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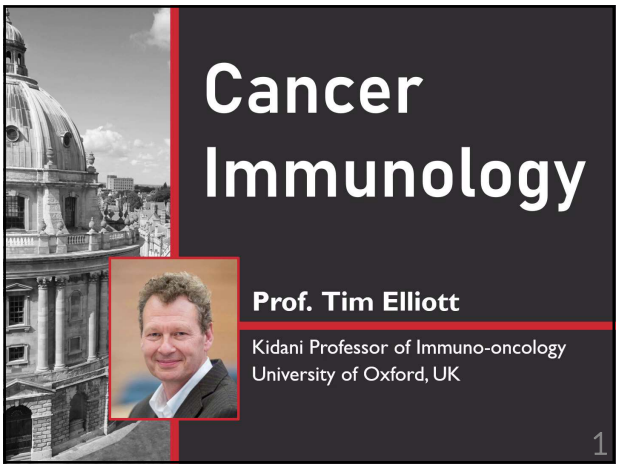


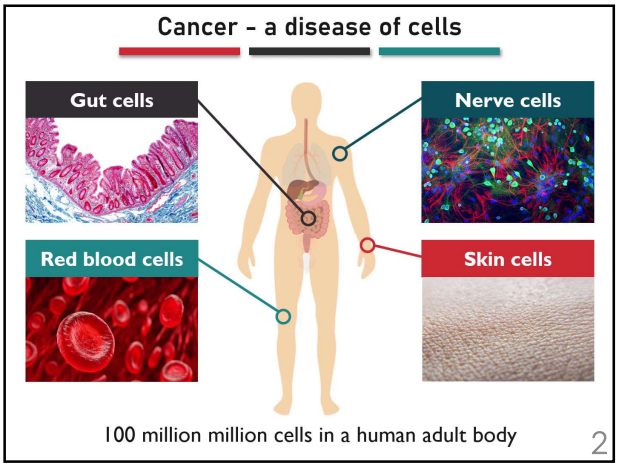


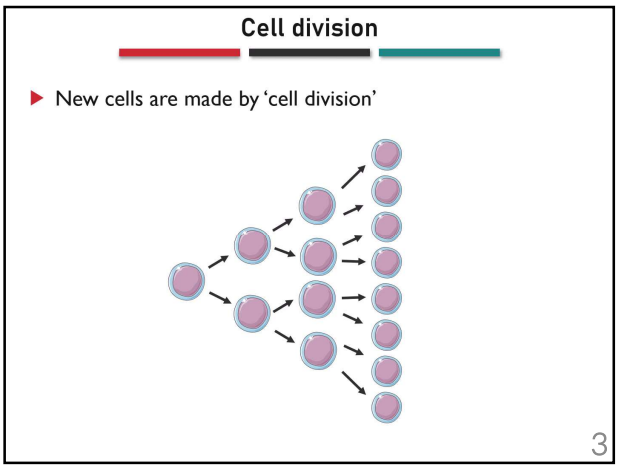

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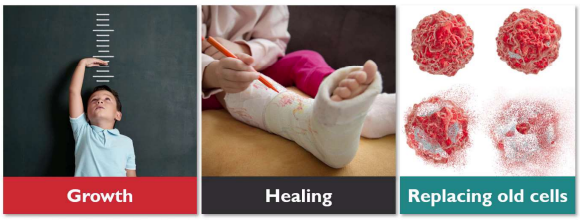
## Cell division

- Cell division is needed for:

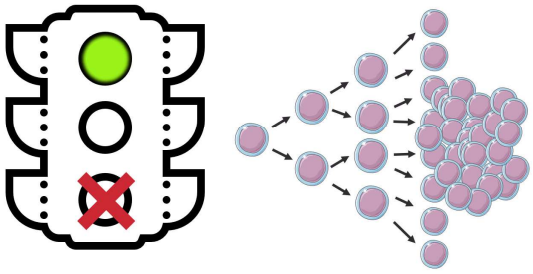



## In fact, in the past minute…

Your body has made…


4

## Cell division is normally tightly controlled



But this goes wrong in cancer

5

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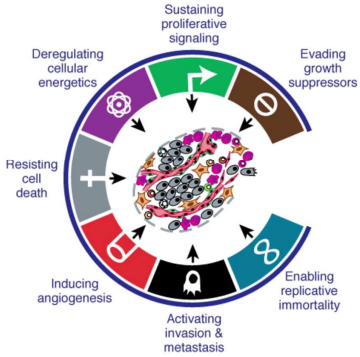
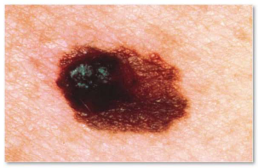
## The hallmarks of cancer




