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A GPU-Accelerated Model of Neuroblastoma to Predict Disease Outcome and Find Drug Targets

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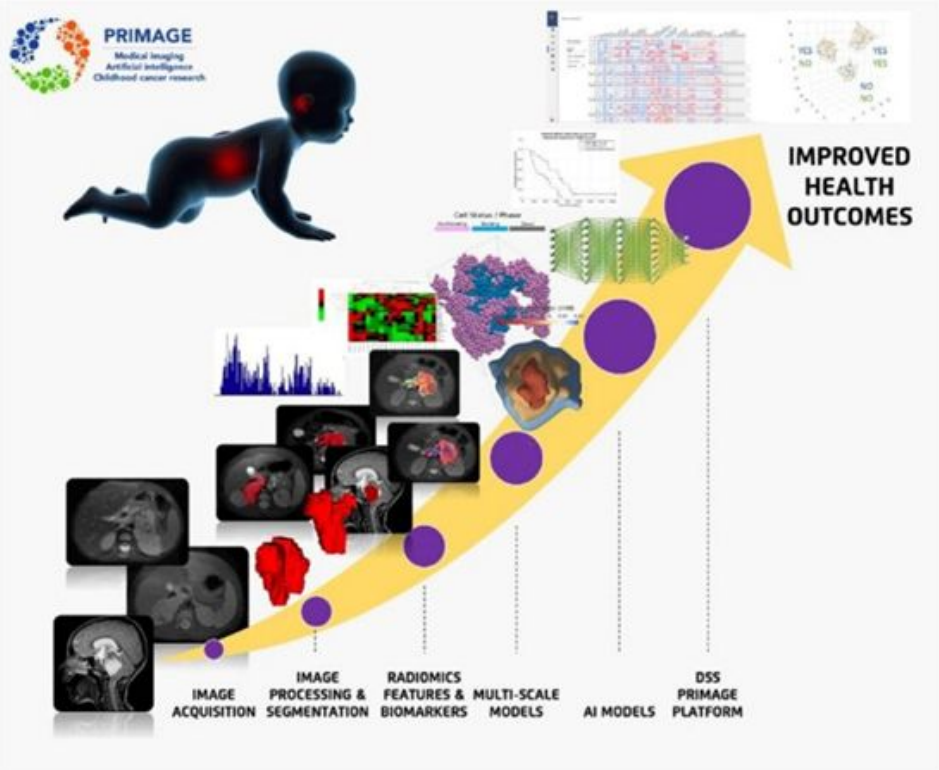
The University Of Sheffield.

PRIMAGE
Medical imaging
Artificial intelligence
Childhood cancer research

Horizon 2020
European Union Funding
for Research & Innovation

Objectives

1. PRIMAGE project.
2. Neuroblastoma.
3. First multicellular model of neuroblastoma.
4. Calibration.
5. Clonal competition.
6. MYCN enigma.
7. Targeted therapies.



Martí-Bonmatí, Luis, et al. "PRIMAGE project: predictive in silico multiscale analytics to support childhood cancer personalised evaluation empowered by imaging biomarkers." *European radiology experimental* 4.1 (2020): 1-11.

Decision support system for the clinical management of malignant solid tumours.

PRIMAGE project: predictive *in silico* multiscale analytics to support childhood cancer personalised evaluation empowered by imaging biomarkers

Luis Martí-Bonmatí[1*], Ángel Alberich-Bayarri[2], Ruth Ladenstein[3], Ignacio Blanquer[4], J. Damian Segrelles[4], Leonor Cerdá-Alberich[5], Polyxeni Gkontra[5], Barbara Hero[6], J. M. García-Aznar[7,8], Daniel Keim[9], Wolfgang Jentner[9], Karine Seymour[10], Ana Jiménez-Pastor[2], Ismael González-Valverde[2], Blanca Martínez de las Heras[11], Samira Essiaf[12], Dawn Walker[13], Michel Rochette[14], Marian Bubak[15], Jordi Mestres[16], Marco Viceconti[17], Gracia Martí-Besa[5], Adela Cañete[11], Paul Richmond[13], Kenneth Y. Wertheim[13], Tomasz Gubala[15], Marek Kasztelnik[15], Jan Meizner[15], Piotr Nowakowski[15], Salvador Gilpérez[18], Amelia Suárez[18], Mario Aznar[18], Giuliana Restante[19] and Emanuele Neri[19]

I contributed the first multicellular model of neuroblastoma to the project.

This talk is about what I did with the model <u>outside PRIMAGE.</u>

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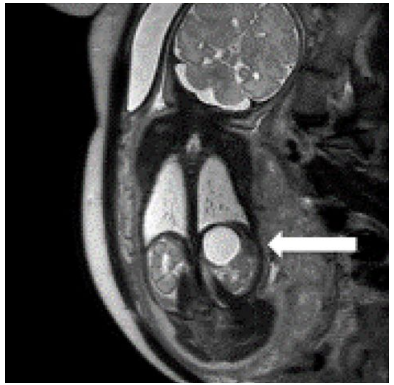
Neuroblastoma

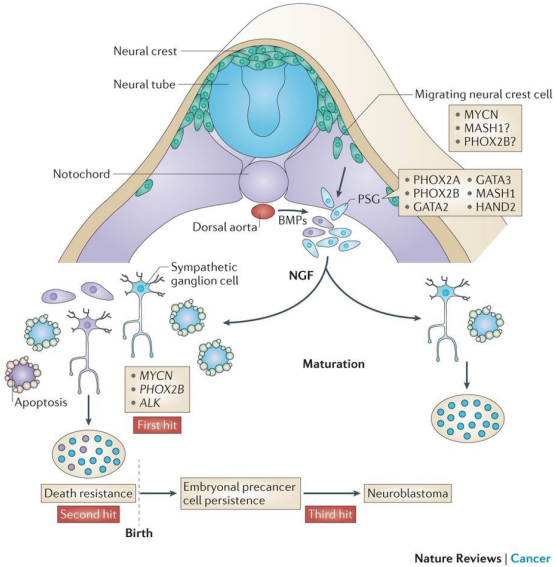


Louis, Chrystal U., and Jason M. Shohet. "Neuroblastoma: molecular pathogenesis and therapy." *Annual review of medicine* 66 (2015): 49.

1. Adrenal medulla is the usual primary site.

2. Most common extracranial solid tumour in children.

3. 15 % of cancer-related deaths in this population.



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

1. Neural crest, transient in the embryo.

2. Differentiate into different cell types.

3. Sympathetic nervous system.

4. MYCN amplification and ALK activation turn them into neuroblastoma cancer stem cells.

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.

