Mathematical modelling and investigation of macrophages heterogeneity and their impact on the evolution of cancer

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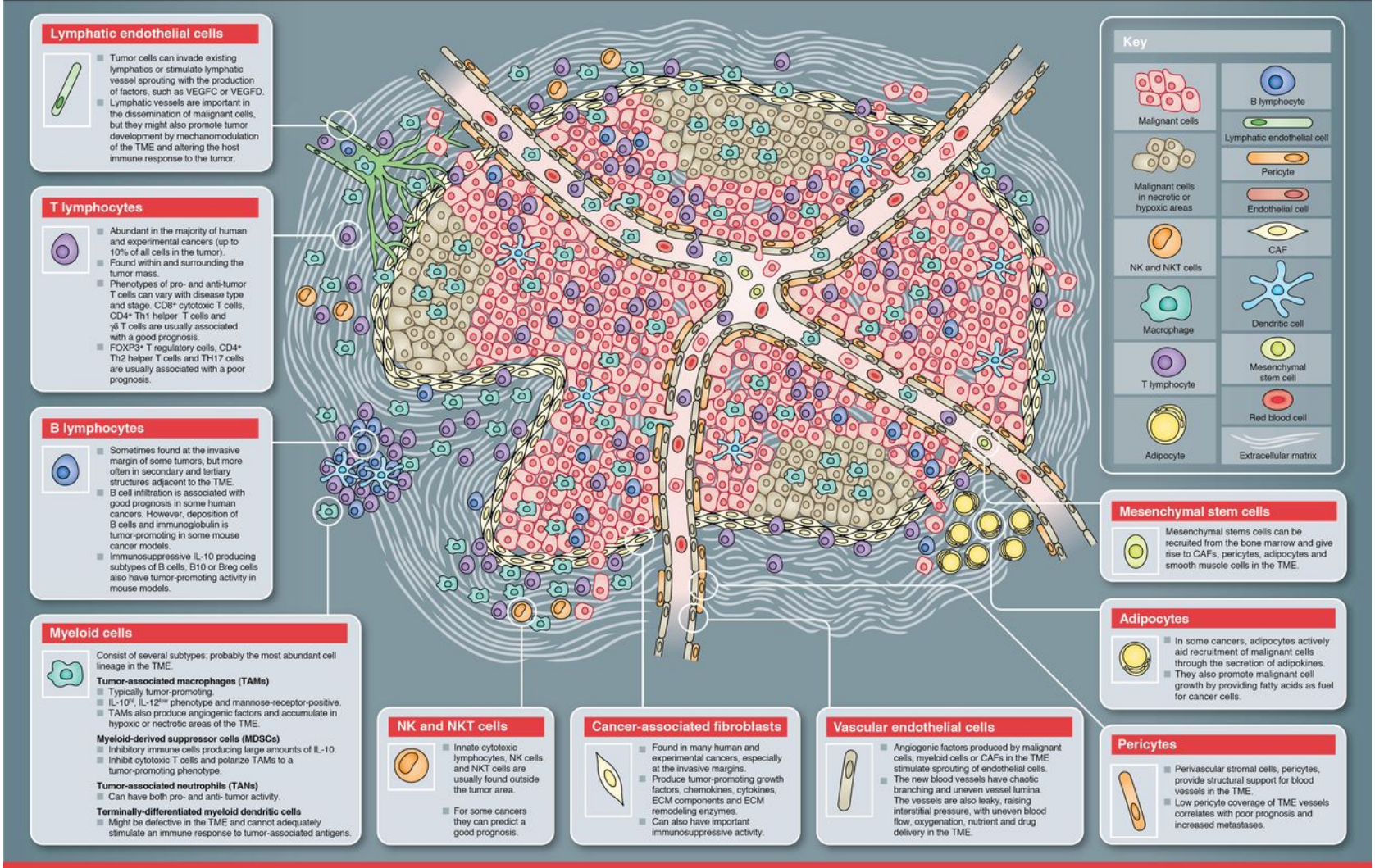
The Tumor Microenvironment at a Glance

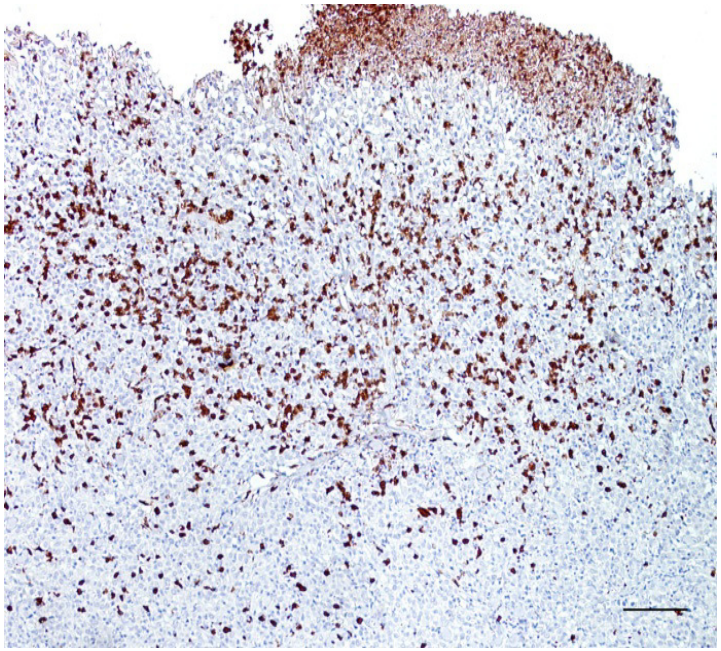
Frances R. Balkwill, Melania Capasso and Thorsten Hagemann



Abbreviations: Breg cells, regulatory B cells; CAF, cancer-associated fibroblast; ECM, extracellular matrix; IL, interleukin; NK cells, natural killer cells; NKT cells, natural killer T cells; TME, tumor microenvironment; VEGF, vascular endothelial growth factor.

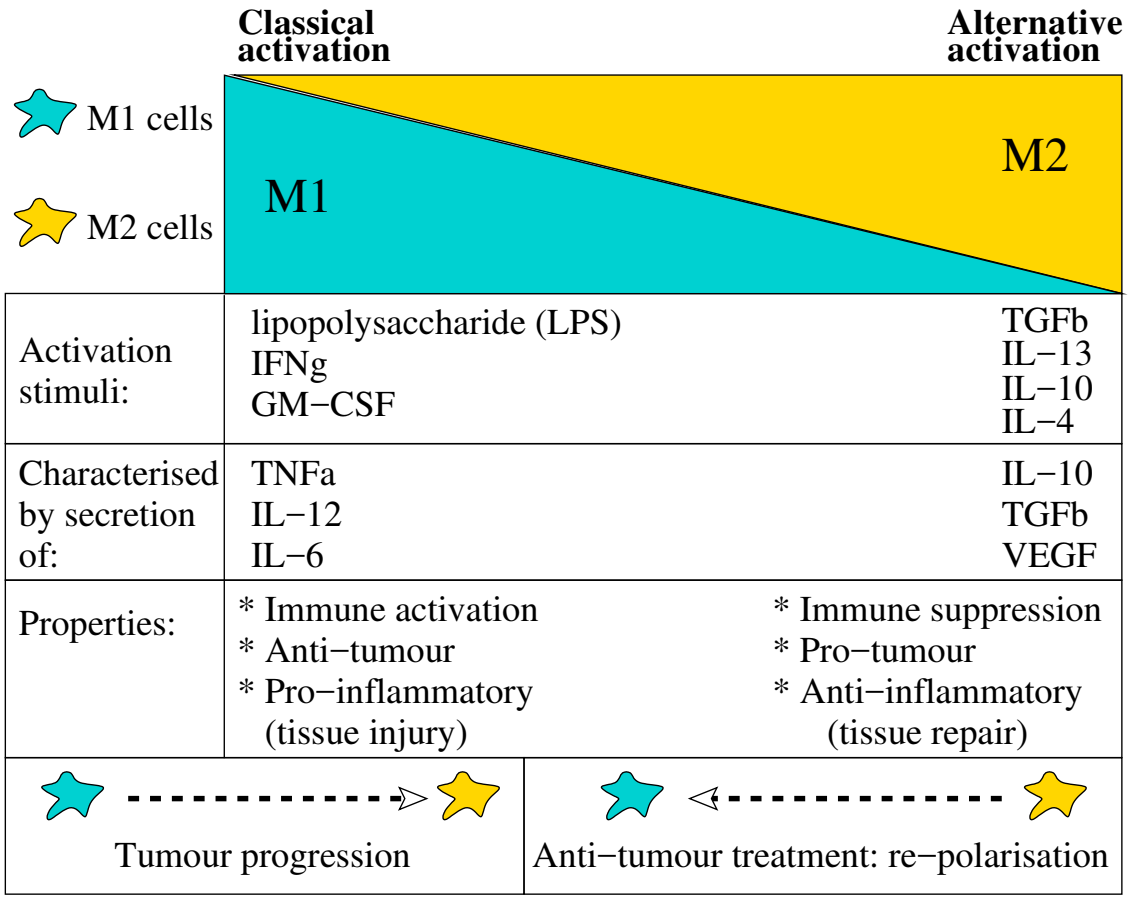
An important component of the tumour microenvironment: macrophages

- Macrophages play a central role in regulating both innate & adaptive immune responses (Th1&Th2 responses, modulate NK cells,...)
- Macrophages are one of the most common cell types in solid tumours, sometimes forming up to 40% of total tumour mass
- Activated macrophages can kill cancer cells by themselves (directly: TNF, NO, phagocytosis), or in an indirect manner through recruitment of other immune cells (e.g., CTLs)
- Increased macrophage infiltration of tumours is generally associated with poor patient prognosis
- Macrophages are a heterogeneous population of cells ...



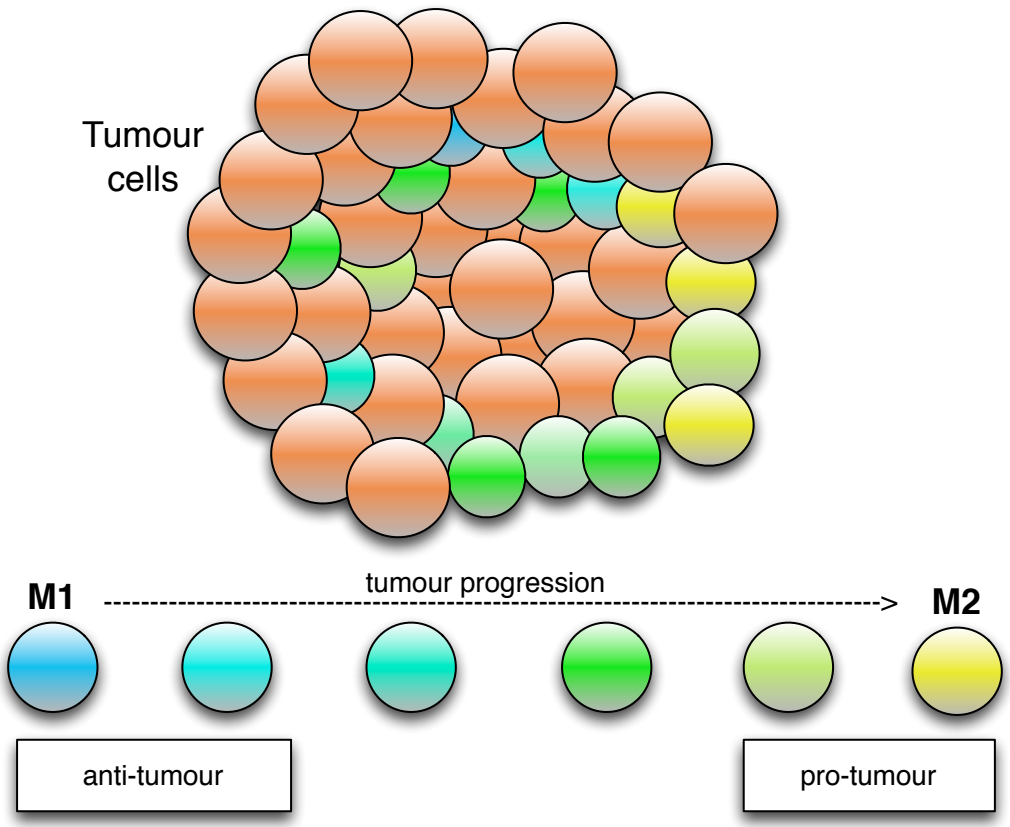
Pires et al, (2011). Ch. 10 in
"Melanoma in the clinic: Diagnosis, management and complications of malignancy"

