M1 Development of Drug and Health products– TU05

université PARIS-SACLAY
FACULTÉ DE PHARMACIE
Inserm
La science pour la santé From science to health

Tumor heterogeneity, microenvironment, antitumor immunity

Christian POÜS
Faculté de Pharmacie, Orsay
Inserm U1193

E. Jacquin 2022-2023
C. Poüs 2024

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Outline

Tumors are heterogeneous
- Cancer cells and tumor microenvironment
- Factors influencing tumor heterogeneity
- Cancer cell heterogeneity - Clonal selection
- Cancer cell heterogeneity - Cancer stem cells (CSCs)
  - *Stem cells*
  - *The two hypotheses of CSC formation*
  - *CSCs, resistance to treatment and relapse*
  - *CSC identification and biomarkers*
  - *Target CSCs for cancer treatment*
- Tumor microenvironement (TME)
  - *Tumor infiltrating cells*
  - *Immune cells infiltrating tumors influence cancer progression*

Cancer immunoediting
- Elimination
  - *Elimination of cancer cells by cytotoxic CD8+ T cells*
  - *Categories of tumor antigens*
  - *Tumor Mutational Burden (TMB)*
  - *T cell-mediated antitumor immunity*
- Equilibrium
  - *Selection for less immunogenic tumor cell clones*
  - *T cell inhibition : the PD-L1/PD-1 axis*
- Escape
  - *Tumor cells avoid immune destruction*
  - *Tumor cell-intrinsic mechanisms*
  - *Immune cell-mediated mechanisms*
    - IFN-Y stimulates PD-L1 expression by tumor cells
    - Immunosuppressive functions of Tregs
    - Tumor-associated Macrophages (TAM)
    - Myeloid-derived suppressive cells (MDSCs)
  - *Inflammation and cancer*

Cancer immunotherapies
- *Multiple immunotherapy strategies*
- *Tumor microenvironment classification*
- *PD-L1/PD-1 axis, an immune checkpoint*
- *Immune checkpoint inhibitors: anti-PD-1 and anti-PD-L1 antibodies*
- *Future perspectives*

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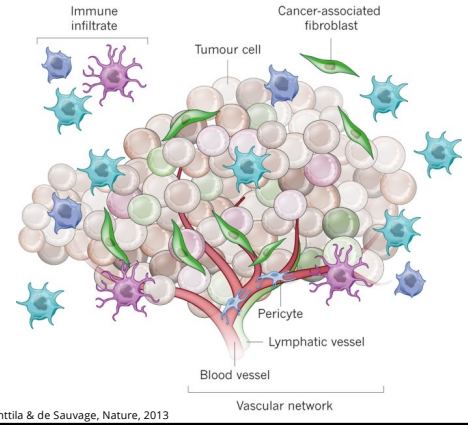
Tumors are heterogeneous



Junttila & de Sauvage, Nature, 2013

Tumors =

Cancer cells = heterogeneous population
- **Cancer cell clones**
- **Cancer stem cells**

Tumor microenvironment (TME)
- Cancer-associated fibroblasts (CAFs)
- Vascular network
- **Various infiltrating immune cells**

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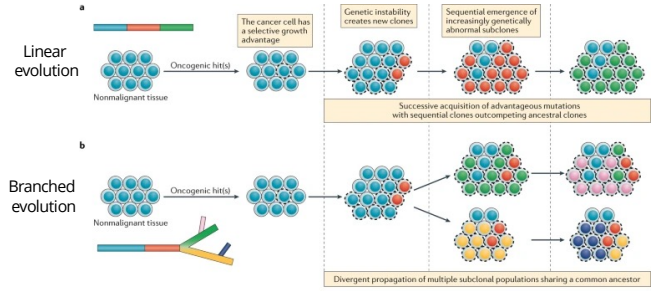
Cancer cell heterogeneity

Clonal selection: acquisition of successive mutations that confer survival/growth advantage



Emerging clone successfully outcompetes the preceding. Surviving dominant clone harbors ancestral mutation