1. Low risk, spontaneous regression.

2. High risk, 50 % relapse.

3. MYCN amplification is a bad sign.

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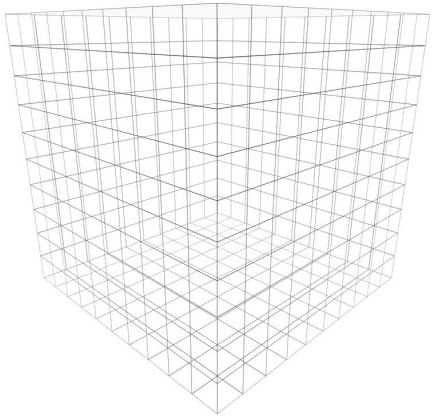
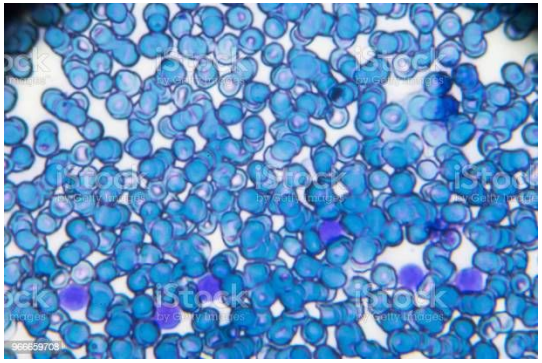
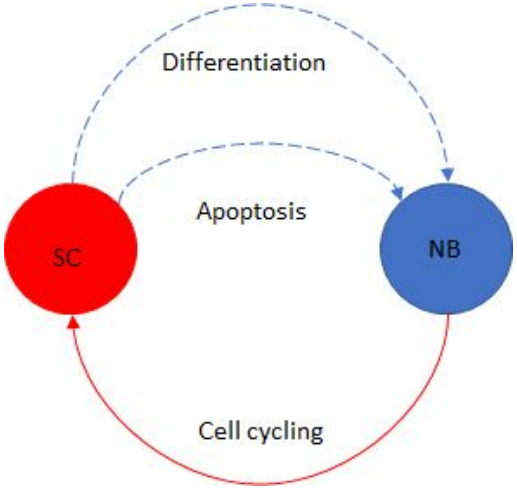
Multicellular model







Continuous automaton to voxelate the microenvironment.

1. Spatial distributions of cells and extracellular matrix.
2. Concentration dynamics of drugs and nutrients (uniform).

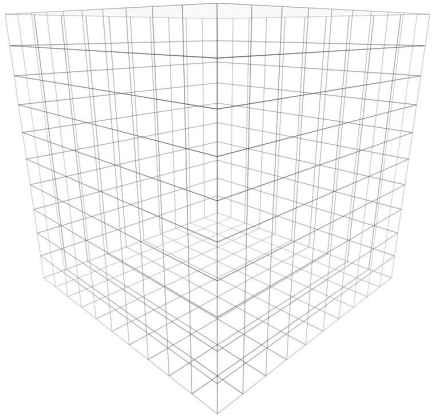
Discrete agents.

1. Neuroblasts and Schwann cells.
2. Cell cycling and death.

Agent attributes.

1. Mutations.
2. DNA status.
3. Gene expression levels.

Multicellular model



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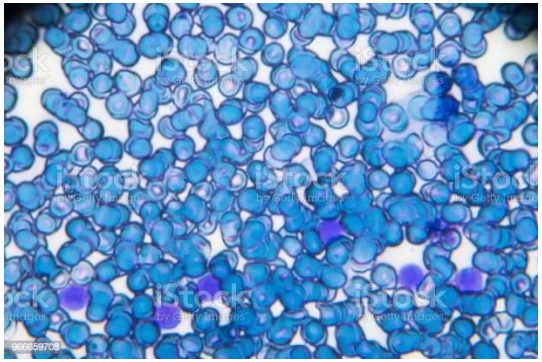

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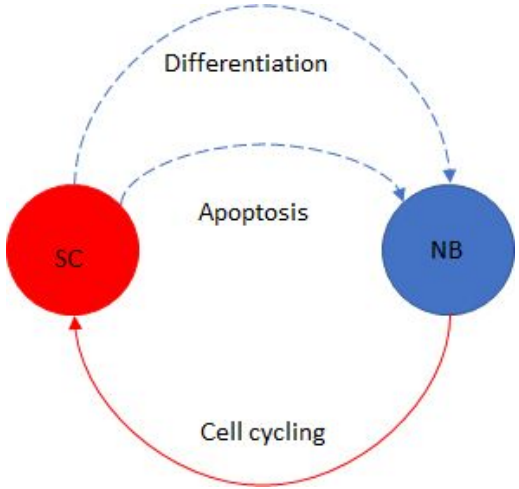
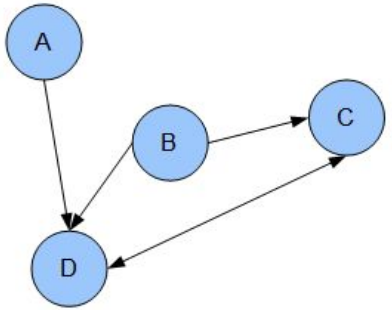
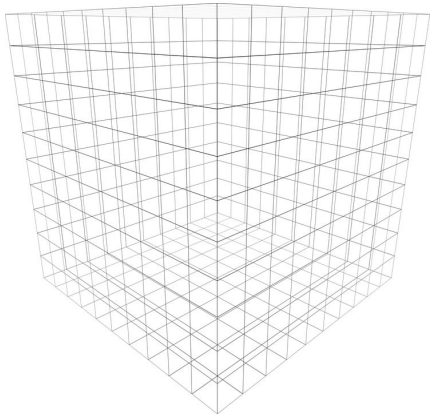




20 gene products.Telomerase, ALT, MYCN, MAPK/RAS pathway, JAB1, CHK1, CDS1, CDC25C, ID2, IAP2, HIF, BNIP3, VEGF, p53, p73, p21, p27, Bcl-2/Bcl-xL, BAK/BAX, and CAS.

Multicellular model



Continuous automaton to voxelate the microenvironment.

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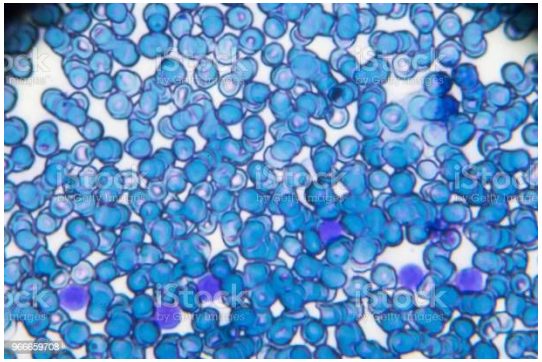

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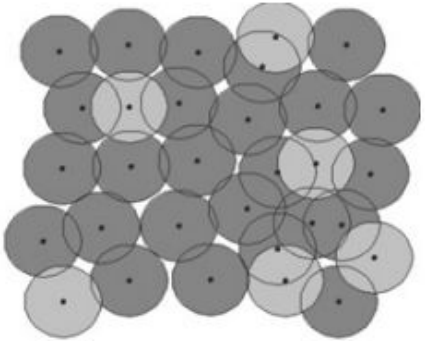

Centre-based mechanical model.

1. Resolve agent-agent overlap and contact inhibition.

2. Linear force law.

3. Equation of motion.

Stochastic simulation algorithm

1. Each agent senses the microenvironment and its neighbouring agents, modifies its behaviour, and updates its attributes.
2. Resolve agent-agent overlap using the mechanical model.
3. Modify the microenvironment by considering the agents collectively.
4. Back to step 1.

A series of Bernoulli trials. For example, is the MAPK/RAS pathway active?

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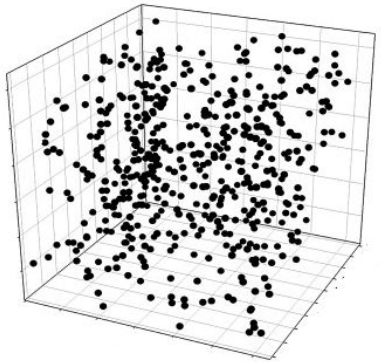
Calibration

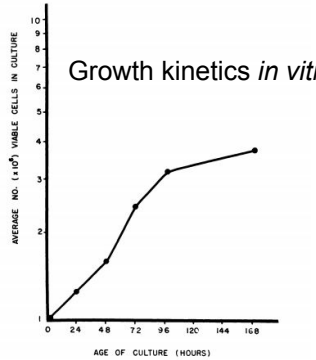


Latin hypercube sampling.