Macrophages plasticity: mediated by microenvironment signals



"These are extremes in a continuum of polarization states in an universe of diversity." (Mantovani, Locati, 2013)

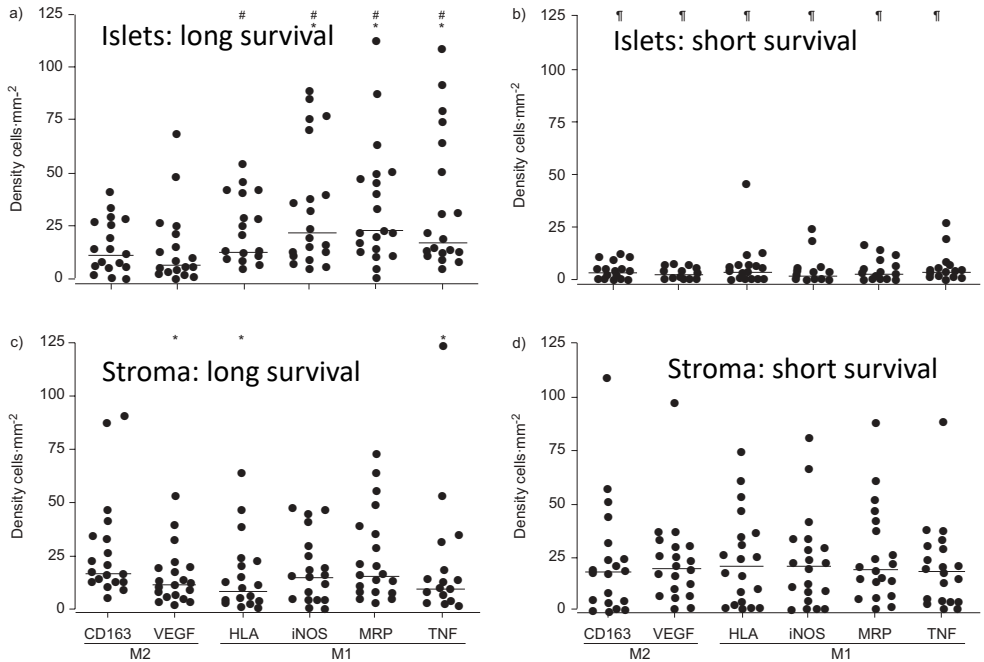
Most tumour-associated macrophages (TAMs) are “M2-like" in established tumours, but “M1-like" cells have also been observed in **early** as well as **advanced** tumours ...



Questions surrounding the density of M1&M2 inside tumour islets/stroma...

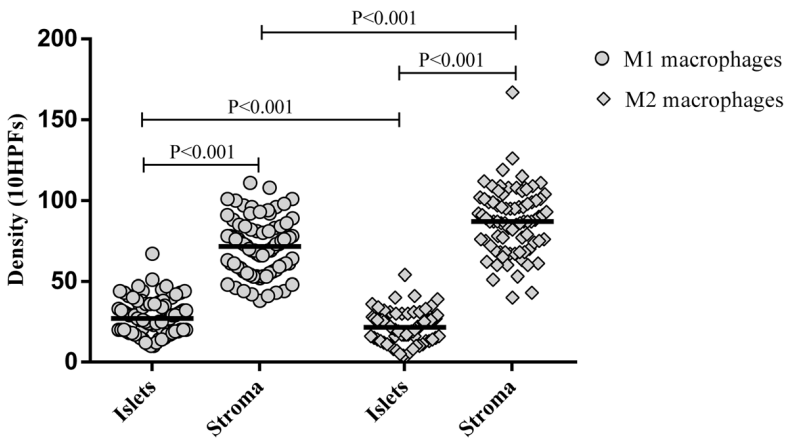
Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival

C.M. Ohri*,#, A. Shikotra*,#, R.H. Green*, D.A. Waller* and P. Bradding*,#



Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer

Jurgita Jackute[1*], Marius Zemaitis[1], Darius Pranys[2], Brigita Sitkauskiene[3], Skaidrius Miliauskas[1], Simona Vaitkiene[1] and Raimundas Sakalauskas[1]



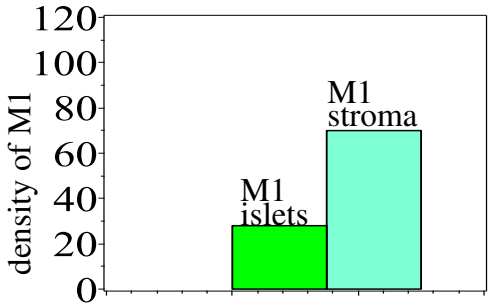
The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time

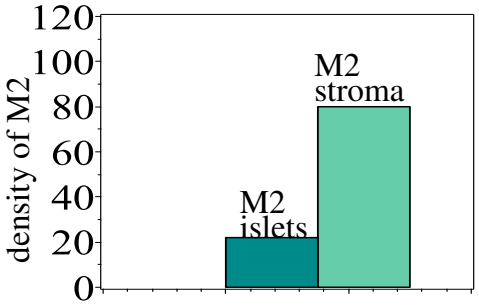
Junliang Ma[1], Lunxu Liu[1*], Guowei Che[1], Nanbin Yu[1,2], Fuqiang Dai[1,3], Zongbing You[4*]

Table 2 Density and microlocalization of macrophages in non-small cell lung cancer

	Long survival			Short survival		
MΦ Form	Islets	Stroma	I + S	Islets	Stroma	I + S
M1	70.1 (0 - 255.3)	33.6 (0 - 257.1)	70.4 (0 - 255.7)	7.3 (0 - 74.9)	13.1 (0 - 129.9)	17.2 (0 - 132.2)
M2	77.6 (0 - 356.9)	78.4 (0 - 327.9)	97.9 (0 - 299.2)	113.4 (0 - 311.5)	79.5 (0 - 234.3)	109.5 (0 - 257.5)

Data from Jackute et al (2018) – patients with Non Small Lung Cancer (NSCLC)

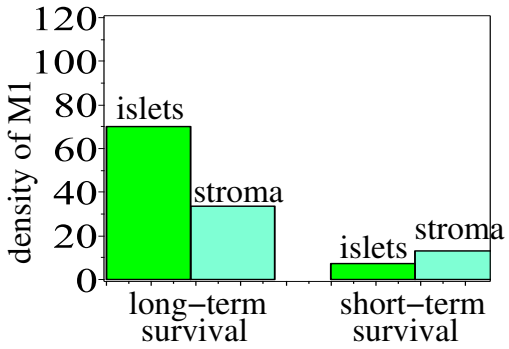


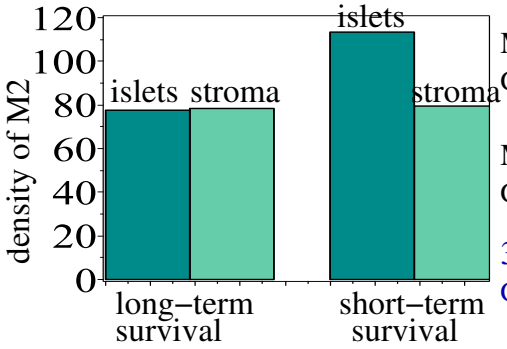


M1 markers:
CD68,iNOS

M2 markers:
CD68,CD163

Data from Ma et al (2010) – patients with Non Small Cell Lung Cancer (NSCLC)



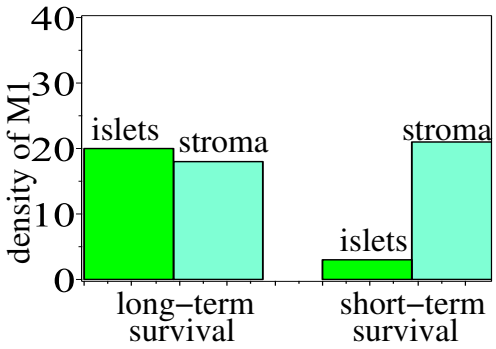


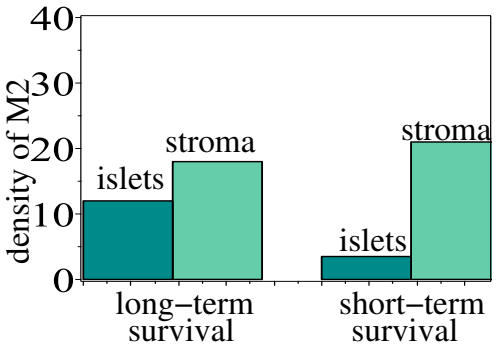
M1 markers:
CD68,HLA–DR

M2 markers:
CD68,CD163

3.1%macroph.
CD163&HLA–DR

Data from Ohri et al (2009) – patients with Non Small Cell Lung Cancer (NSCLC)





M1 markers:
HLA–DR,
iNOS, TNFa
MRP 8/14

M2 markers:
CD163,VEGF

6.45% macroph.
CD163+HLA–DR

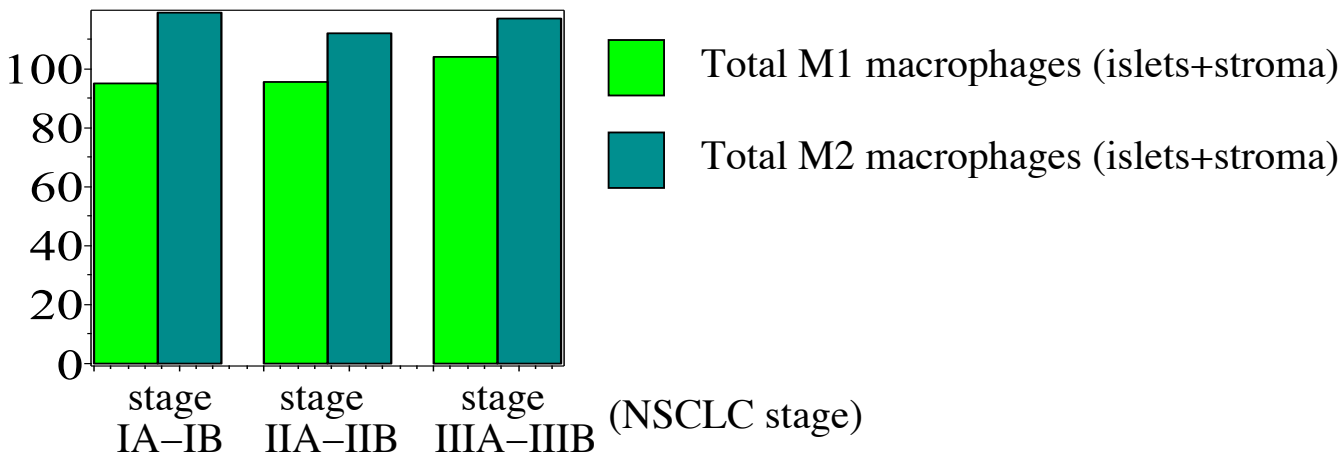
Jackute *et al. BMC Immunology* (2018) 19:3
DOI 10.1186/s12865-018-0241-4

BMC Immunology

RESEARCH ARTICLE Open Access

Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer

Jurgita Jackute[1*], Marius Zemaitis[1], Darius Pranys[2], Brigita Sitkauskiene[3], Skaidrius Miliauskas[1], Simona Vaitkiene[1] and Raimundas Sakalauskas[1]



No data on the association between NSCLC stages & macrophage infiltration in the papers by Ma et al (2010) or Ohri et al (2009) …

- Patients with stage I or II NSCLC
- Ex-vivo phenotype of TAMs vs.