Multiple clones emerge from common ancestral clone, some diverging from the main axis before others

More or less cancer cell heterogeneity

Dagogo-Jack & Shaw et al, Nature Rev Clin Oncol, 2017

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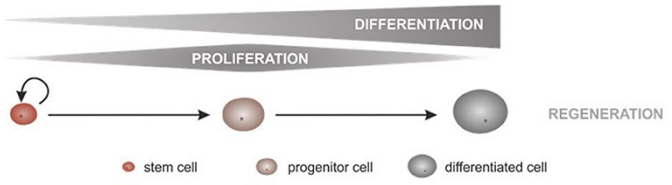
Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

Stem cells

- Unlimited cell proliferation capacity
- Asymmetric division: two different daughter cells
  - One ensures the self-renewal of stem cells
  - The other has transient high proliferative capacity and then differentiates



Walcher et al, Front. Immunol., 2020

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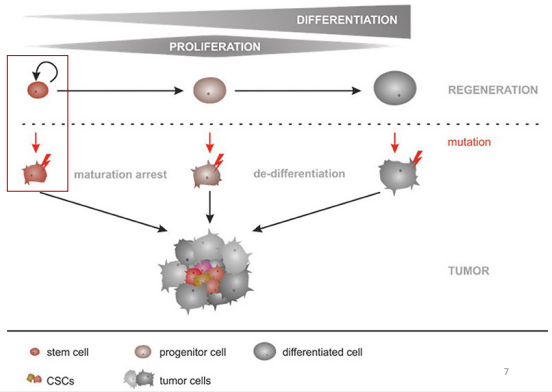
Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

Cancer stem cells formation upon tumor initiation, two hypotheses

1) Transformation of tissue-resident stem cells following mutation acquisition



Walcher et al, Front. Immunol., 2020

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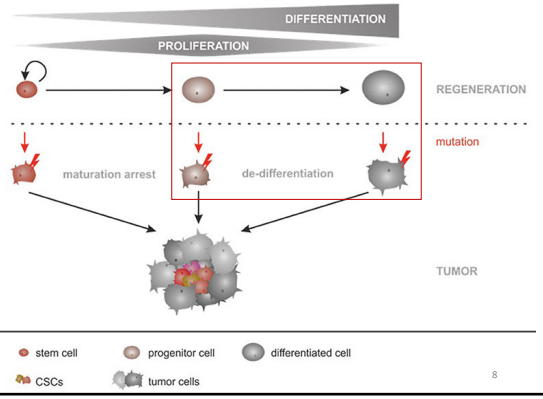
Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

Cancer stem cells formation upon tumor initiation, two hypothesis

2) Accumulation of mutations in differentiated cells or progenitors leading to their **transformation and de-differentiation** (stem cell phenotype acquisition)



Walcher et al, Front. Immunol., 2020

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Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

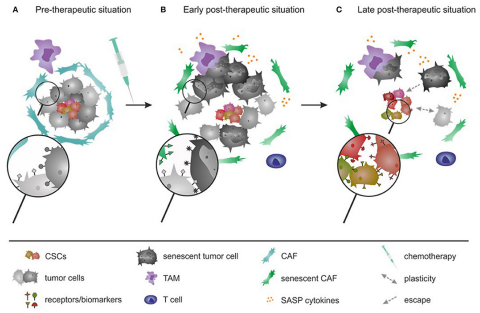
Cancer stem cells, resistance to treatment and relapse

Tumor with cancer stem cells: aggressive and therapy-resistant tumors

A: Heterogeneous tumor, before treatment

B: Chemotherapy/radiotherapy:
proliferating tumor cells and CAFs die
or become senescent and attract immune cells (inflammation)
Cancer stem cells (CSCs) survive

C: Uncleared senescent cells and sustained inflammation stimulate CSC maintenance and proliferation → resistance or relapse