1. 3000 combinations of 20 fitting parameters.

2. Minimised differences between simulation results and *in vitro* data.

3. Refined calibrated parameters for *in vivo* use.



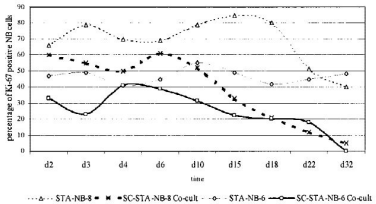
Growth kinetics *in vitro*.

Tumilowicz, Joseph J., et al. "Definition of a continuous human cell line derived from neuroblastoma." *Cancer research* 30.8 (1970): 2110-2118.

	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

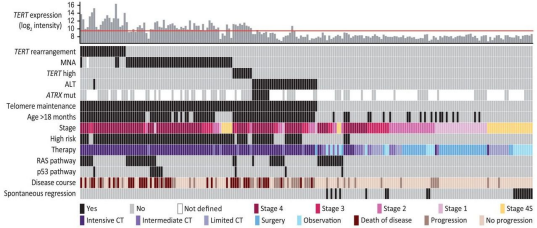
Warren, Daniel R., and Mike Partridge. "The role of necrosis, acute hypoxia and chronic hypoxia in 18F-FMISO PET image contrast: a computational modelling study." *Physics in Medicine & Biology* 61.24 (2016): 8596.

Extent of necrosis during hypoxia *in vitro*.



Ambros, Ingeborg M., et al. "Neuroblastoma cells provoke Schwann cell proliferation in vitro." *Medical and Pediatric Oncology: The Official Journal of SIOP—International Society of Pediatric Oncology (Societé Internationale d'Oncologie Pédiatrique)* 36.1 (2001): 163-168.

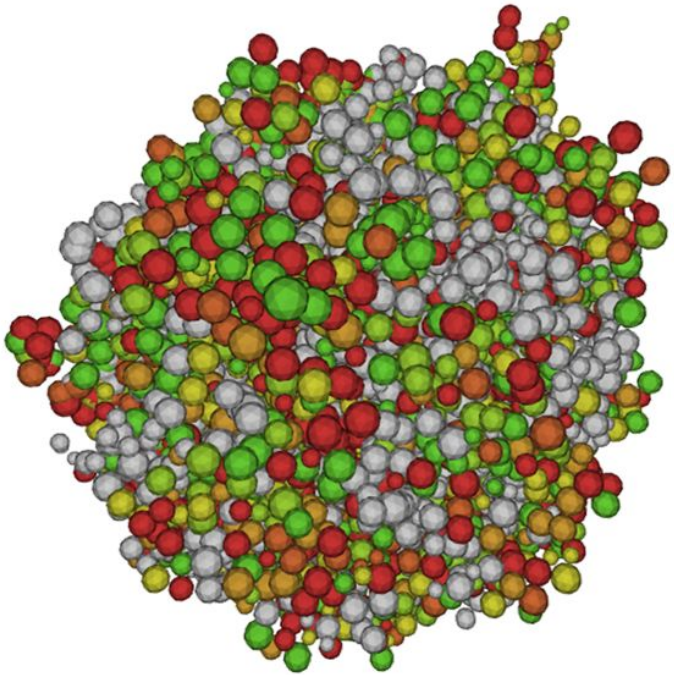
Interactions between neuroblastic and Schwann cells *in vitro*.



Ackermann, Sandra, et al. "A mechanistic classification of clinical phenotypes in neuroblastoma." *Science* 362.6419 (2018): 1165-1170.

Clinical outcomes associated with different mutations.

Calibration



Costly simulations.
1. Millions of agents.
2. Four months in a patient's life.
3. Stochastic simulations.

Simulations on GPUs.

1. FLAMEGPU and FLAMEGPU2 were used to generate optimised CUDA code.

2. 3000 time steps took up to 10 minutes.

3. Calibration took 40 days in total.

Hardware: 2 TITAN V GPUs, 1 TITAN XP GPU, and 1 TITAN RTX GPU.

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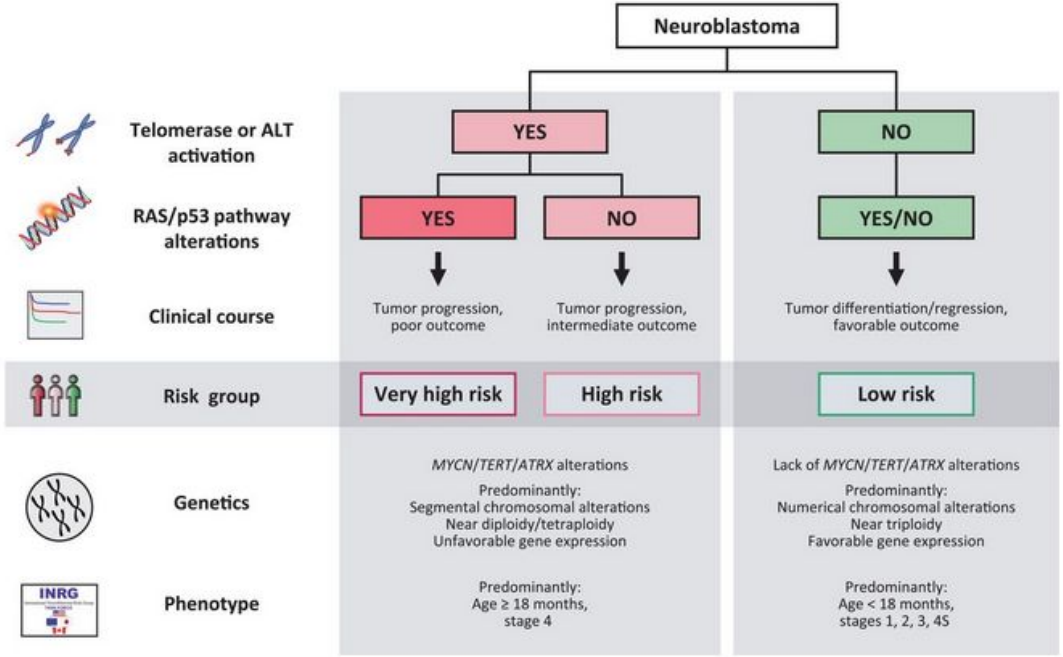
Clonal competition

Clonal composition.
1. Four clones.
2. Each clone has six subclones.

Clones: MYCN amplification, TERT rearrangement, ATRX inactivation, and wild type.

Subclones: combinations of p53 inactivation and ALK activation.

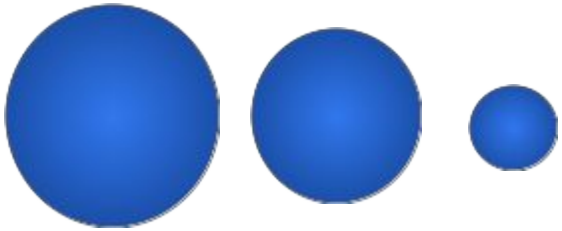
Macroscopic features.
1. Oxygen level.
2. Abundance of Schwann cells.