M1 & M2 macrophages differentiated in vitro

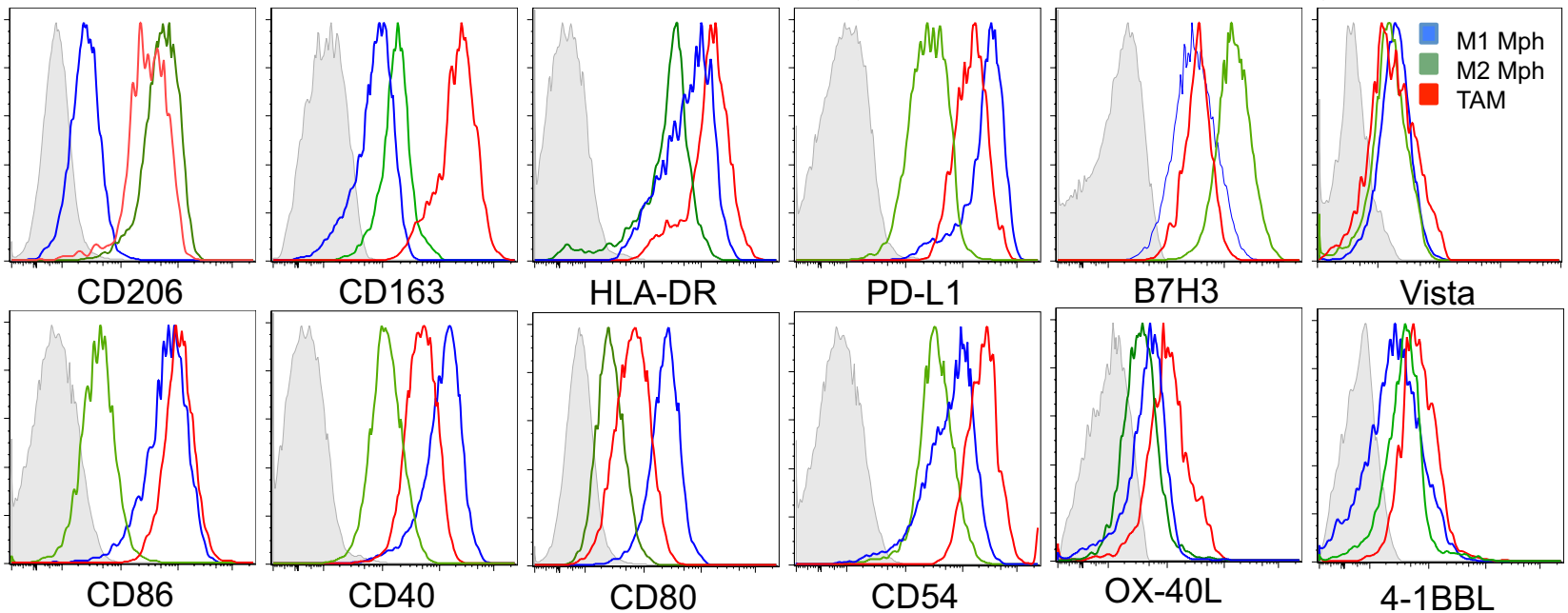
Fig. 2 Mixed phenotype of NSCLS-associated macrophages

CANCER

Human tumor-associated monocytes/macrophages and their regulation of T cell responses in early-stage lung cancer

Sunil Singhal[1], Jason Stadanlick[1], Michael J. Annunziata[1], Abhishek S. Rao[1], Pratik S. Bhojnagarwala[1], Shaun O'Brien[2], Edmund K. Moon[2], Edward Cantu[3], Gwenn Danet-Desnoyers[4], Hyun-Jeong Ra[4], Leslie Litzky[5], Tatiana Akimova[5,6], Ulf H. Beier[7], Wayne W. Hancock[5,6], Steven M. Albelda[2], Evgeniy B. Eruslanov[1]*

E



(**E**) Flow cytometric analysis of the expression of macrophage markers on TAMs and M1/M2 macrophages differentiated in vitro.

(Singhal et. al, Science Translational Medicine, 2019)

“Although monocyte-derived M1 and M2 macrophages showed clear differences in expression of respective markers, TAMs simultaneously expressed both M2 and M1 markers, and sometimes to an even higher degree than in vitro-differentiated M2 and M1 macrophages (Fig.2E). ***Hence, the phenotype of macrophages in early-stage tumours is “mixed"*** *and not predominantly biased toward either M1 or M2 classical macrophage phenotypes".*

(Singhal et. al, Science Translational Medicine, 2019)

“Although monocyte-derived M1 and M2 macrophages showed clear differences in expression of respective markers, TAMs simultaneously expressed both M2 and M1 markers, and sometimes to an even higher degree than in vitro-differentiated M2 and M1 macrophages (Fig.2E). ***Hence, the phenotype of macrophages in early-stage tumours is “mixed"*** *and not predominantly biased toward either M1 or M2 classical macrophage phenotypes".*

... still many unknowns about the classification TAMs (based on markers) in NSCLC... and in many other cancers...

... and many unknowns about the role of mixed-phenotype macrophages on tumour progression...

Goal...

Use modelling/computational approaches to investigate the effect of macrophages heterogeneity on tumour dynamics:

distinct M1 & M2 phenotypes vs. mixed M1-M2 phenotypes

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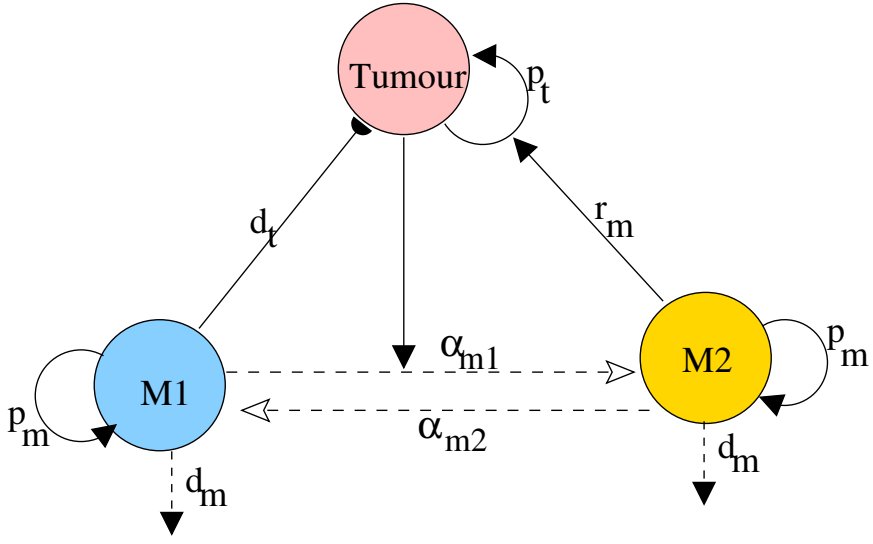
Use modelling/computational approaches to investigate the effect of macrophages heterogeneity on tumour dynamics:

distinct M1 & M2 phenotypes vs. mixed M1-M2 phenotypes

[Eftimie, Math. Biosci. 2020]

- Model for the anti-tumour/pro-tumour roles of the 2 extreme macrophage phenotypes: M1, M2
- Model for the anti-tumour/pro-tumour roles of a phenotype-structured population of macrophages

Modelling tumour–macrophages interactions: a discrete-phenotype ODE model



$$\text{Tumour:} \quad \frac{du_T}{dt} = p_t u_T \left(1 - \frac{u_T}{K_T}\right)(1 + r_m u_{M2}) - d_t u_T u_{M1},$$

$$\text{M1 cells:} \quad \frac{du_{M1}}{dt} = p_m u_{M1} \left(1 - \frac{u_{M1} + u_{M2}}{K_M}\right) - d_m u_{M1} - \alpha_{m1} u_{M1} \frac{u_T}{K_T^* + u_T} + \alpha_{m2} u_{M2}$$

$$\text{M2 cells:} \quad \frac{du_{M2}}{dt} = p_m u_{M2} \left(1 - \frac{u_{M1} + u_{M2}}{K_M}\right) - d_m u_{M2} + \alpha_{m1} u_{M1} \frac{u_T}{K_T^* + u_T} - \alpha_{m2} u_{M2}$$

TAM-targeted therapeutic strategies for cancer

[Frontiers in Bioscience, Elite, 7, 334-351, January 1, 2015]

Tumour-associated macrophage polarisation and re-education with immunotherapy

Debra H. Josephs[1,2], Heather J. Bax[1,2], Sophia N. Karagiannis[1]

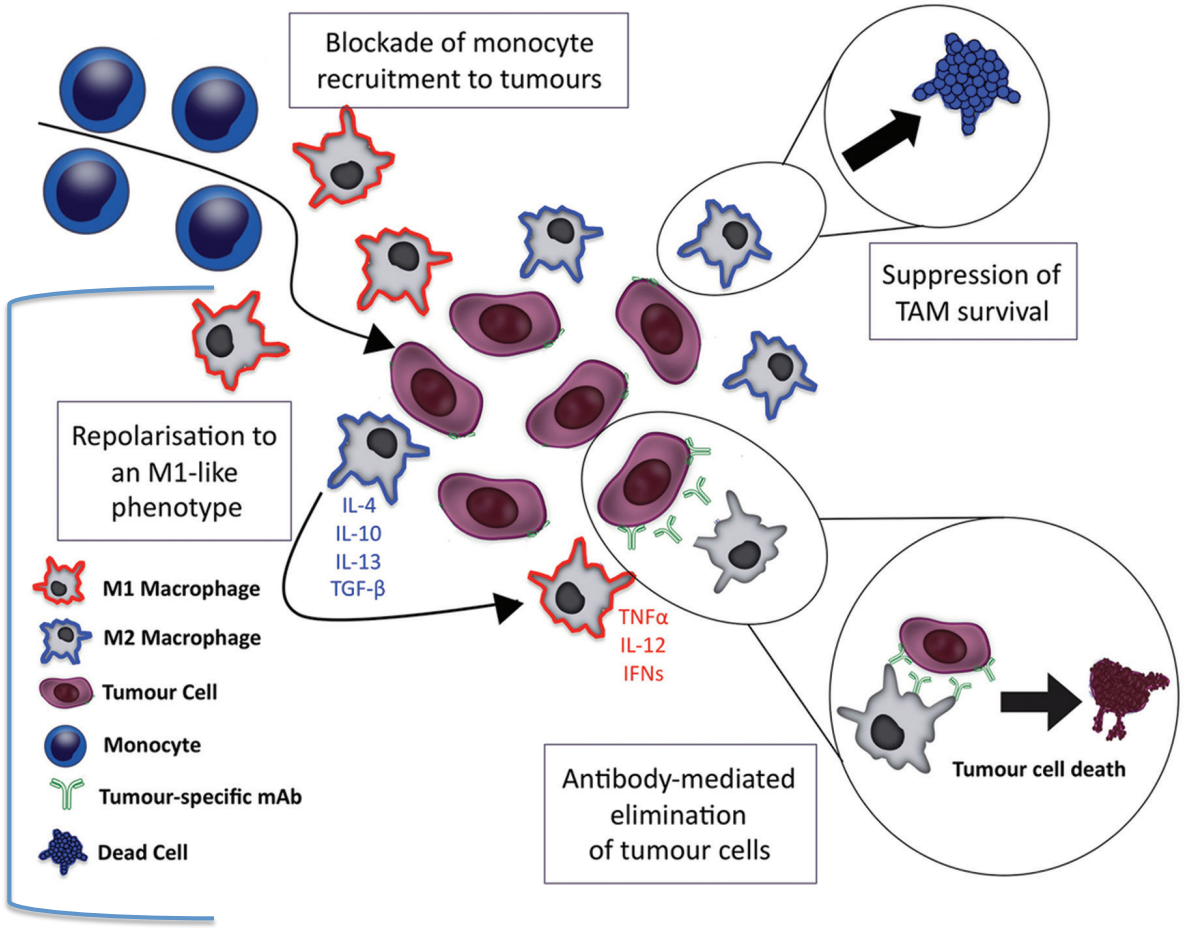


Figure 1. Tumour-associated macrophage (TAM) -targeted therapeutic strategies for cancer. The pro-tumorigenic functions of TAMs depend on their accumulation and survival within tumours and their M2-like polarisation status. Current TAM-targeted treatment strategies include: (i) blockade of monocyte/macrophage recruitment; (ii) suppression of TAM survival; (iii) repolarisation of TAMs towards an M1-like phenotype; and (iv) antibody-mediated elimination of tumour cells by monocytes/macrophages. Cytokines listed are the key cytokines required for M1- or M2- skewing of macrophages.