Walcher et al, Front. Immunol., 2020

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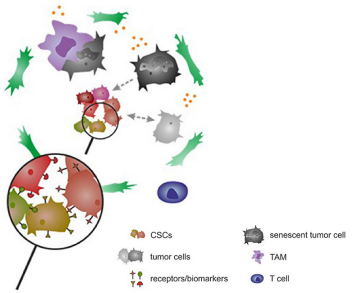
Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

Cancer stem cell identification and biomarkers

- CSC detection to evaluate resistance to treatment and predict relapse?
- **Senescence biomarkers** under evaluation:
  - β-galactosidase activity
  - p53 level and nuclear localization
- **CSC biomarkers** under evaluation:
  - solid tumors: CD44, CD133
  - hematological cancers: CD44, CD123, CD33



Walcher et al, Front. Immunol., 2020

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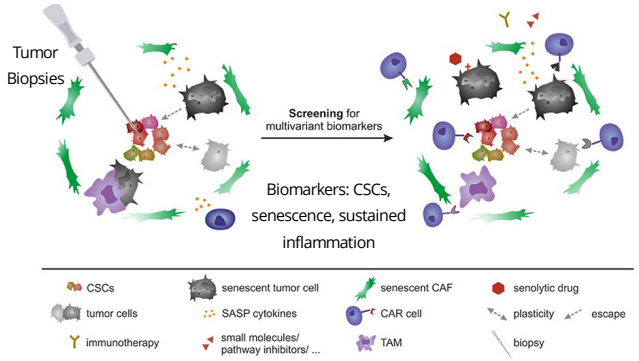
Cancer cell heterogeneity – Cancer Stem Cells (CSCs)

Prospects: Target cancer stem cells (CSCs) to improve cancer treatment



2nd line of treatment:

- CAR T cell therapies targeting CSCs ($CD123^+$, CD133+)
- Senolytic drugs (Quercetin, Dasatinib) to deplete senescent cells

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Cancer cell heterogeneity – Take home messages

- Cancer cells contribute to tumor heterogeneity due to genetic instability and co-existence of cancer cell clones.
- Cancer stem cells (CSC) contribute to tumor heterogeneity.
- CSC form upon tumor initiation.
- CSC are resistant to conventional cancer treatments and favor relapse.
- CSC maintenance and proliferation is favored by other cell senescence and inflammation.
- Biomarkers of CSC could be exploited to predict relapse.
- The targeting of CSCs to improve cancer treatment is currently under investigation.

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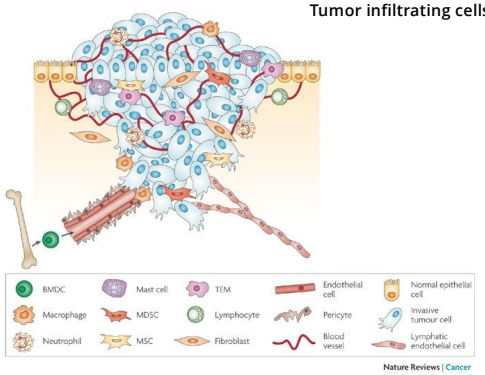
Tumor microenvironment (TME)

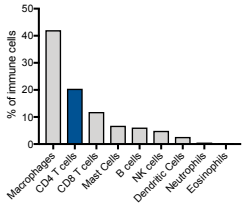
Tumor infiltrating cells



Joyce & Pollard, Nature Reviews Cancer, 2009

Immune cells infiltrating tumors

29 cancer types (n=10490 patients)



Thorsson *et al.* Immunity, 2018

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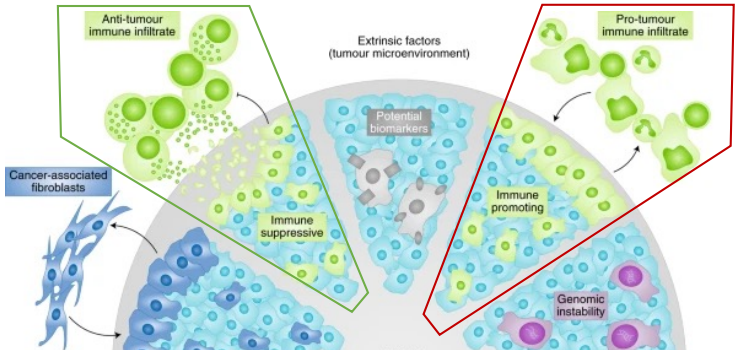
Tumor microenvironment (TME)