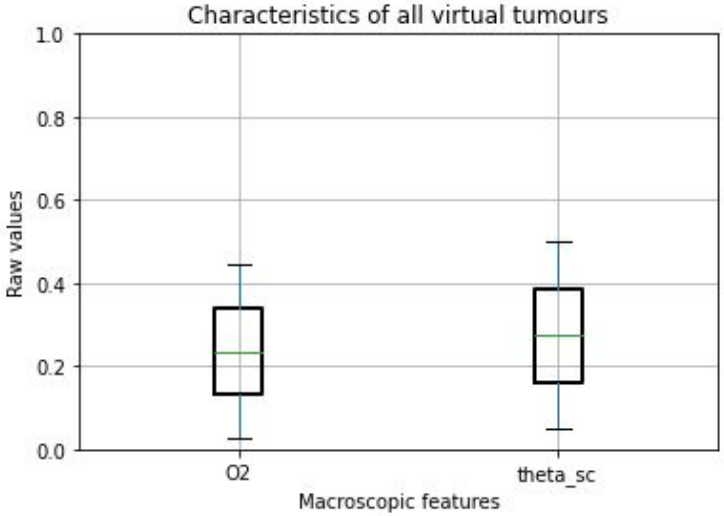
Ackermann, Sandra, et al. "A mechanistic classification of clinical phenotypes in neuroblastoma." *Science* 362.6419 (2018): 1165-1170.

Created 1200 virtual tumours with arbitrary clonal compositions and macroscopic features.

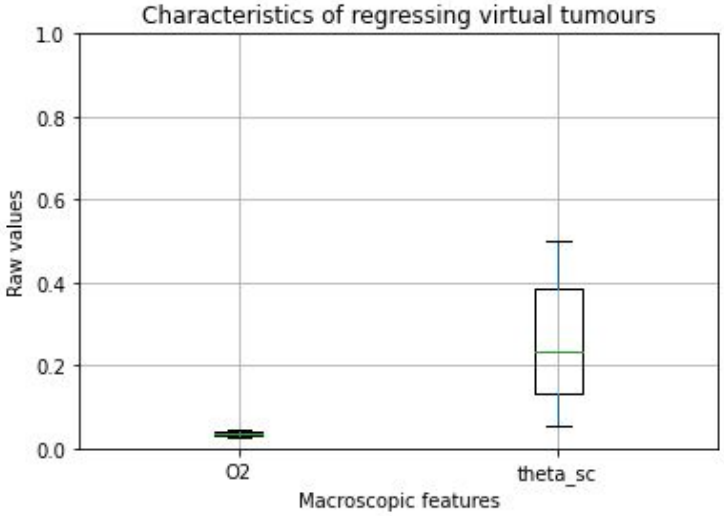
Clonal competition



Outcome 1: regression.
1. Driven by hypoxia.
2. Clonal composition did not influence the outcome (data not shown).



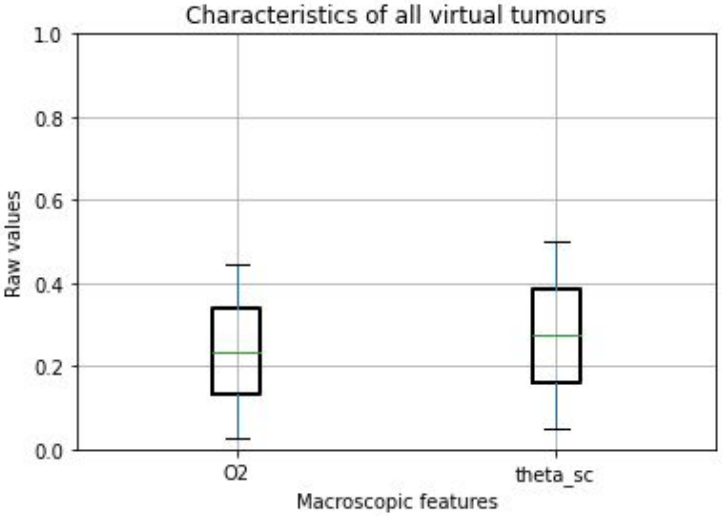
All 1200 virtual tumours (control group).



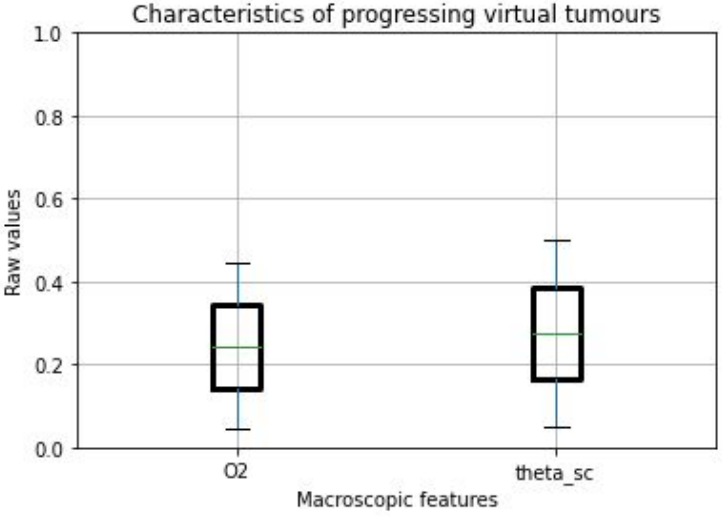
45 regressing cases.

Clonal competition

Outcome 2: progression.
1. Sufficient oxygen.
2. Clonal composition did not influence the outcome (data not shown).



All 1200 virtual tumours (control group).

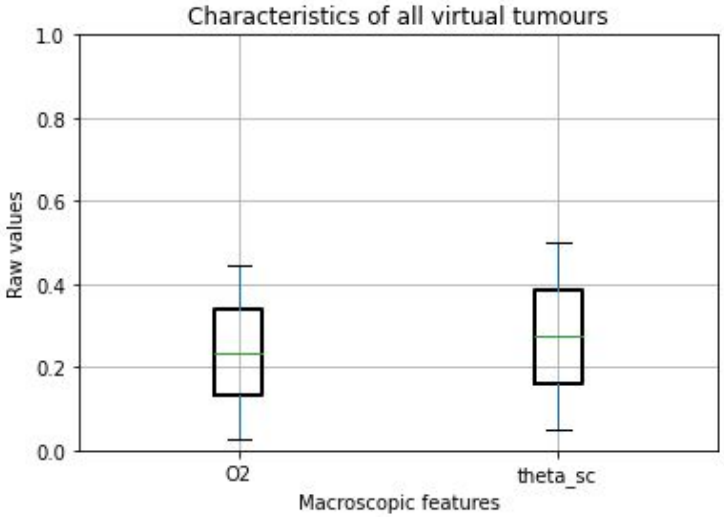


1155 progressing cases.

Clonal competition

Jin, Zegao, et al. "Development of differentiation modulators and targeted agents for treating neuroblastoma." *European journal of medicinal chemistry* 207 (2020): 112818.

Outcome 3: differentiation.
1. Unobserved.
2. Differentiation is rare in high-risk cases.
3. Schwann cells do not matter in this parametric regime, which describes high-risk neuroblastoma.