Hanahan D and Weinberg RA. *Cell*. 2011; 144: 646-674

6

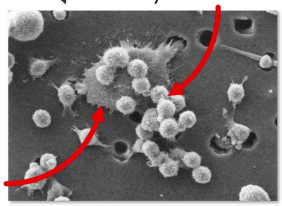
## Cancer is regulated by the immune system



Our immune system is able to recognise and destroy transformed cells

×1000

Cytotoxic T cell



Cancer cell

7

## Immune surveillance

One of the functions of the immune system is to identify and kill tumour cells

**Immunosurveillance**

Cancers which come to medical attention are only those that have managed to evade the immune system

8

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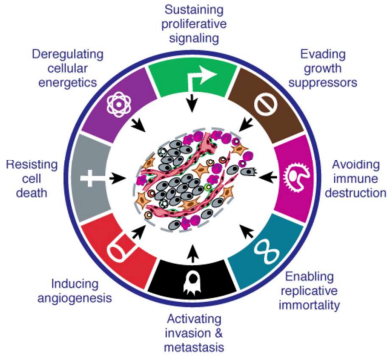
## The hallmarks of cancer include immunoevasion




Hanahan D and Weinberg RA. *Cell.* 2011; **144**: 646-674

9

## Evidence for immunosurveillance

- The high frequency of cancers in immunosuppressed patients
  - Extremes of age
  - Primary[1] and secondary immunodeficiency[2]
    - Immunosuppression that arises from HIV infection

[1]Mayor PC. *et al., J Allergy Clin Immunol.* 2018; **141**: 1028-1035
[2]Marcus JL. *et al., Cancer Epidemiol Biomarkers Prev.* 2015; **24**: 1167

10

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[3]Vial T and Descotes J. *Toxicology.* 2003; **185**: 229-40

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- Increased incidence of tumours in neonatal thymectomised[4] and immunocompromised mice[5]
  - Implies that T cells are important in immunosurveillance

[4]O'Gara RW and Ards J. *J National Cancer Institute*. 1961; **27**: 299-309
[5]Huang P. *et al., Comp Med*. 2011; **61**: 227-234

## Evidence for immunosurveillance

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- Increased incidence of tumours in neonatal thymectomised[4] and immunocompromised mice[5]
  - Implies that T cells are important in immunosurveillance
- Genetically modified mice that lack cytotoxic machinery, such as perforin-deficient mice, have a higher incidence of spontaneous tumours[6]

[6]Smyth MJ. *et al., J Exp Med*. 2000; **192**: 755-60

## Evidence of anti-tumour T-cell responses

- In people with cancer, we can find evidence that the immune system has responded, albeit not very effectively[1,2]

[1]Vesely MD. *et al., Ann Rev Immunol*. 2011; **29**: 235-271
[2]Finn OJ. *Ann Oncol*. 2012; **23(suppl 8)**: VIII6-VIII9

11

HSTalks

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## Evidence of anti-tumour T-cell responses

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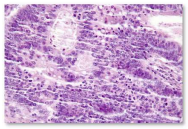
  - Tumour-specific T-cells[4]
  - High frequency of tumour-infiltrating lymphocytes[5]



[3]Reuschenbach M. *et al., Cancer Immunol Immunother.* 2009; **58**: 1535-44
[4]Godet Y. *et al., Clin Cancer Research.* 2012; **18**: 2943-2953 [5]Mlecnik B. *et al., Cancer Metastasis Rev.* 2011; **30**: 5-12

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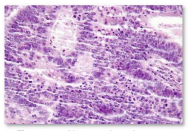
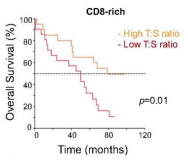
- In many tumour types, infiltration by CD8$^+$ effector T cells correlates with better prognosis[6,7]
  - Especially true for CD103$^+$ $T_{RM}$ CTL[8]



Tumour infiltrating lymphocytes in colorectal carcinoma




[6]Jochems C. *et al., Exp Biol Med (Maywood).* 2011; **236**: 567-79
[7]Galon J. *et al., Science.* 2006; **313**: 1960-4 [8]Ganesan A. *et al., Nat Immunol.* 2017; **18**: 940-950