Test computationally some of these therapeutic strategies:

$$\text{Tumour:}\quad \frac{du_T}{dt} = p_t u_T\left(1-\frac{u_T}{K_T}\right)(1+r_m u_{M2}) - d_t u_T u_{M1},$$

$$\text{M1 cells:}\quad \frac{du_{M1}}{dt} = p_m u_{M1}\left(1-\frac{u_{M1}+u_{M2}}{K_M}\right) - d_m u_{M1} - \alpha_{m1} u_{M1}\frac{u_T}{K_T^*+u_T} + \alpha_{m2} u_{M2}$$

$$\text{M2 cells:}\quad \frac{du_{M2}}{dt} = p_m u_{M2}\left(1-\frac{u_{M1}+u_{M2}}{K_M}\right) - d_m u_{M2} + \alpha_{m1} u_{M1}\frac{u_T}{K_T^*+u_T} - \alpha_{m2} u_{M2}$$

- Antibody-mediated phagocytosis of tumour cells: increase d_t
- Re-polarisation to an M1-like phenotype: increase α_{m2}
- Blockade of monocyte recruitment to tumours: decrease p_m
- Suppression of TAM survival: increase d_m

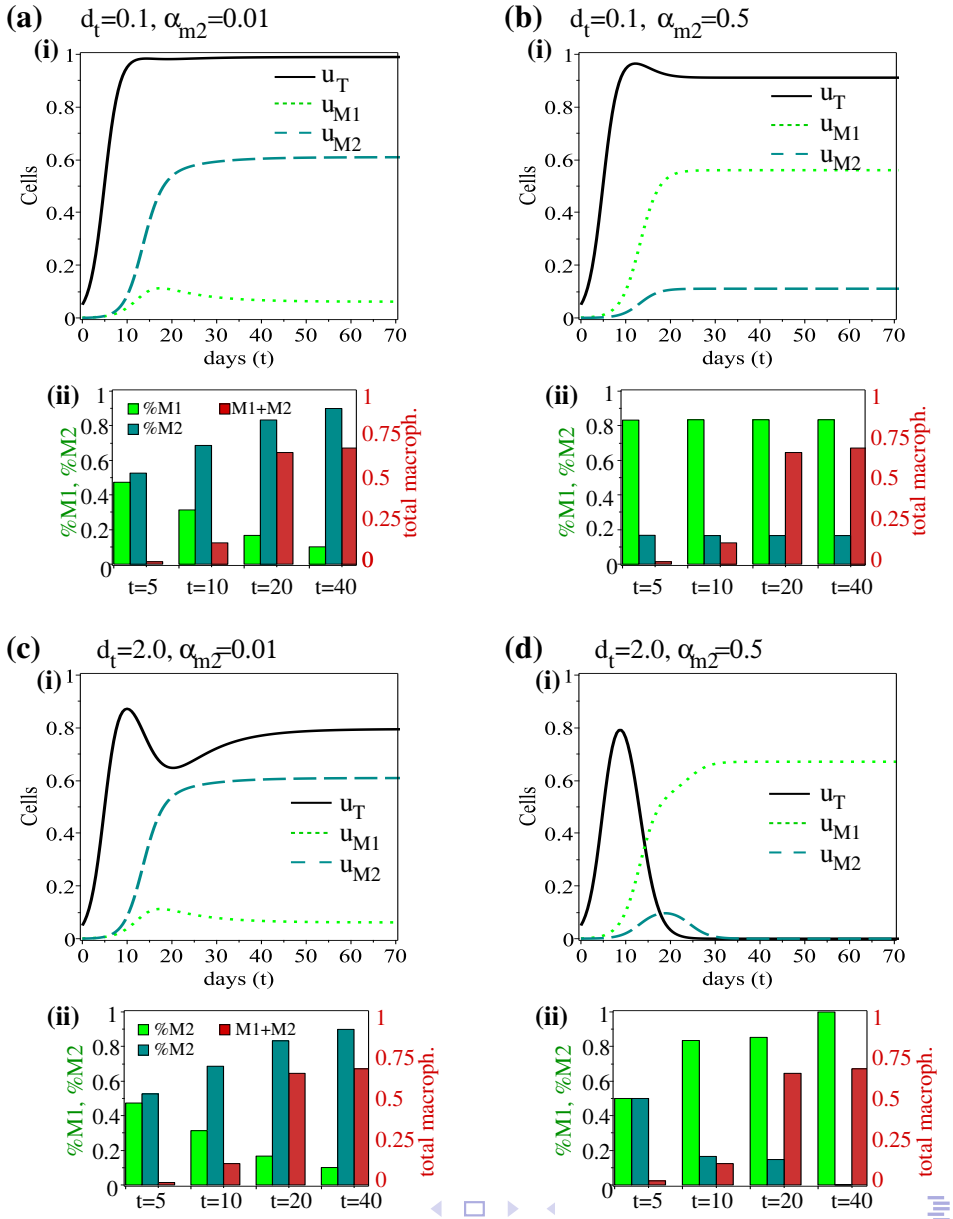
Short-term dynamics:

- (a),(c): increase d_t

  (phagocytosis of tumour cells)

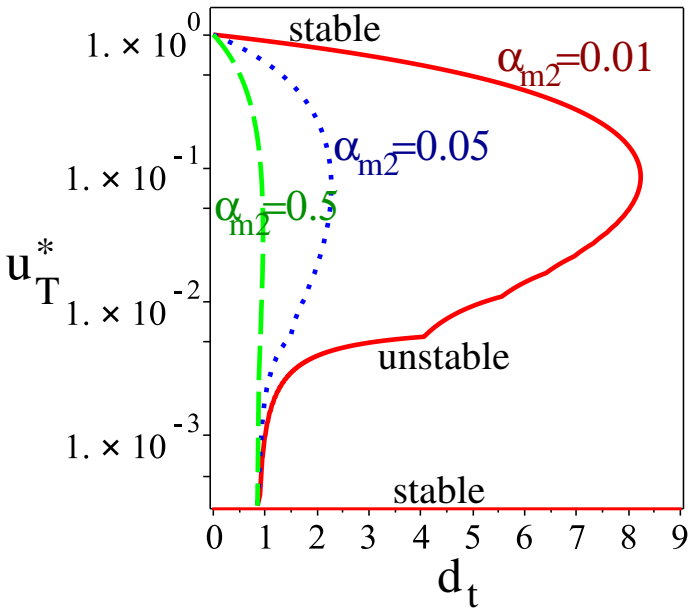
- (b),(d): increase α_{m2}

  (re-polarisation towards M1)



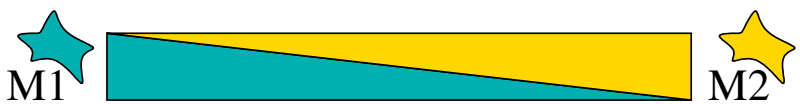
Long–term dynamics:

- hysteresis phenomenon:
  - tumour–free steady state
  - coexistence steady state

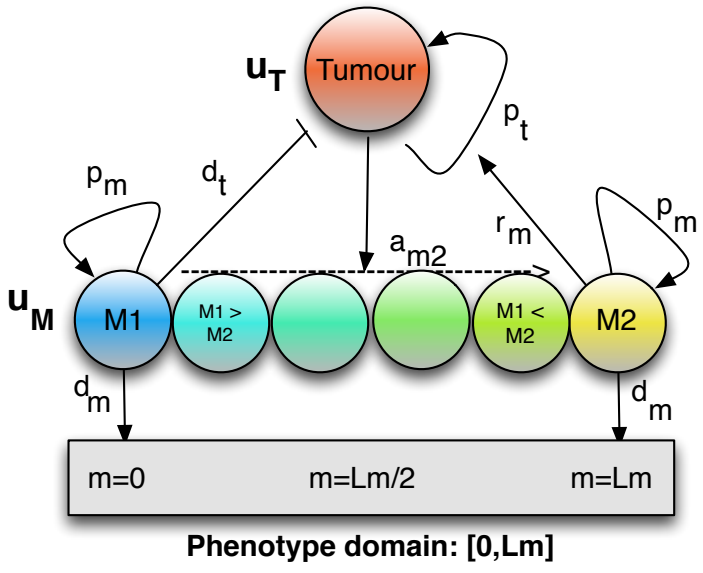


Increasing α_{m2} (while keeping d_t fixed & large) pushes the system towards the stable tumour-free state

What is the effect of a continuous phenotype structure for the macrophages population, in the context of persistence/elimination of tumour cells?



A phenotype-structured continuous model

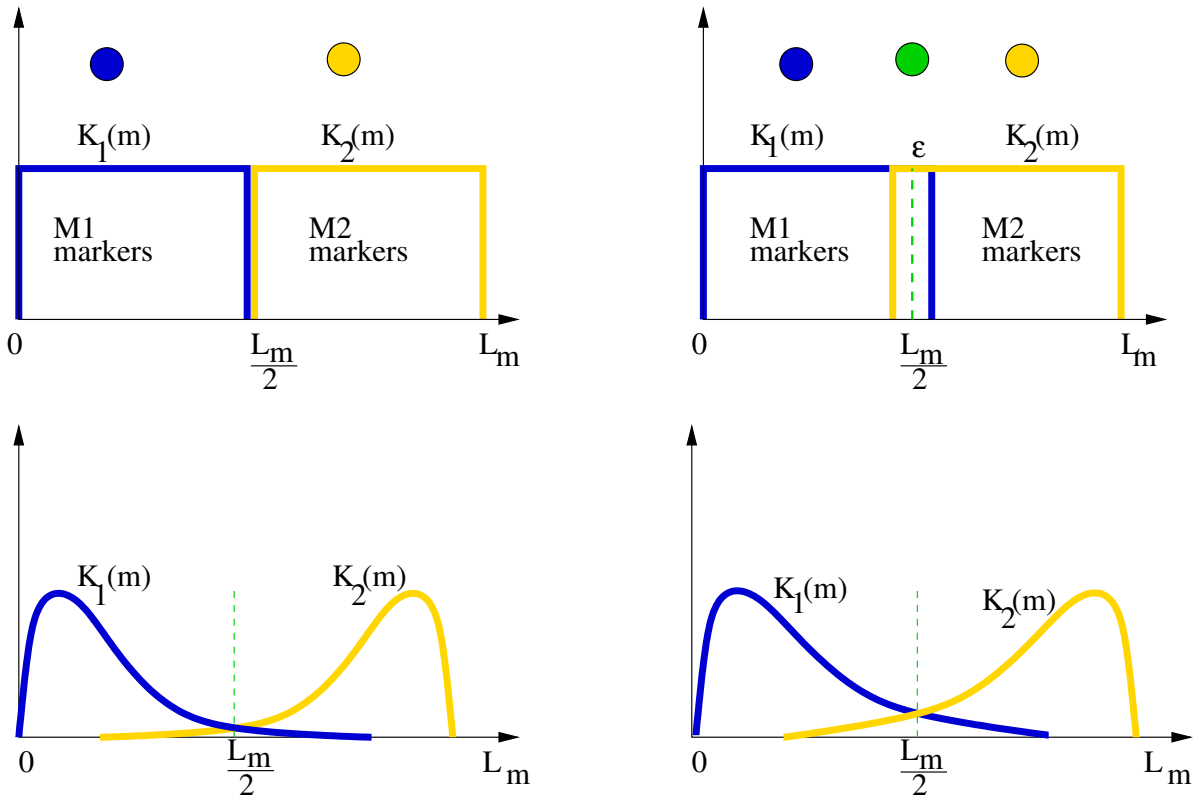


$$\frac{du_T}{dt} = p_t u_T\left(1-\frac{u_T}{K_T}\right)\left(1+r_m\int_0^{Lm} K_2(m)u_M(m,t)dm\right) - d_t u_T\int_0^{Lm} K_1(m)u_{M1}(m,t)dm,$$