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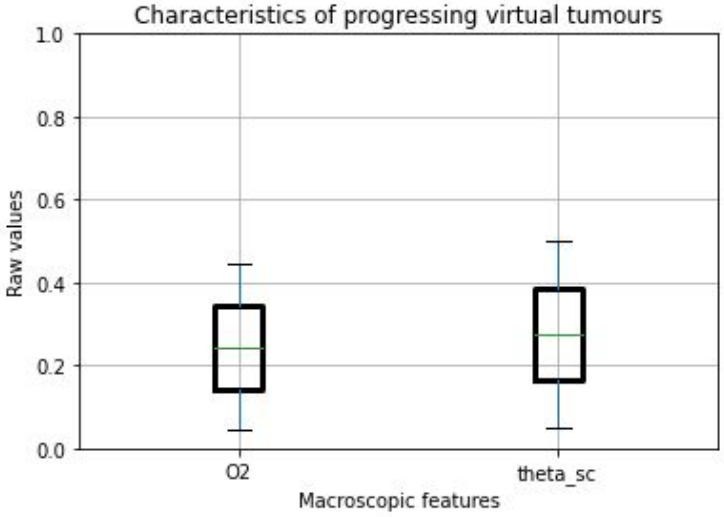

1155 progressing cases.

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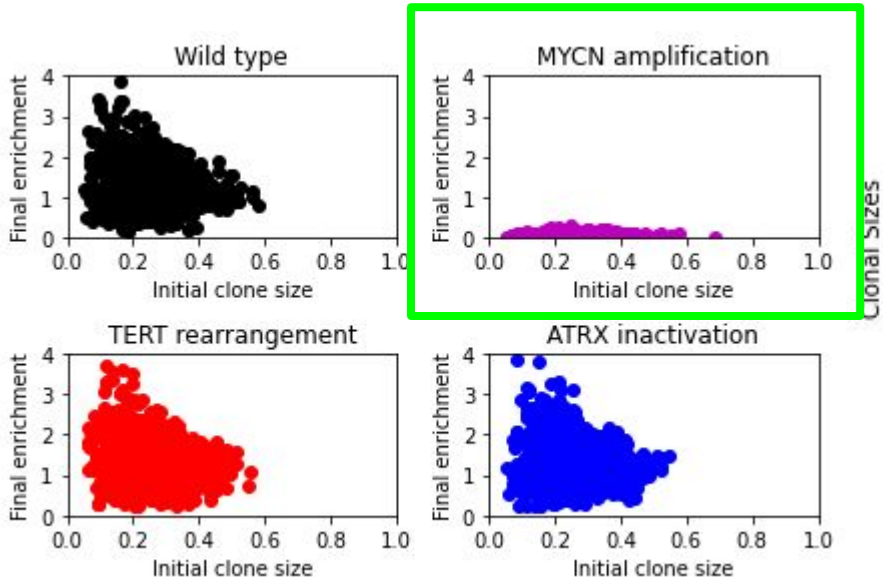
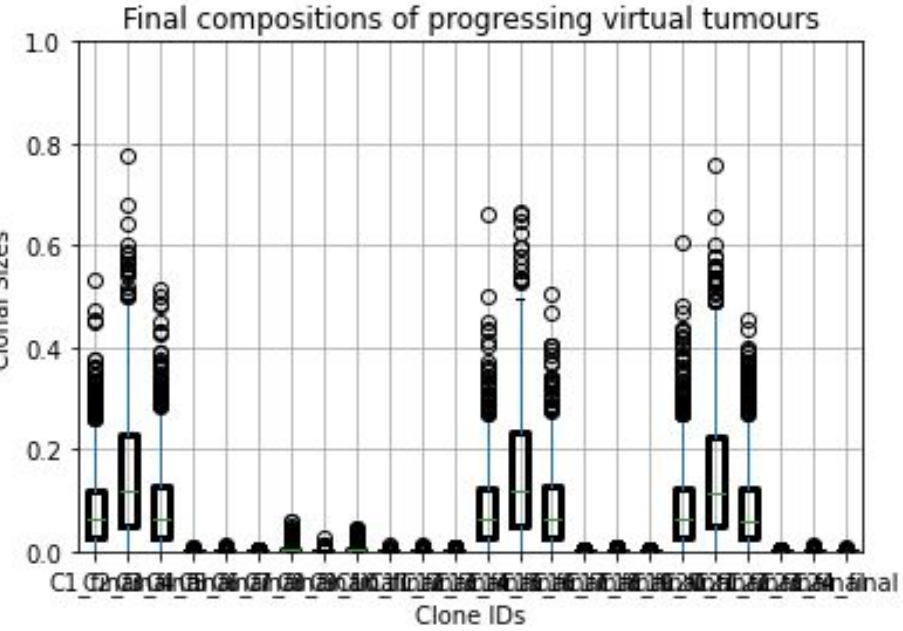
Clonal competition





MYCN-amplified clone died!

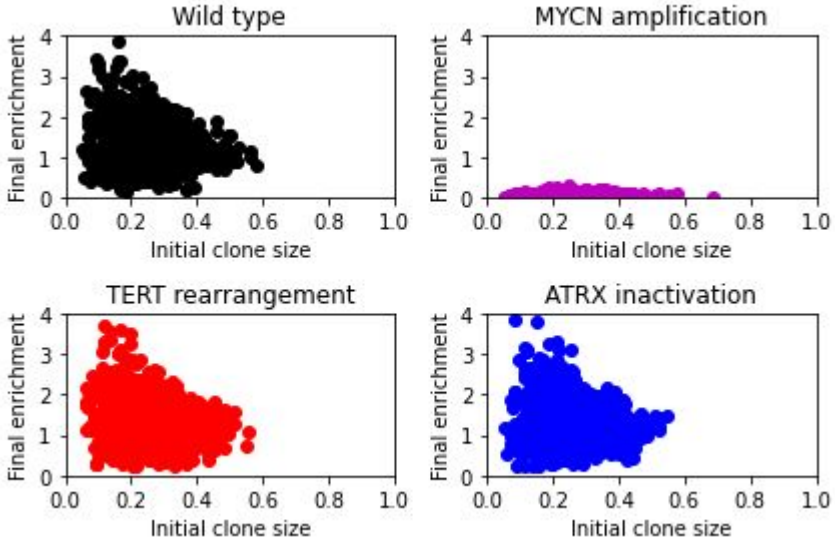
The other three expanded similarly.

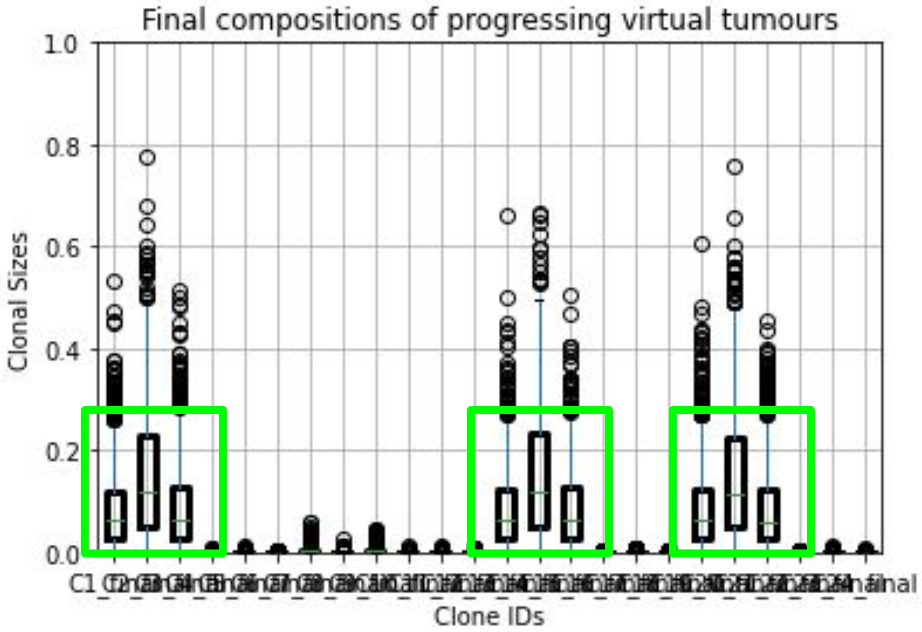
MA versus WT: p-value < 0.1 %
1. Student's t-test.
2. Permutation test.