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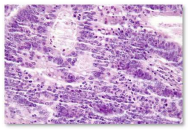
  - Especially true for CD103$^+$ $T_{RM}$ CTL[8]
- We sometimes see spontaneous regression of advanced and metastatic cancers[9]



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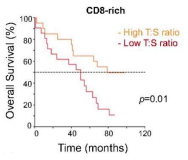


Lung squamous-cell carcinoma

[9]Kalialis LV. *et al., Melanoma Res.* 2009; **19**: 275-82 Peranzoni E. *et al., PNAS.* 2018; **115**: e4041-e4050

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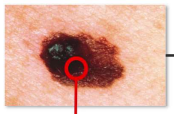
## NGS evidence of anti-tumour immunity

Tumour



Improvements in next generation transcriptomic profiling of tumour RNA has shown:

**Common gene signature:**

**Chemokines associated with lymphocytes**
- CXCL9
- CXCL10
- CCL5

**Th1 signalling**
- IFN-γ
- IL12B
- CD8α and β
- TBX21 (Tbet)
- STAT 1
- IRF 1

**Effector mechanisms**
- Perforin
- Granzyme A, B, H
- Granulysin

→ Destructive autoimmunity

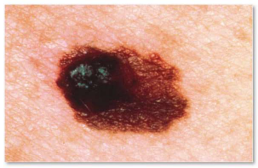
→ Acute pathogen clearance

→ Acute graft rejection

→ Graft *versus* host disease

12

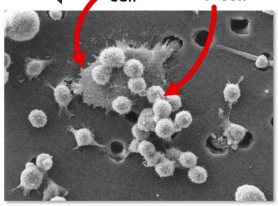
## How do T-cells recognise cancer cells?



×1000

Cancer cell

Cytotoxic T cell



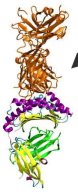
×1000

**Molecular recognition**

T cell receptor

Short peptide

MHC class I

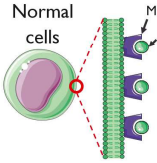


13

## How do T-cells recognise tumour cells?

- Need to distinguish normal from tumour cells
  - Tumour associated antigens
  - Tumour specific antigens

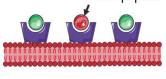
Normal cells



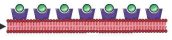
Class I MHC

Self peptide

Viral peptide

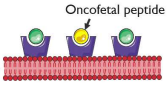


**Virally-induced cancers**
e.g., HPV-1 E6/7



**Normal proteins that become overexpressed**
e.g., HER2neu

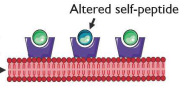
Oncofetal peptide



**Embryonic gene expression in adult cells**
e.g., MAGE-1

Majority of neoepitopes are derived from passenger mutations - specific to an individual's tumour

Altered self-peptide



**Mutation generates new peptide in class I MHC molecules**
'Neoepitopes'

14

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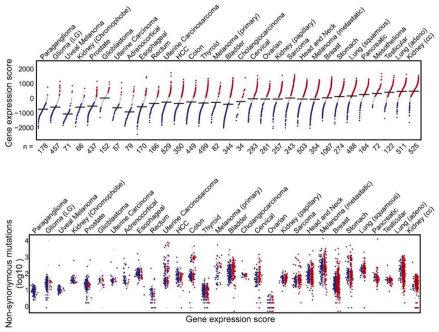
## Tumour neoantigens

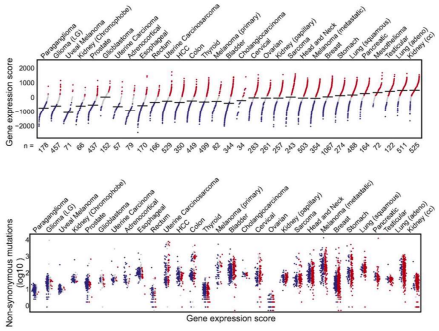



Spranger S. *et al., PNAS.* 2016; **113**: E7759-E7768

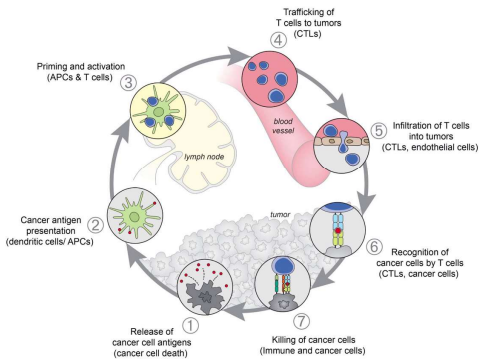
15

## Tumour neoantigens

Some tumours like melanoma and lung cancer generate more neoepitopes likely to be recognised by T cells




