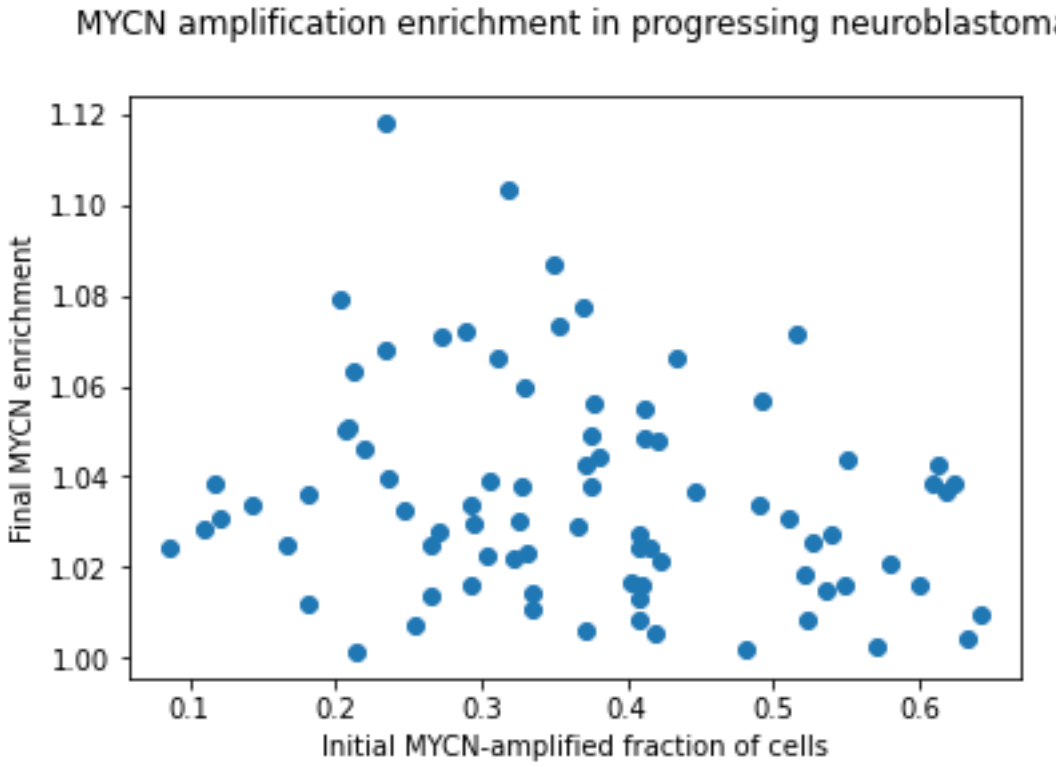




Enrichment more obvious in this dataset. Macroscopic features favouring progression also select the MYCN-amplified clone.

x-axis: initial clone size.
y-axis: final clone size/initial clone size.
A regressing tumour has no living neuroblasts at the end; dataset not shown.

Conclusions

1. PRIMAGE project aims to build a cloud-based decision-making platform for the clinical management of malignant solid tumours.

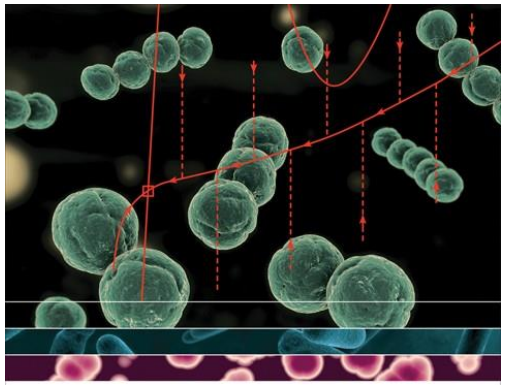
2. Built and calibrated the first multicellular model of neuroblastoma.