ANOVA: p-value > 25 %
1. F-test.
2. Permutation test.

1155 progressing cases.

Clonal competition





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The nine growing subclones all had their p53 intact!

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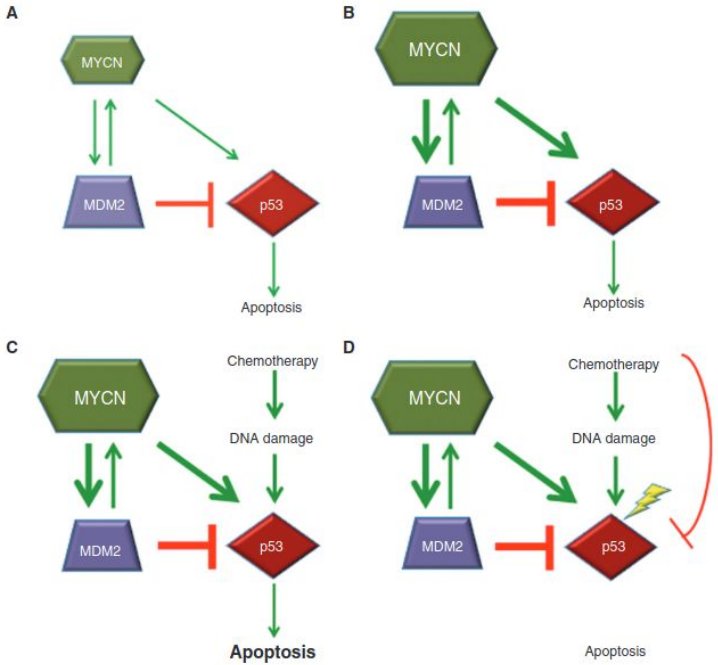
MYCN enigma



Huang, Miller, and William A. Weiss. "Neuroblastoma and MYCN." *Cold Spring Harbor perspectives in medicine* 3.10 (2013): a014415.

MYCN amplification is associated with p53 inactivation. **This is in the model.**

Gamble, Laura D., et al. "MYCN sensitizes neuroblastoma to the MDM2-p53 antagonists Nutlin-3 and MI-63." *Oncogene* 31.6 (2012): 752-763.

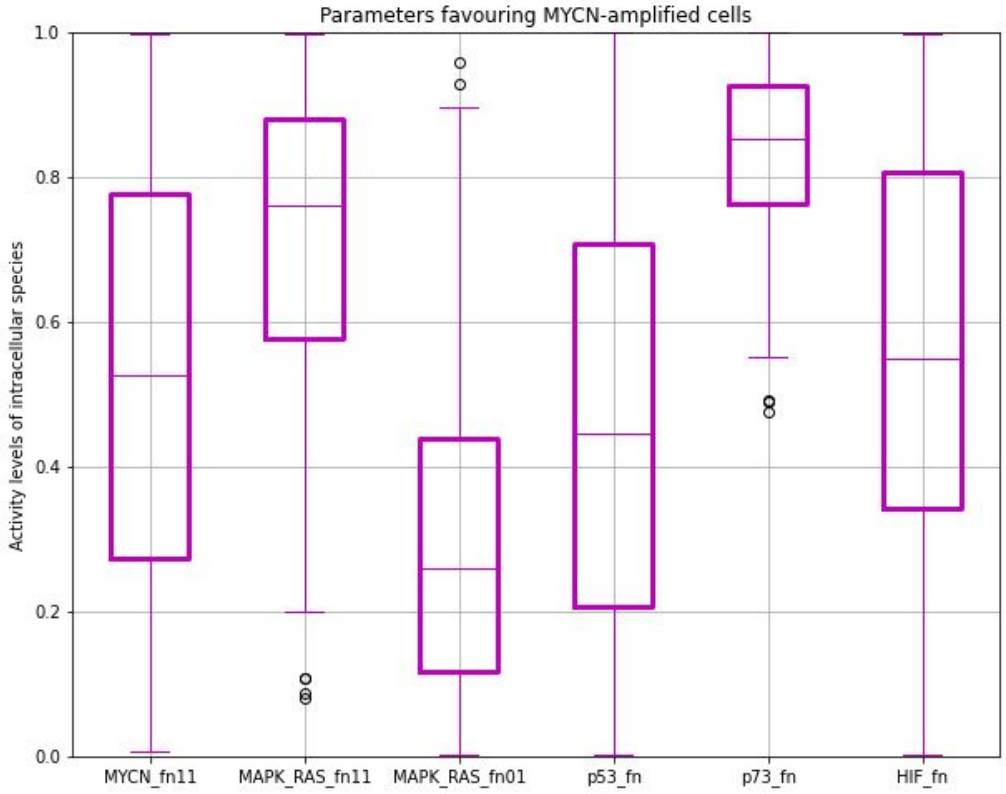
It is true that p53 triggers apoptosis, but it also repairs damaged DNA, among other things.

MYCN and p53 have a **non-linear relationship** with each other, and with the disease outcome.

Performed a sensitivity analysis on the gene expression levels of one virtual tumour.

MYCN, MAPK/RAS, p53, p73, and HIF.

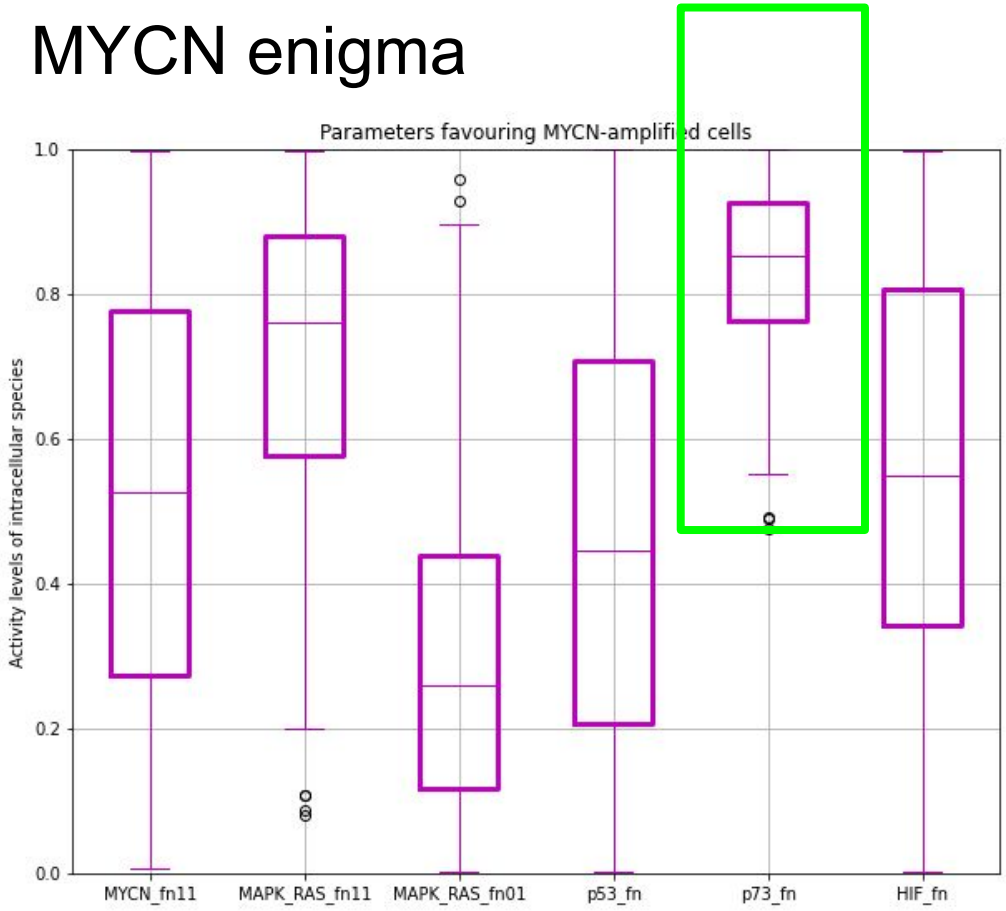
MYCN enigma



1000 combinations of gene expression levels.