## Tumour antigens lead to a cycle of immune attack




Chen DS and Mellman I. *Immunity.* 2013; **39**(1): 1-10

16

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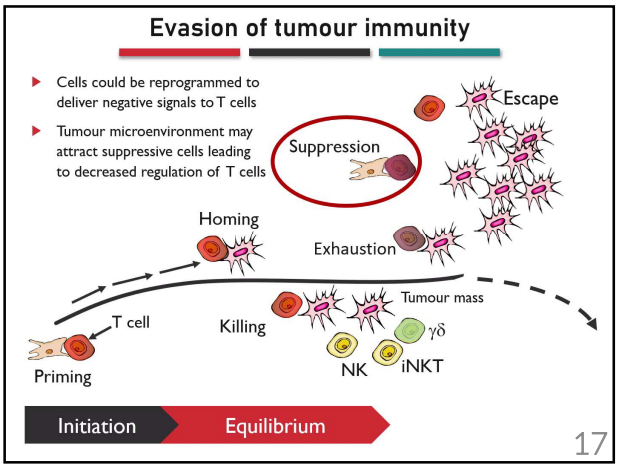


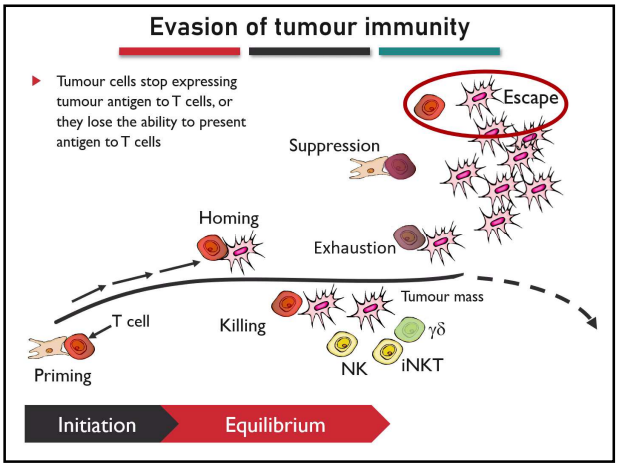


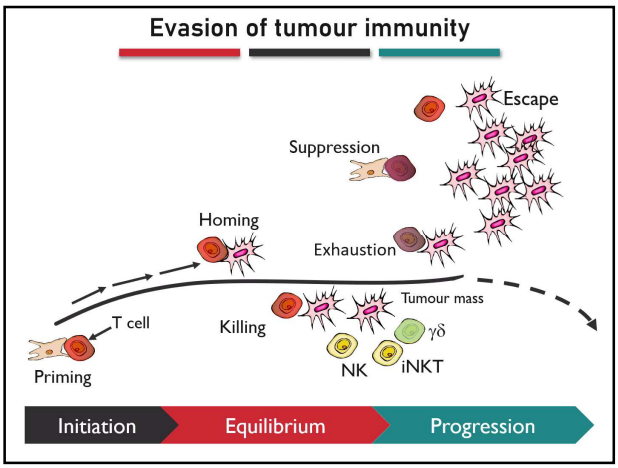

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## NGS evidence for evasion of tumour immunity 18

Extended signature for ICR includes genes for immune escape, suppression and exhaustion

**Common gene signature:**

**Chemokines associated with lymphocytes**
- CXCL9
- CXCL10
- CCL5

**Th1 signalling**
- IFN-γ
- IL12B
- CD8α and β
- TBX21 (Tbet)
- STAT 1
- IRF 1

**Effector mechanisms**
- Perforin
- Granzyme A, B, H
- Granulysin

ICR = immunologic constant of rejection

**Immune escape**
- β-2m
- MHC I
- MHC II
- Antigen processing

**Immune suppression**
- FoxP3
- IDO

**Immune exhaustion**
- PD1
- PD-L1
- TIM-3
- TIGIT
- LAG3

## Immune escape

- MHC I antigen processing signature correlates with ICR signature and are frequently mutated in multiple cancer types[1]
  - Structural proteins HLA-A/B
  - β-2 microglobulin
  - TAP1/2
  - Tapasin
- Cancer cells evolve to escape HLA restriction through mutation of HLA class I genes[2]