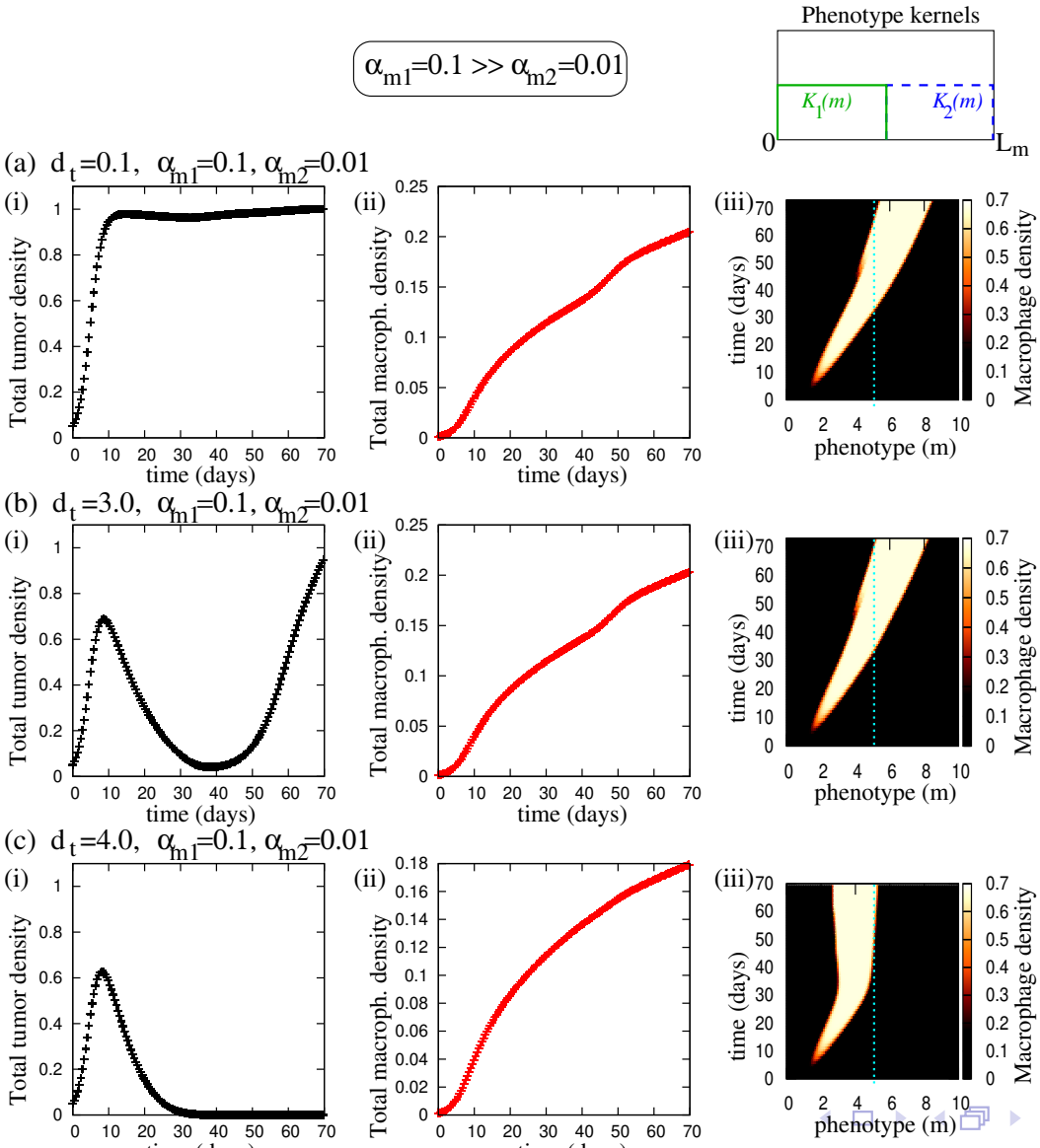
$$\frac{\partial u_M(m,t)}{\partial t} = -\frac{\partial\left(\gamma(t)u_M F(u_M,u_T)\right)}{\partial m} + p_m u_M\left(1-\frac{u_M}{K_M}\right) - d_m u_M,$$

with $F(u_T,u_M) = \alpha_{m1}\dfrac{u_T}{K_T^* + u_T} - \alpha_{m2}\displaystyle\int_0^{Lm} K_1(m)u_M(m,t)dm, \qquad \gamma(t) = e^{-g_0\int_0^{Lm} K_2(m)u_M(m,t)dm}$

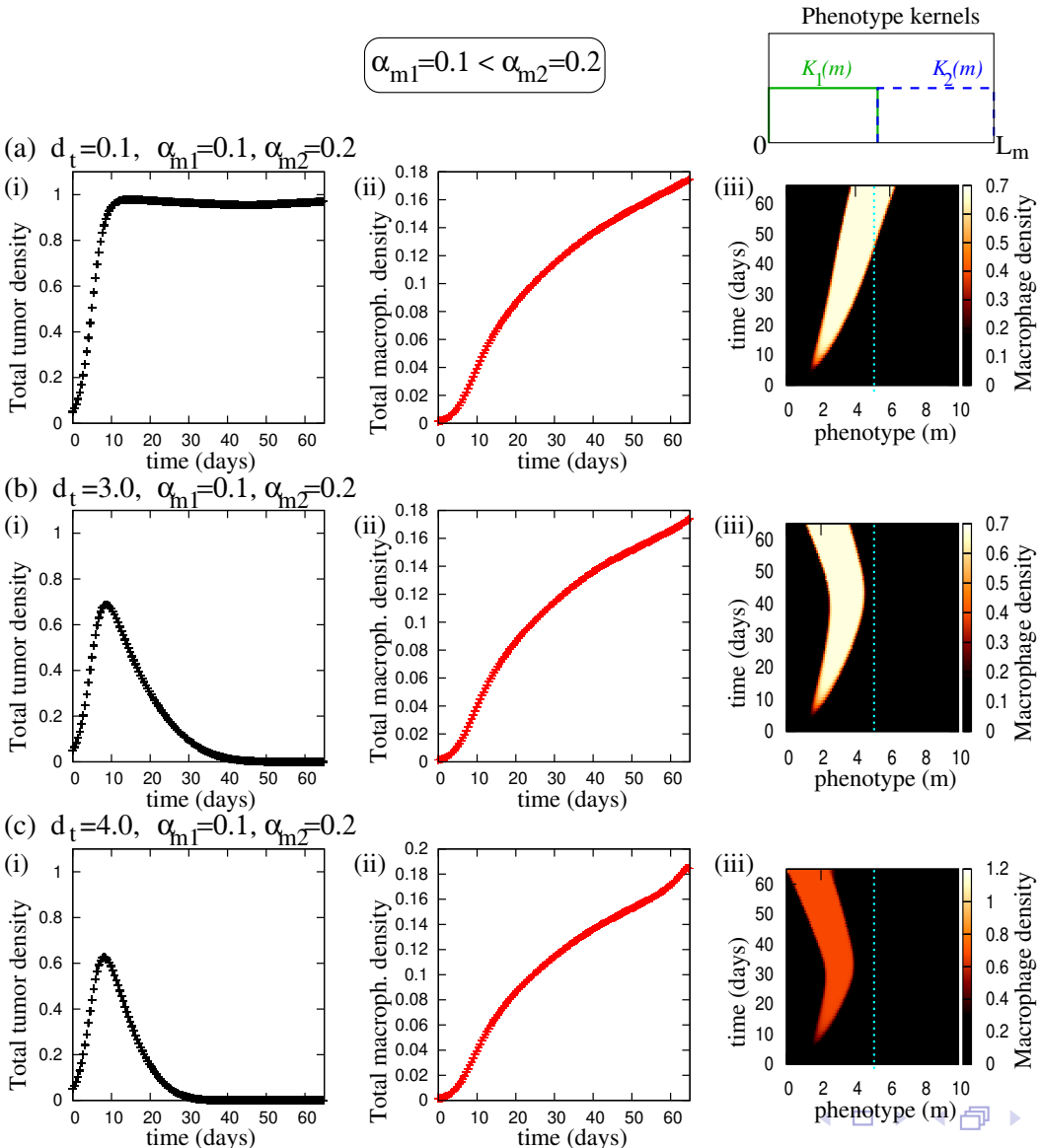
Examples of macrophages phenotype kernels



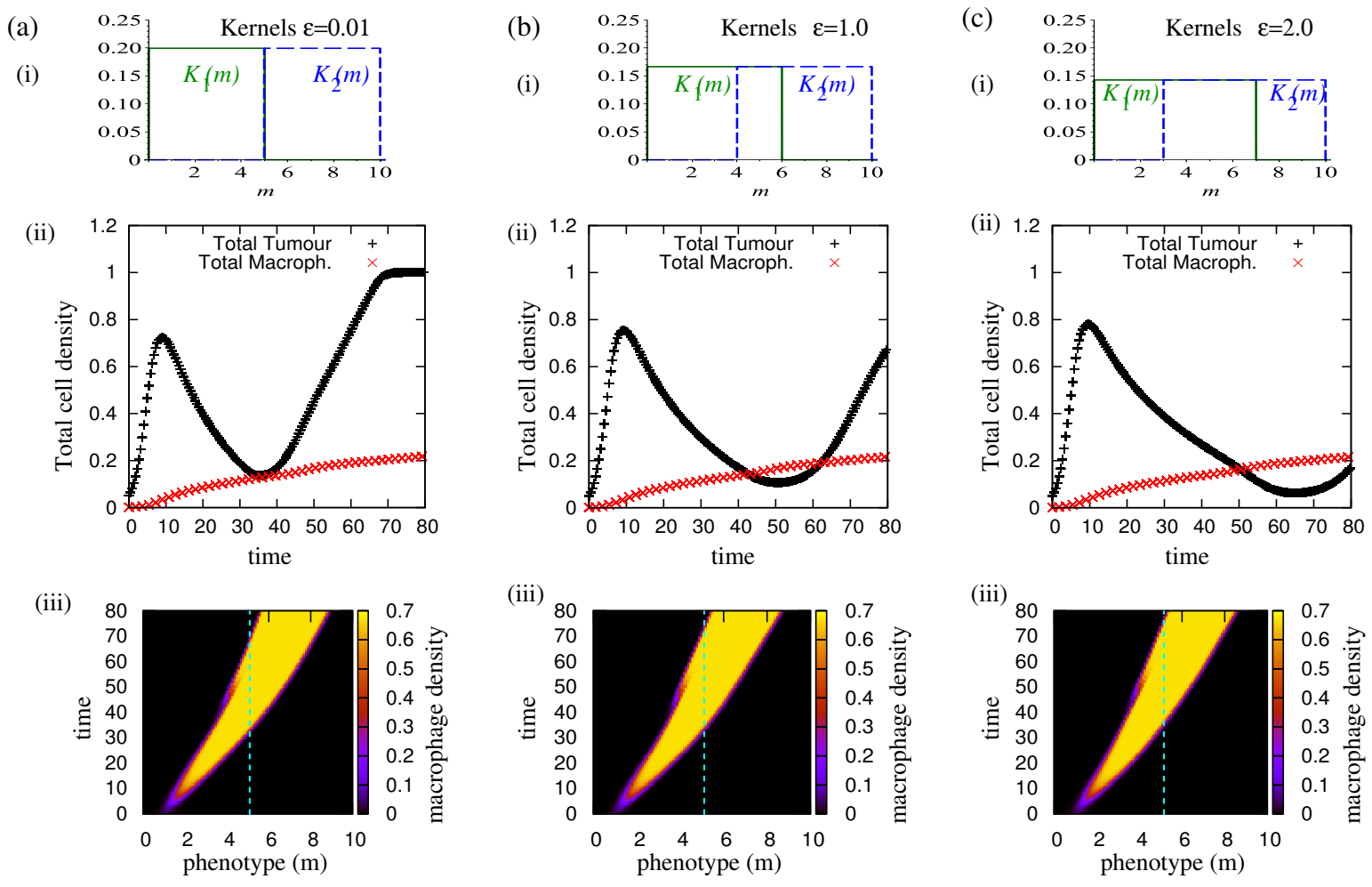
Numerical results: increase the tumour elimination rate d_t