Immune cells infiltrating tumors influence cancer progression



Lawson et al, Nature Cell Biology, 2018

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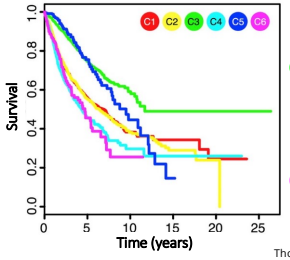
Tumor microenvironment (TME)

Immune cells infiltrating tumors influence cancer progression

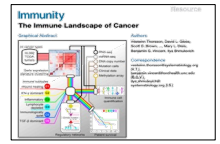
Cancer Genome Atlas (TCGA): genomic and transcriptomic data from cancer samples

Immune profile definition (C1 to C6)



C3 → Good prognosis

C6 → Bad prognosis



Survival (C3 profile):

- Infiltrating "inflammatory" macrophages
- Abundant infiltrating T lymphocytes
- Tumor neoantigen diversity

Thorsson *et al.* Immunity, 2018

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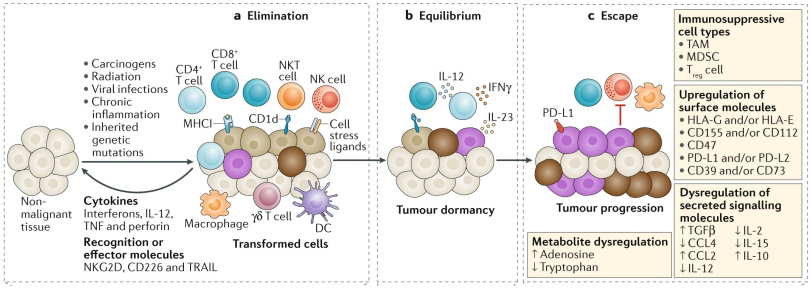
Cancer immunoediting



(a) Elimination
Detection and elimination of transforming cells by immune cells

(b) Equilibrium
Between tumor cells surviving elimination phase and immune system pressure
Clone emergence

(c) Escape
Unrestrained growth of less immunogenic tumor cell clones
Tumor clinically detectable

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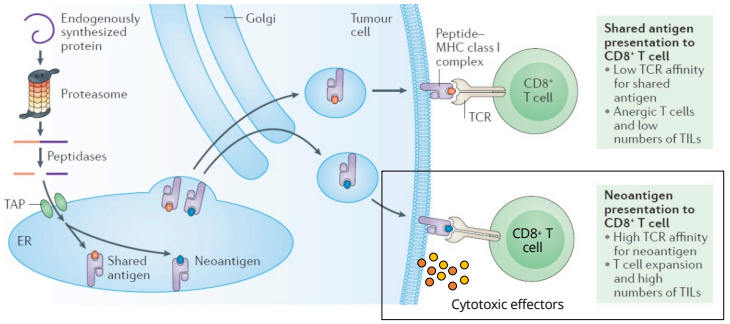
Cancer immunoediting - Elimination

Elimination of cancer cells by cytotoxic CD8⁺ T cells



Tumor neoantigens presented by MHC-I molecules on tumor cells are recognized by cytotoxic CD8⁺ T cells

Yarchoan et al, Nat Rev Cancer, 2017

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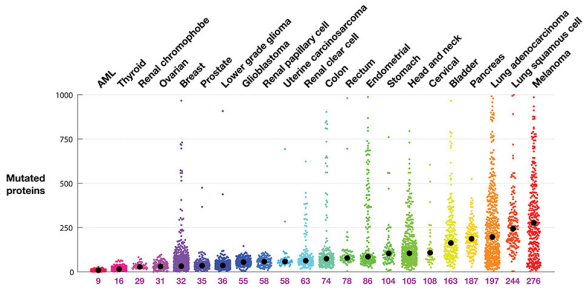
Cancer immunoediting - Elimination