283 cases where the **MYCN-amplified clone expanded drastically.**

MYCN enigma



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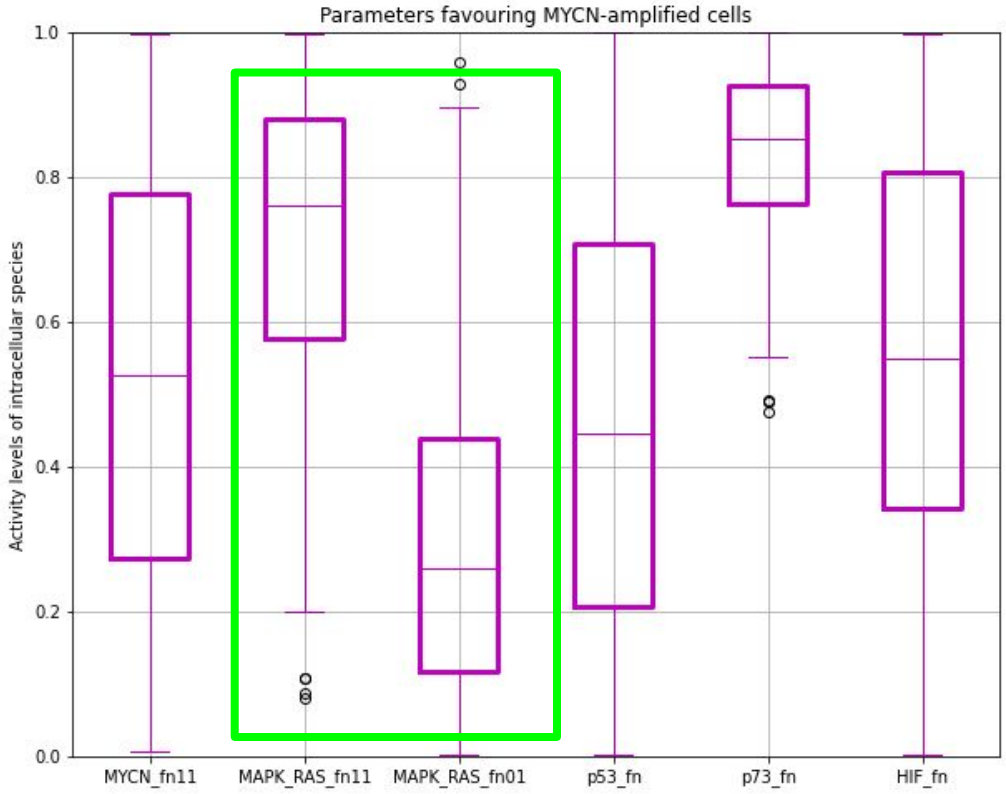
1. **p73,** which belongs to the same family, must be active enough to **compensate** for the inactivated p53.

MYCN enigma



1000 combinations of gene expression levels.

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1. p73, which belongs to the same family, must be active enough to compensate for the inactivated p53.

2. **MYCN amplification** must **boost** MAPK/RAS signalling (**cell cycling**) more than ALK activation.

MYCN enigma

Final MYCN-amplified clone sizes given different initial sizes

	Low O2	High O2
0	0	0
0.25	0.2521	0.252
0.5	0.5028	0.5026
0.75	0.7521	0.7519
1	1	1

Picked a set of parameters favouring the MYCN-amplified clone and tested different sizes.

Numerical advantage did not translate to a reproductive advantage.

The genetic tumor background is an important determinant for heterogeneous *MYCN*-amplified neuroblastoma

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Amplification of *MYCN* is the signature genetic aberration of 20–25% of neuroblastoma and a stratifying marker associated with aggressive tumor behavior. The detection of heterogeneous *MYCN* amplification (hetMNA) poses a diagnostic dilemma due to the uncertainty of its relevance to tumor behavior. Here, we aimed to shed light on the genomic background which permits hetMNA in neuroblastoma and tied the occurrence to other stratifying markers and disease outcome. We performed SNP analysis using Affymetrix Cytoscan HD arrays on 63 samples including constitutional DNA, tumor, bone marrow and relapse samples of 26 patients with confirmed hetMNA by *MYCN*-FISH. Tumors of patients ≤18m were mostly aneuploid with numeric chromosomal aberrations (NCAs), presented a prominent MNA subclone and carried none or a few segmental chromosomal aberrations (SCAs). In older patients, tumors were mostly di- or tetraploid, contained a lower number of MNA cells and displayed a multitude of SCAs including concomitant 11q deletions. These patients often suffered disease progression, tumor dissemination and relapse. Restricted to aneuploid tumors, we detected chromosomes with uniparental di- or trisomy (UPD/UPT) in almost every sample. UPD11 was exclusive to tumors of younger patients whereas older patients featured UPD14. In this study, the MNA subclone appears to be constraint by the tumor environment and thus less relevant for tumor behavior in aggressive tumors with a high genomic instability and many segmental aberrations. A more benign tumor background and lower tumor stage may favor an outgrowth of the MNA clone but tumors generally responded better to treatment.

Outgrowth of the MYCN-amplified clone in a heterogeneous tumour depends on its genetic background.

ARTICLE
Genetics and Genomics

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

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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.

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MYCN-amplified clone does not always enjoy a selective advantage.

Size of MYCN-amplified clone does not affect disease outcome.