Numerical results: increase the M2→M1 re-polarisation rate a_{m1}

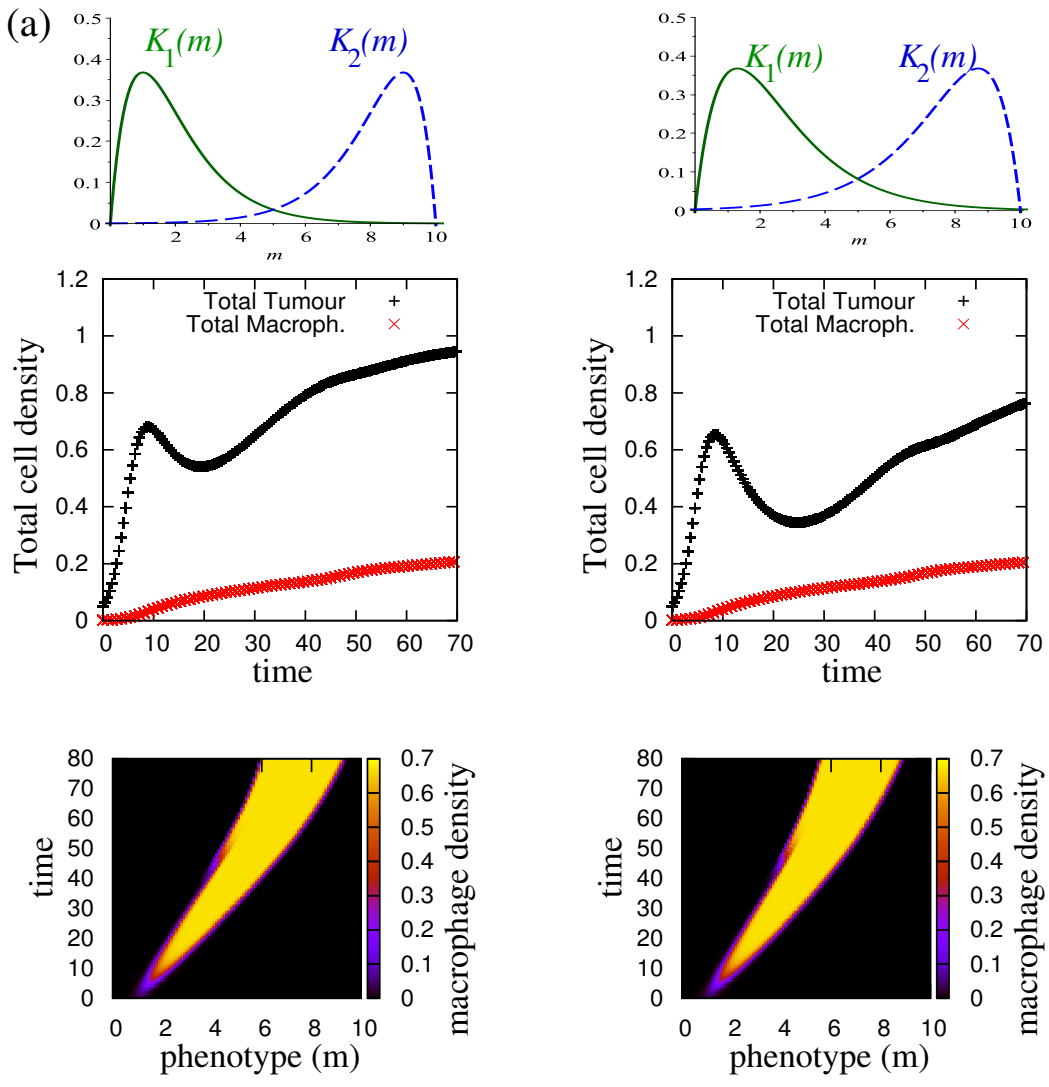


Increasing the overlap between the M1 and M2 phenotypes



Increasing the overlap of M1 & M2 $\Rightarrow$ delay tumour relapse

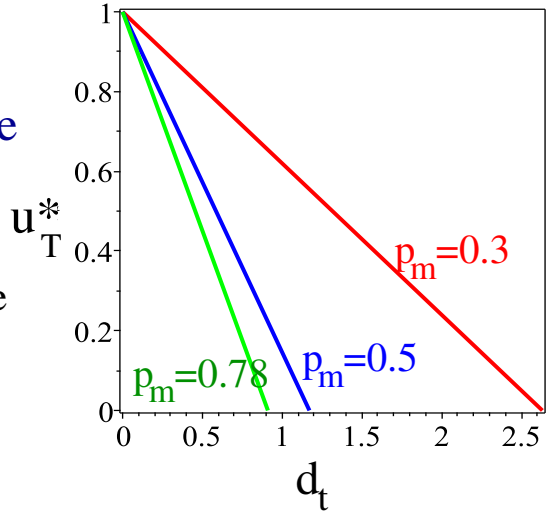
Increasing the overlap between the M1 and M2 phenotypes...



Long-term dynamics: **phenotype-homogeneous** steady states

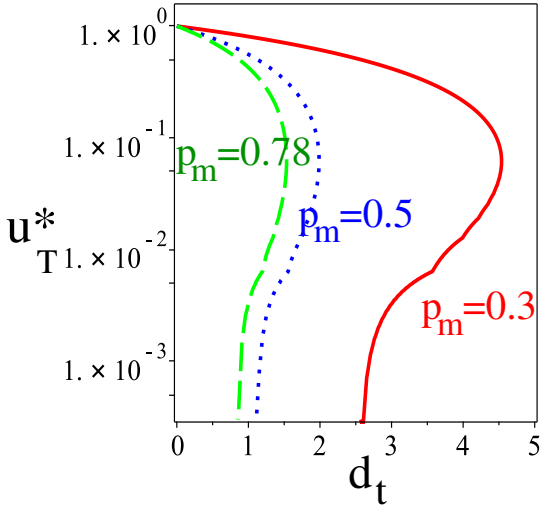
Co–existence homogeneous steady state (PDE):

p_m=Macroph. prolif. rate
d_t=M1 tumour–kiling rate



VS.

Co–existence homogeneous steady state (ODE)



Long-term dynamics: **phenotype-heterogeneous** steady states

$$u_T^* = \frac{K_T}{p_t}\left[\frac{p_t\left(1 + r_m \int_0^{Lm} K_2(m)u_M^*(m)dm\right) - d_t \int_0^{Lm} K_1(m)u_M^*(m)dm}{1 + r_m \int_0^{Lm} K_2(m)u_M^*(m)dm}\right],$$

$$\frac{du_M^*(m)}{dm} = \frac{u_M^*(m)}{\gamma^* F(u_T^*, u_M^*)}\left[(p_m - d_m) - \frac{p_m}{K_M}u_M^*(m)\right],$$

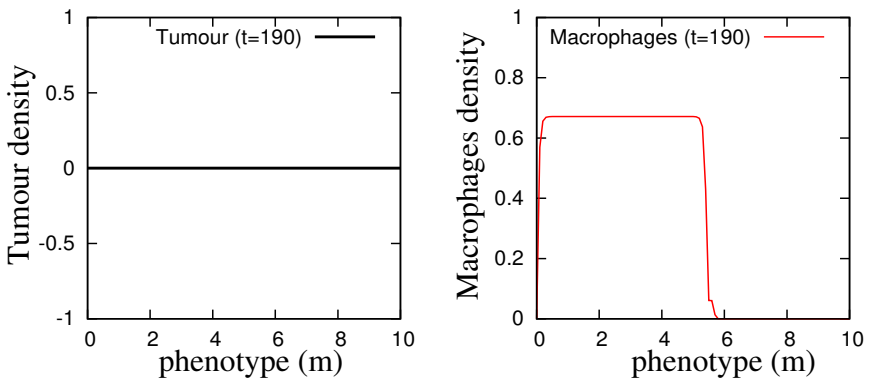
where

$$\gamma^* = e^{-g_0 \int_0^{Lm} H_2(m)u_M^*(m)dm},$$

and

$$F(u_T^*, u_M^*) = \alpha_{m1}\frac{u_T^*}{u_T^* + K_T^*} - \alpha_{m2}\int_0^{Lm} K_2(m)u_M^*(m)dm.$$

For $u_T^* = 0$:



Summary...

- Tumour is eliminated/controlled in the presence of a large M1/M2 ratio & large d_t
- Tumour can persist in the presence of
  - large M1/M2 ratio and small d_t
  - large M2/M1 ratio

Summary...

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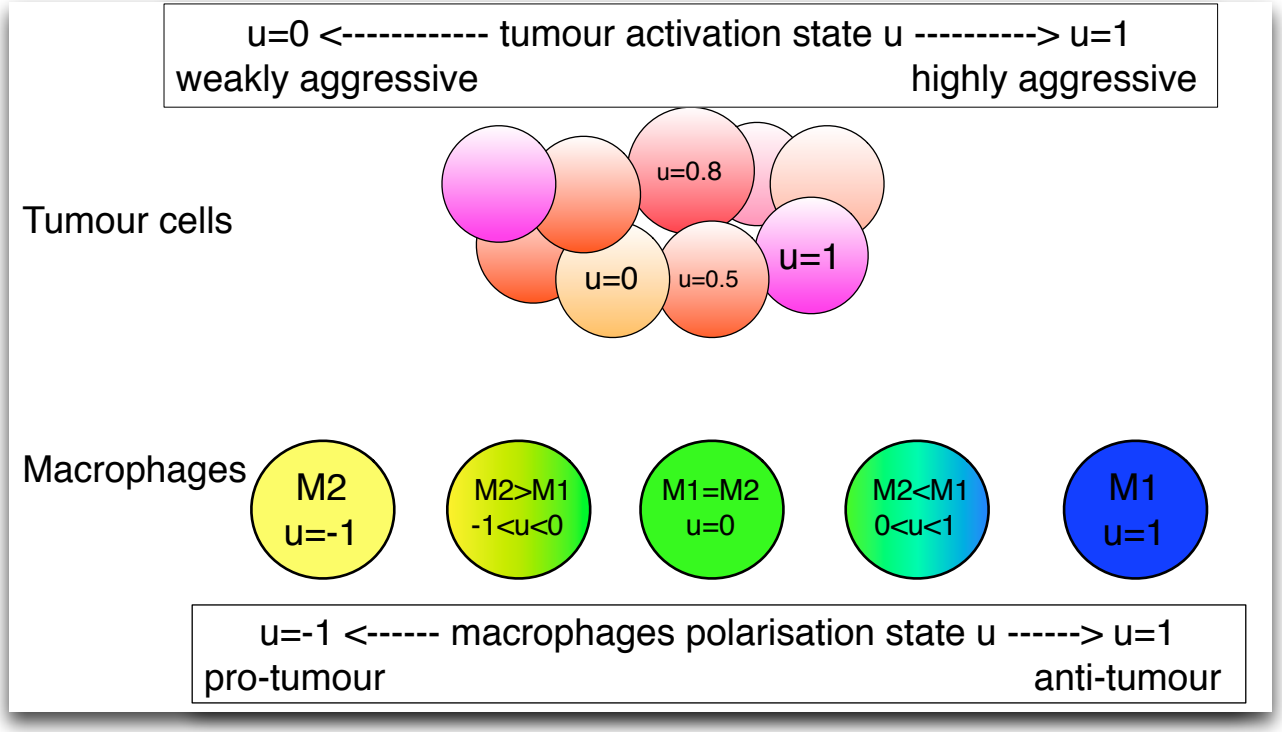
- Tumour can persist in the presence of
  - large M1/M2 ratio and small d_t
  - large M2/M1 ratio
- For these models: the long term behaviour of ODE system more complex than for the PDE system
- The type of phenotype kernel chosen for the PDE system can lead to different predictions regarding tumour elimination (i.e., faster/slower)
- The effect of M1-M2 overlap (i.e. mixed macrophages):
  - No M1-M2 overlap: relatively similar tumour dynamics for discrete & continuous models
  - Increasing the overlap between M1 & M2 phenotype (i.e., more macrophages with mixed M1-M2 markers) leads to a delay in tumour reduction & subsequent relapse

Macrophage phenotypic heterogeneity & tumour clonal heterogeneity...