[1]McGranahan N. *et al., Cell.* 2017; **171**: 1259-1271
[2]Tran E. *et al., N Engl J Med.* 2016; **375**: 2255-2262

19

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  - Tapasin
- Cancer cells evolve to escape HLA restriction through mutation of HLA class I genes[2]
- The dominant oncogenic mutations in individual cancers tend to occur in peptides that are poorly presented by the HLA allotypes present in the patient[3]

[3]Marty R. *et al., Cell.* 2017; **171**: 1272-1283

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## Immune escape

- Homozygosity at HLA class I associates with poor response to immunotherapy[4]
  - The more diverse the HLA class I proteins expressed by tumour cells, the higher the cancer of neoepitopes
  - Therefore, loss of homozygosity reduces the number of potential neoepitopes for recognition

[4]Chowell D. *et al., Science.* 2018; **359**: 582-587

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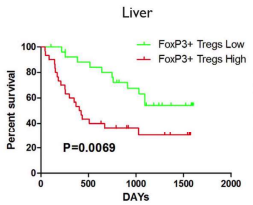
  - Therefore, loss of homozygosity reduces the number of potential neoepitopes for recognition
- Inverse relationship between tumour associated antigen expression and $CD8^+$ CTL responses in mouse models[5] and some human cancers[6]

[5]Schirrmacher V. *et al.*, Invasion Metastasis. 1981; 1: 175-194
[6]Jager E. *et al., Int J Cancer.* 1996; **66**: 470-6

## Immune suppression

### Regulatory T cells (Treg)

- $CD4^+$ T cells that express FoxP3
- Recruited by chemokine CCL22 secreted by tumour cells (CCR4 on Tregs)
- Secrete inhibitory cytokines (TGF-β, IL-35, IL-10)
- Metabolically disruptive to Teff (CD39/73 generated adenosine)
- Express high levels of IL-2R, acts as a local sink for the T cell growth factor IL-2
- Induce indoleamine 2,3-dioxygenase production by DC via CTLA-4 (depletes tryptophan which T cells need)




Patients with low FoxP3+ Tregs had better survival outcome than those with high FoxP3+ Treg

Tu JF. *et al.*, Sci Rep. 2016; **6**: 35056

20

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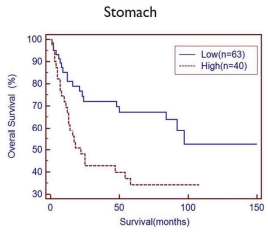
## Immune suppression

### Tumour associated macrophages (TAM)

- Bone-marrow origin, polarised to M2 under influence of IL-4
- Secrete inhibitory cytokines (TGF-β, IL-10)
- Secrete arginase-1 which generates (immunosuppressive) L-arg
- Express indoleamine 2,3-dioxygenase
- Express checkpoint molecules PD-L1/2, CD86
- Secrete CCL5, 20, 22 which attract Treg



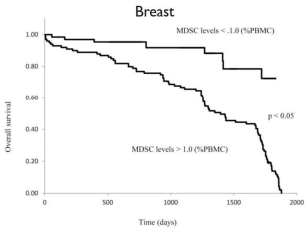

Kaplan-Meier showing data for low TAM density and high TAM density

Wu M. *et al., Plos One.* 2015; **10**: e0134122

## Immune suppression

### Myeloid derived suppressor cells (MDSC)

- $CD14^{+}CD11b^{+}CD33^{+}$ differentiated under the influence of GM-CSF, G-CSF and IL-6
- Secrete arginase-1 which generates (immunosuppressive) L-arg
- Secrete inhibitory cytokines (TGF-β, IL-10)
- Express checkpoint molecules PD-L1/2, CD86



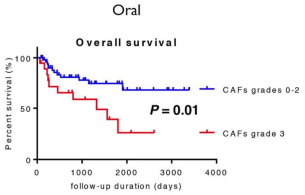

Gonda K. *et al., Oncol Lett.* 2017; **14**: 1766-1774

21

## Immune suppression

### Cancer-associated fibroblasts (CAF)

- Develop from fibroblasts in the presence of TGF-β
- Express markers SMA, FAP, Tenascin-C, Periostin
- Stimulate angiogenesis and block T cells from attacking tumour
- Induce (TAM)




Takahashi H. *et al., Oncotarget.* 2017; **8**: 8633-8647

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