3. Macroscopic features of neuroblastoma are better predictors of disease outcome than clonal composition.

4. Biology is as quantitative as physics.

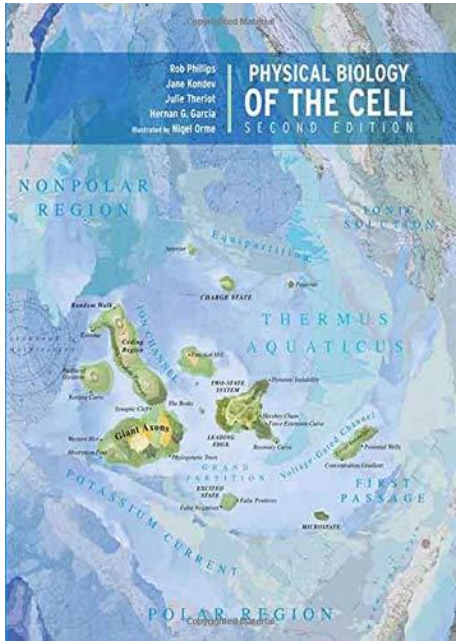
Society for Mathematical Biology

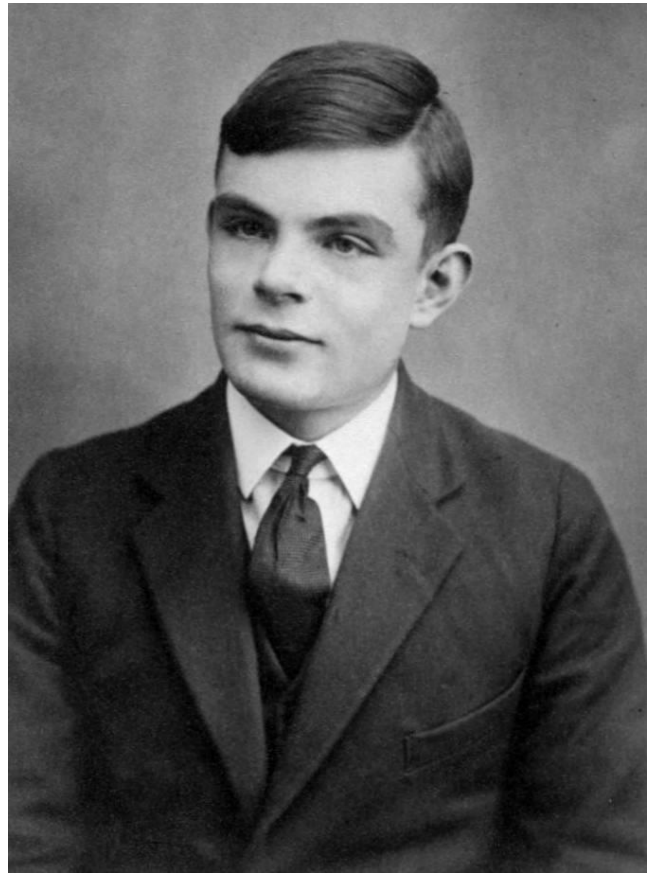
ISCB
INTERNATIONAL SOCIETY FOR COMPUTATIONAL BIOLOGY

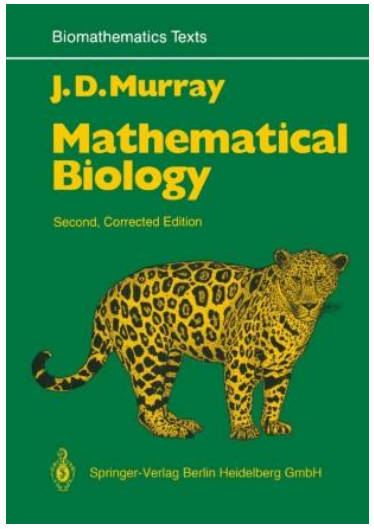


Mathematical Modeling in Systems Biology AN INTRODUCTION

Brian P. Ingalls







PLOS COMPUTATIONAL BIOLOGY

Read my publications.
kywertheim.com