Objectives

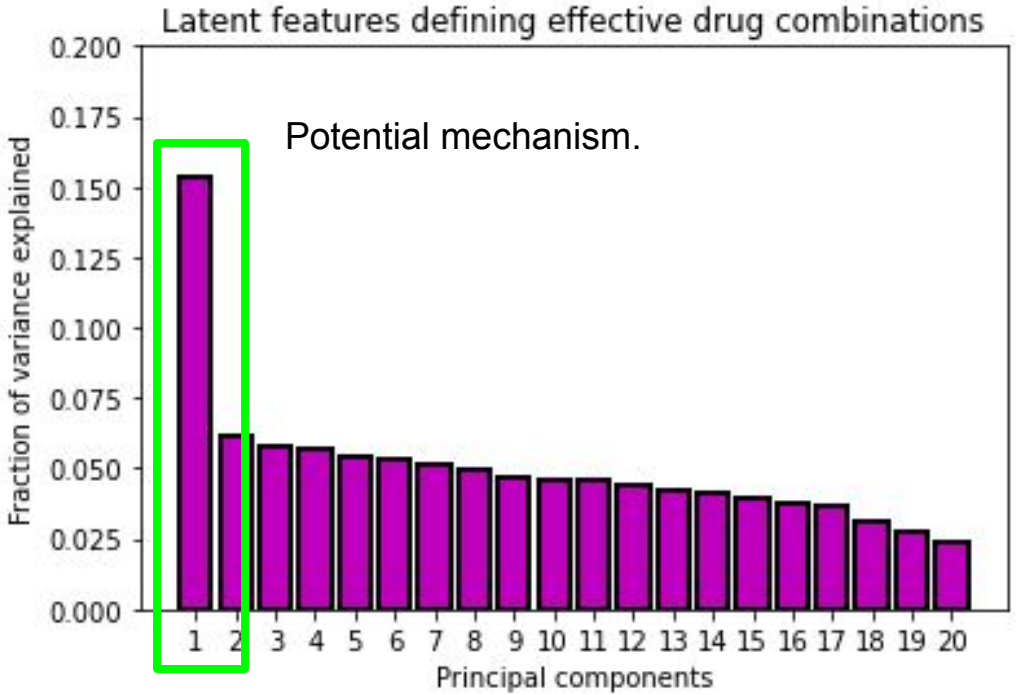
1. PRIMAGE project.
2. Neuroblastoma.
3. First multicellular model of neuroblastoma.
4. Calibration.
5. Clonal competition.
6. MYCN enigma.
7. Targeted therapies.

Targeted therapies

Step 1. Created a virtual tumour with one large MYCN-amplified clone only.

Step 2. Chose gene expression levels favouring the MYCN-amplified clone.

Step 3. Tested 1000 combinations of drugs targeting the 20 gene products.

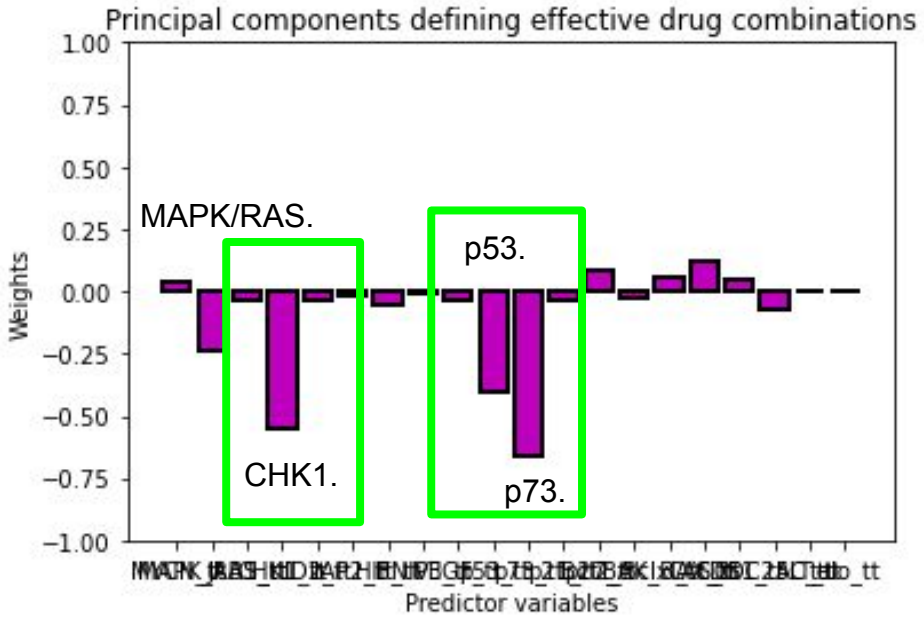


Kept the 305 best and 310 worst drug combinations in terms of shrinking the tumour.

Principal component analysis.

20 gene products.Telomerase, ALT, MYCN, MAPK/RAS pathway, JAB1, CHK1, CDS1, CDC25C, ID2, IAP2, HIF, BNIP3, VEGF, p53, p73, p21, p27, Bcl-2/Bcl-xL, BAK/BAX, and CAS.

Targeted therapies

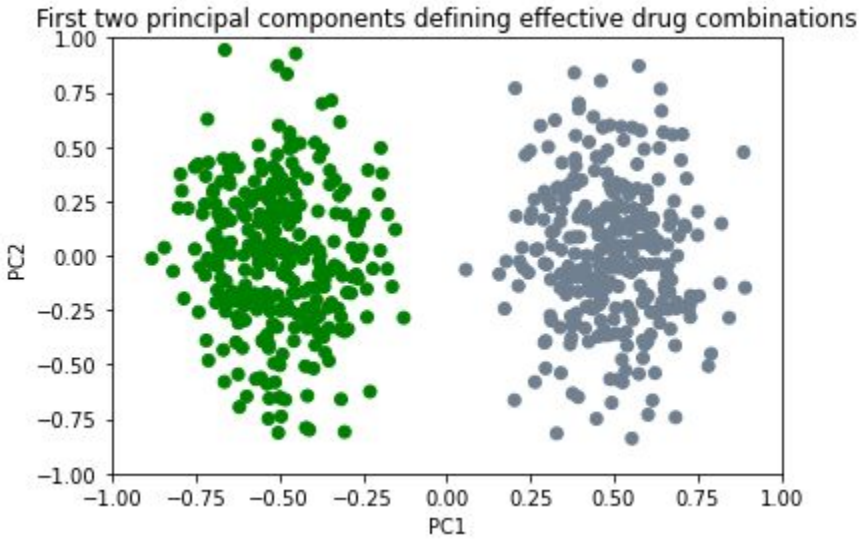


Weights of the first principal component.

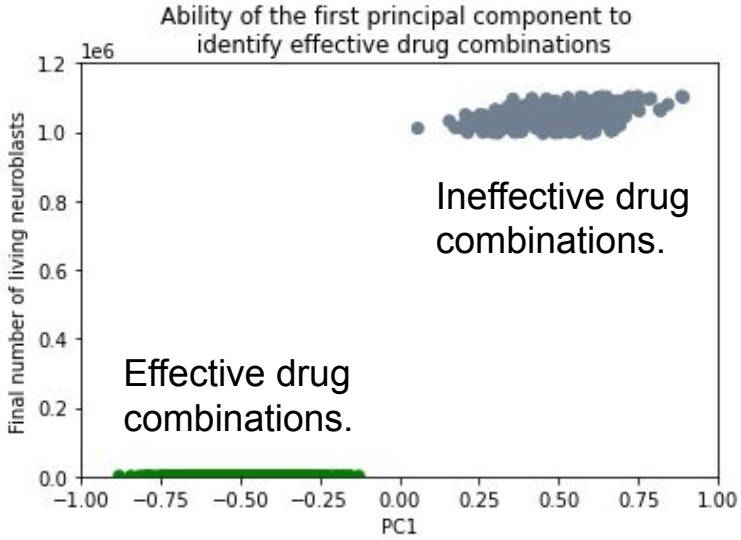
Effective drug combinations targeted CHK1, p53, and p73 in the *in silico* trials.

Consistent with earlier sensitivity analysis of MYCN enigma, as CHK1 switches on p73.

Targeted therapies



Projected the data onto the first principal component (PC1) and clustered the data points along this axis.



The two predicted clusters separate the effective and ineffective drug combinations perfectly.

The first principal component is a valid mechanism.

Inhibiting CHK1, p53, and p73 shrinks the MYCN-amplified clone.

Conclusions

Built, calibrated, and validated the first multicellular model of neuroblastoma.

MYCN-Amplified clone requires p73 and enhanced cell cycling (MAPK/RAS signalling) to thrive.

Drugs targeting CHK1, p53, and p73 are effective against MYCN-amplified clone.