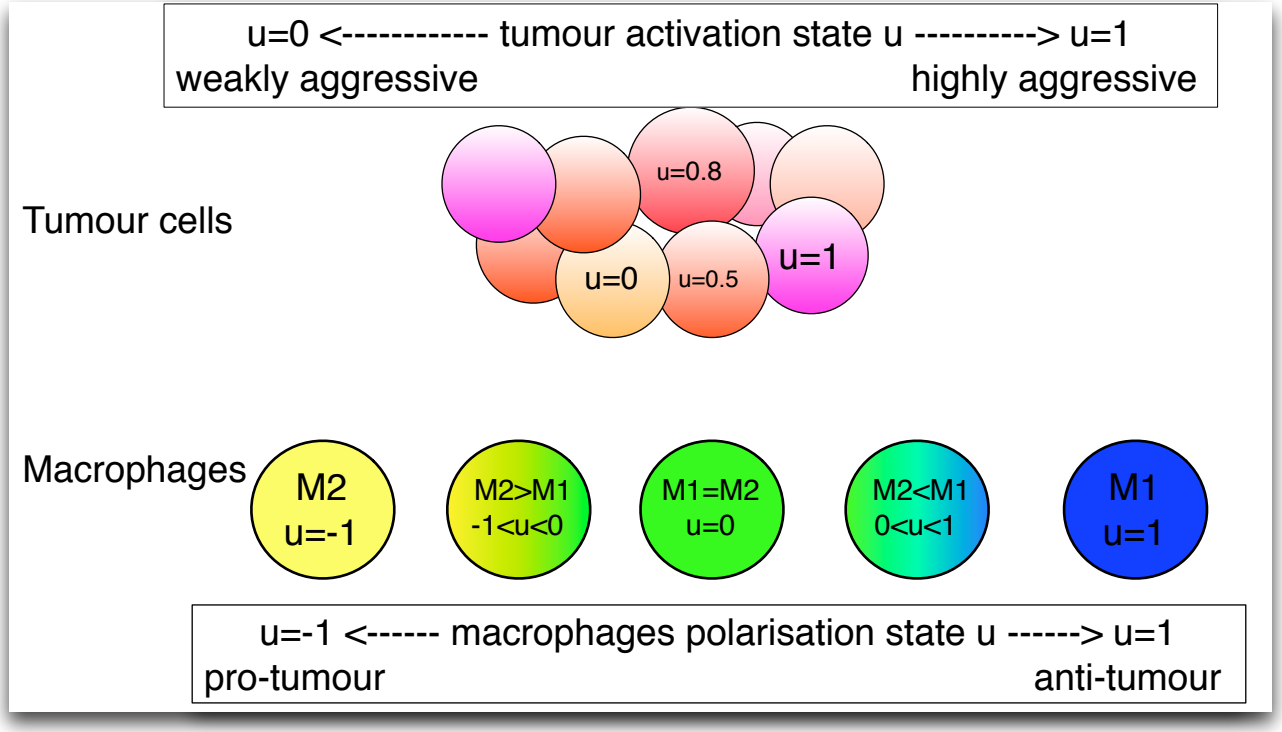
Dr. L. Gibelli (Univ. Edinburgh)

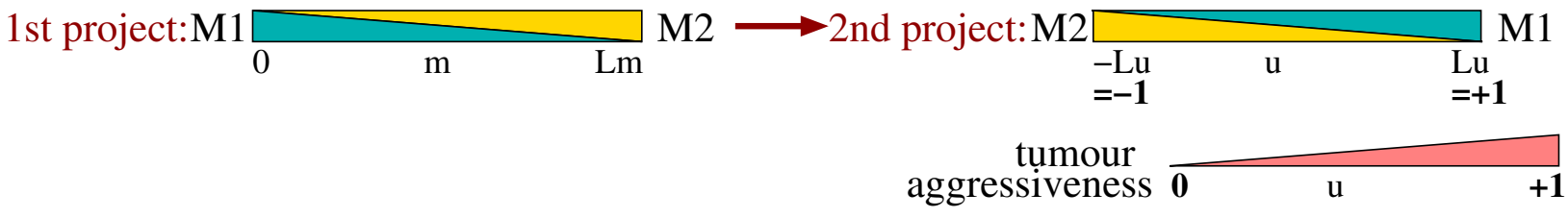
R. E., L. Gibelli, 2020. ***A kinetic theory approach for modelling macrophages heterogeneity and plasticity during cancer progression***.
Mathematical Models and Methods in Applied Sciences (M3AS). In press

Macroph. phenotypic heterogeneity + tumour clonal heterogeneity (E., Gibelli; M3AS, 2020)



Macroph. phenotypic heterogeneity + tumour clonal heterogeneity (E., Gibelli; M3AS, 2020)





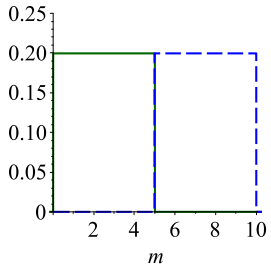
Macrophage phenotypic heterogeneity + tumour clonal heterogeneity

(Eftimie, Gibelli; M3AS, 2020)

$$\text{macrophages:}\quad f_1 = f_1(t,u) : [0,T]\times[-1,1]\to\mathbb{R}_+,$$
$$\text{tumour cells:}\quad f_2 = f_2(t,u) : [0,T]\times[0,1]\to\mathbb{R}_+.$$

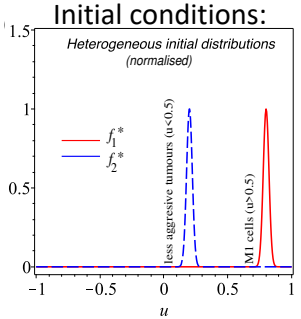
$$\begin{aligned}\partial_t f_1(t,u) =& \lambda_1 \int_{-1}^{1}\int_{0}^{1} \mathcal{B}_{12}(u_*\to u|u_*,u^*) f_1(t,u_*) f_2(t,u^*)\,du^*\,du_* \\ &- \lambda_1 f_1(t,u)\int_0^1 f_2(t,u^*)du^* - \mu^d f_1(t,u) \\ &+ \alpha f_1(t,u)\Big(1-\frac{\int_{-1}^1 f_1(t,u^*)du^*}{K_1}\Big)\int_0^1 f_2(t,u^*)\,du^*,\end{aligned}$$

$$\begin{aligned}\partial_t f_2(t,u) =& \lambda_2 \int_{-1}^{0}\int_{0}^{1} \mathcal{B}_{21}(u_*\to u|u_*,u^*) f_1(t,u^*) f_2(t,u_*)\,du_*\,du^* \\ &- \lambda_2 f_2(t,u)\int_{-1}^0 f_1(t,u^*)du^* - \gamma f_2(t,u)\int_0^1 f_1(t,u^*)\,du^* \\ &+ f_2(t,u)\Big(\beta^n+\beta^f\int_{-1}^0 f_1(t,u^*)\,du^*\Big)\Big(1-\frac{\int_0^1 f_2(t,u^*)du^*}{K_2}\Big).\end{aligned}$$



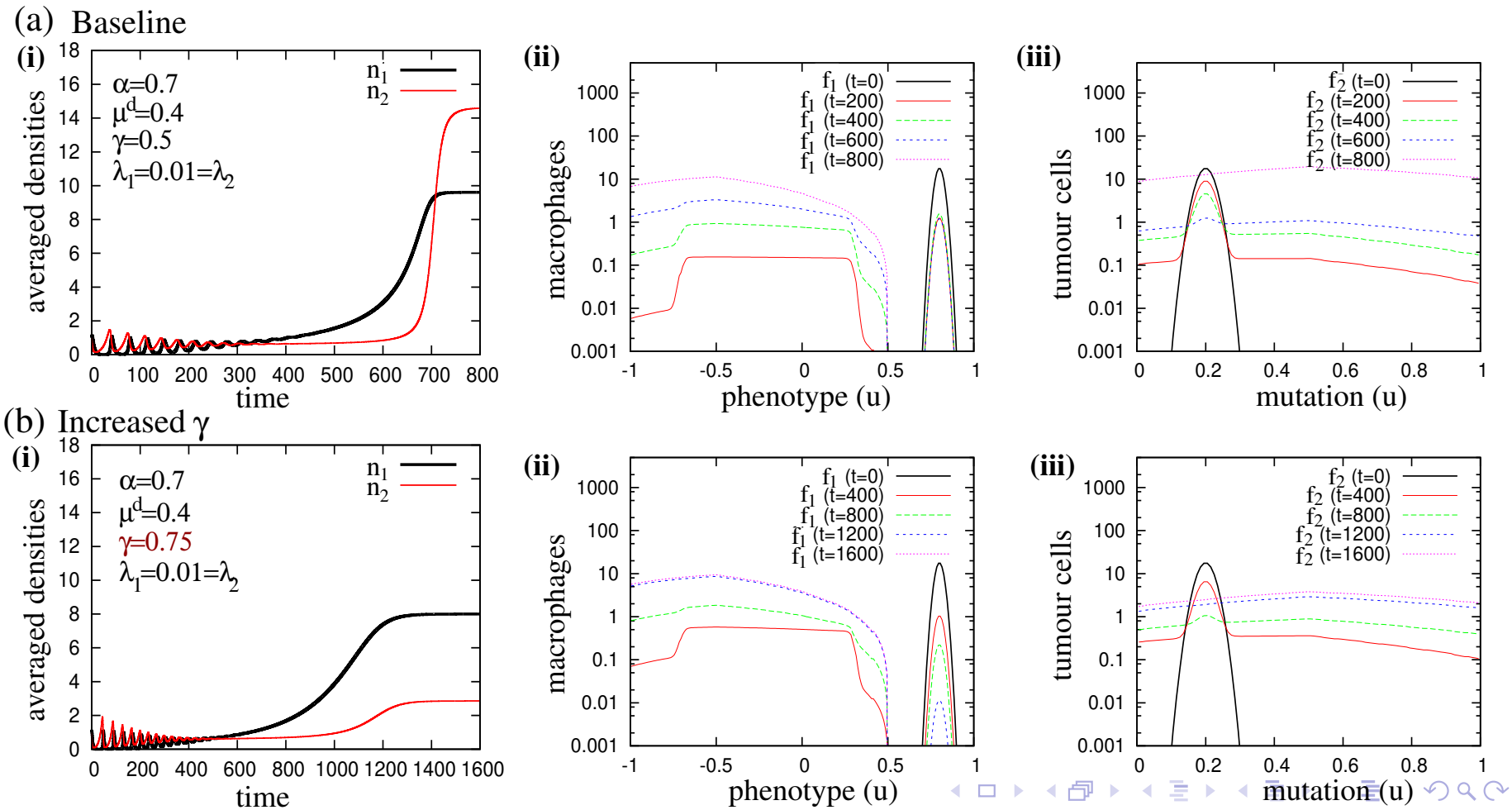
$\mathcal{B}_{1,2}$, $\mathcal{B}_{2,1}$ = normalised step functions

Initial conditions:



Macrophage phenotypic heterogeneity + tumour clonal heterogeneity (E., Gibelli; M3AS, 2020):

n_1 =total macrophages, n_2=total tumour => dormancy ...



Summary...

- dormant tumour dynamics is characterised by an increase in **clonal heterogeneity of tumours** & phenotypic heterogeneity of macrophages
- dormant tumour behaviour was mediated by M1-like macrophages
- tumour relapse was associated with a significant increase (from $f_1 \leq 1$ to $f_1 \approx 10$) in the density of macrophages with the extreme M2 phenotype ($u \approx -1$)

Work in progress: Multi-scale modelling of tumour-macrophage interactions & spatial spread of tumour cells and macrophages...

S. Suveges, PhD student
(Univ. Dundee)

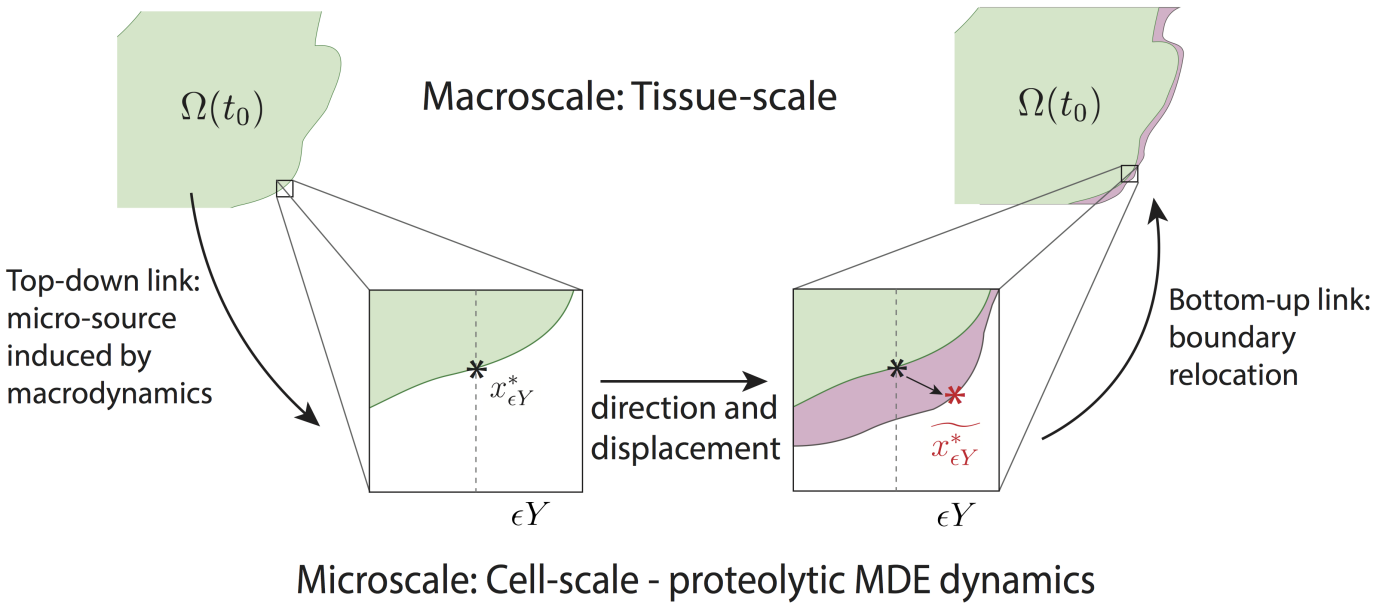
S. Suveges, R. E., D. Trucu, 2020. ***Multi-scale modelling of cancer invasion in the presence of M2 TAMs***. Submitted.

Dr. D. Trucu
(Univ. Dundee)

Multi-scale modelling of tumour-macrophage interactions (Süveges, E., Trucu; submitted, 2020)

Investigate the tumour-macrophages dynamics at 2 spatial scales:

- **Macro-scale:** movement of macrophages & tumour cells (& their interactions with ECM)
  mid/late-stage cancer => focus on M2-like macrophages (migrate & degrade ECM)
- **Micro-scale:** degradation and re-arrangement of ECM fibres, and tumour-boundary movement during invasion



From:

R. Shuttleworth, D. Trucu , (2019), *Multiscale modelling of fibres dynamics and cell adhesion with moving boundary cancer invasion*, BMB, 81, 2176–2219.

Current work: Multi-scale modelling of tumour-macrophage interactions (Süveges, E., Trucu; submitted, 2020)

Macroscale:

- Cancer cells: $\frac{\partial c}{\partial t} = \nabla \cdot [D(M)\nabla c - c\mathrm{A}(\mathbf{u}, \Theta)] + \mu c[1 - \rho(\mathbf{u})][1 + f_1(M)]$

  u=(c(x,yt),F(x,t),l(x,t),M(x,t))

  $\left(\begin{array}{c}\Theta = related\ to\ macroscopic \\ fibre\ orientation, which\ further \\ connects\ to\ the\ micro-dynamics \\ of\ fibres\end{array}\right)$

- ECM fibres: $\frac{\partial F}{\partial t} = -F f_2(c, M)$

- ECM non-fibres: $\frac{\partial l}{\partial t} = -l f_3(c, M) + \alpha M(1 - \rho(\mathbf{u}))$

- M2 cells: $\frac{\partial M}{\partial t} = D_M \nabla^2 M + f_4(c, M)$

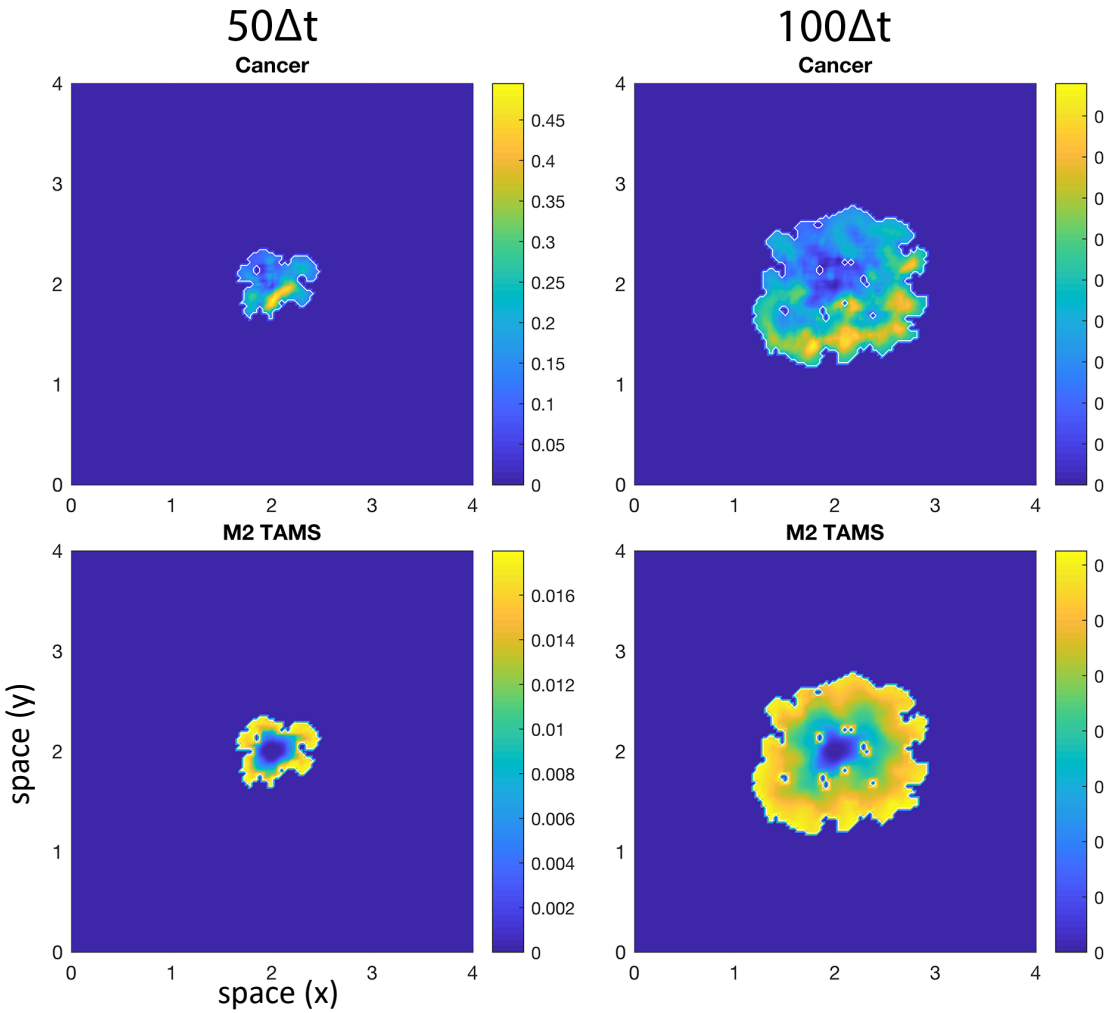
Microscale:

- MDEs density over each micro domain on tumour interface: $\frac{\partial m}{\partial t} = D_m \nabla^2 m + \mathrm{h}[\mathrm{c}, \mathrm{M}]$

Multi-scale modelling of tumour-macrophage interactions (Süveges, E., Trucu, 2020, submitted)

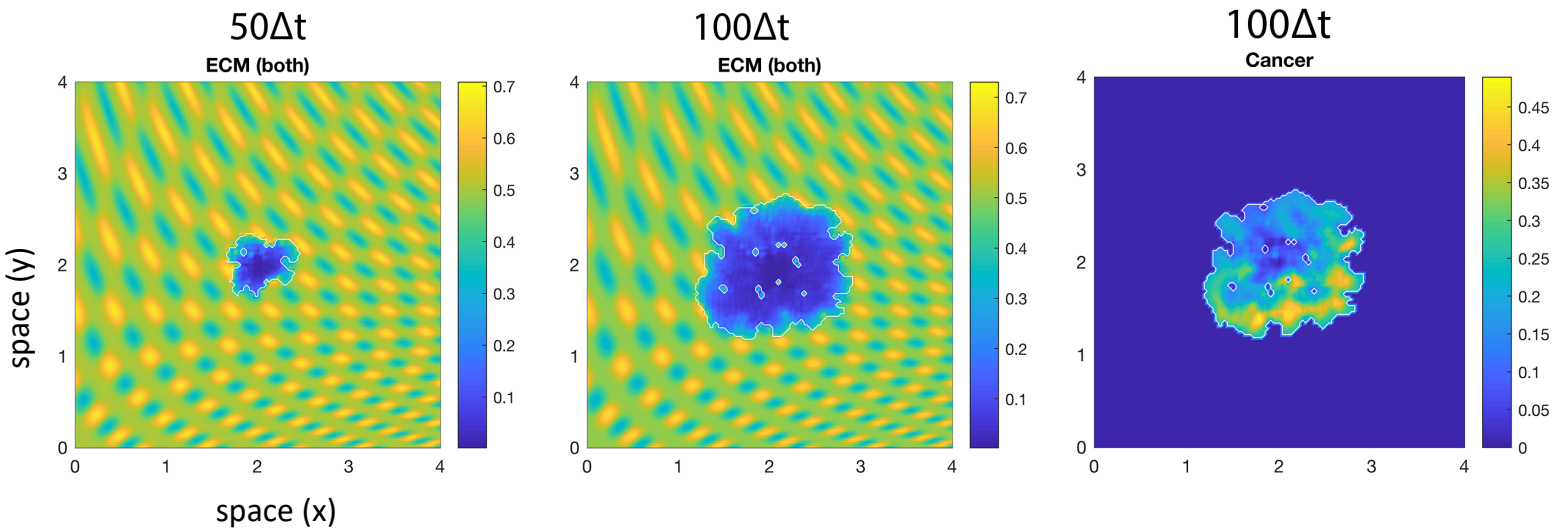
Example of Tumour - M2 macrophages spatial co-migration
(via ECM degradation)

"TAMs are mainly localized in the peripheral tumour stroma and decrease in number towards the center."
(Makela et al., Scientific Reports, 2016)



Multi-scale modelling of tumour-macrophage interactions (Süveges, E., Trucu, 2020, submitted)

ECM Degradation:



Work in progress:

- The role of M1 macrophages on slowing-down tumour spread
- The spatial distribution of M1 & M2 cells inside the tumour…?
- Role of mixed-phenotype macrophages on the spatial spread of tumours…?

To conclude...

- Mathematical/computational approaches can be used to generate (& test) hypotheses regarding the role of mixed M1/M2 phenotype of tumour dynamics
  - models need validation using murine/human data...still to do...
- Mathematical models with discrete and continuous macrophage phenotype (with no overlap!) show similar dynamics
- The overlap in phenotype markers (i.e., macroph. with both M1&M2 markers) can delay the elimination/growth of tumours
- Tumour dormancy is characterised by: (i) increase in tumour clonal heterogeneity, and (ii) increase in macrophage phenotypic heterogeneity
- TAMs contribute to the spatial invasion of cancer cells: the effect of mixed M1-M2 phenotype still unknown...

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