Tumor Mutational Burden (TMB):

total number of mutations (changes) found in the DNA of cancer cells



Tumor with high TMB are more likely to express immunogenic neoantigens that can be recognized by cytotoxic T cells

Castle et al, Front Immunol , 2019

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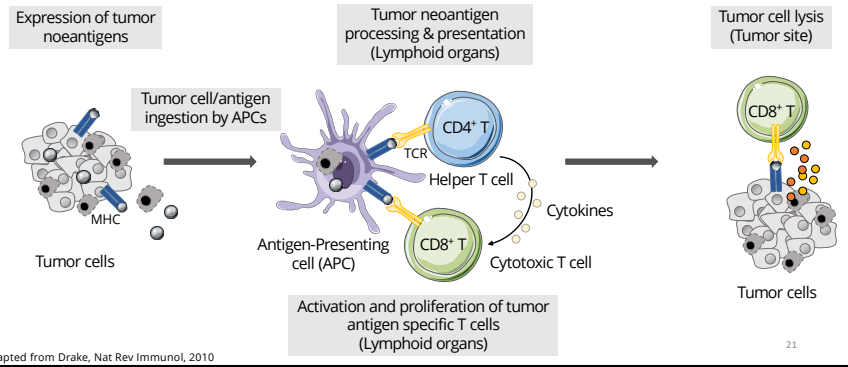
Cancer immunoediting - Elimination

Elimination of cancer cells: T cell-mediated antitumor immunity



Adapted from Drake, Nat Rev Immunol, 2010

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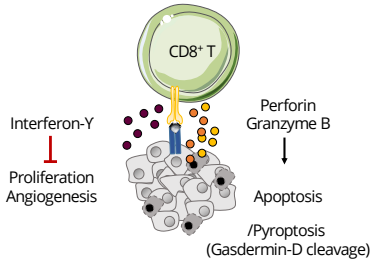
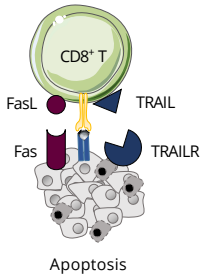
Cancer immunoediting - Elimination

Elimination of cancer cells: T cell-mediated antitumor immunity

Secretion-mediated cytotoxicity: cytotoxic effectors



Extrinsic apoptosis induction: death ligand expression



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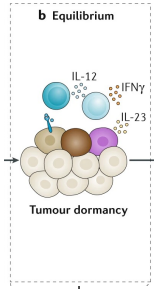
Cancer immunoediting - Equilibrium

Infiltrating immune cells

- Balance between immunosuppressive (Tregs, Myeloid-Derived Suppressor Cells) and immune cells with antitumor activity (CD8+ T, DCs, NK cells)
- Balance between IL-23/10 (pro-tumor) and IL-12/IFN-Y (anti-tumor)



Tumor cells: progressive loss of immunogenicity

- Genetic and epigenetic changes
- Progressive tumor antigen loss
- Progressive MHC-I downregulation
- ↑ PD-L1 expression : suppression of T cell activity

Selection for less immunogenic tumor cell clones

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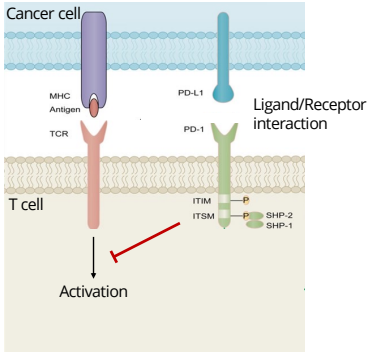
Cancer immunoediting - Equilibrium



T cell inhibition : the PD-L1/PD-1 axis

- Programmed cell death ligand 1 (PD-L1) expression by cancer cells
- Programmed cell death 1 (PD1) receptor expression by T cells
- Ligand/Receptor interaction
- Inhibition of T cell activation
- Suppression of antitumor T cell activity

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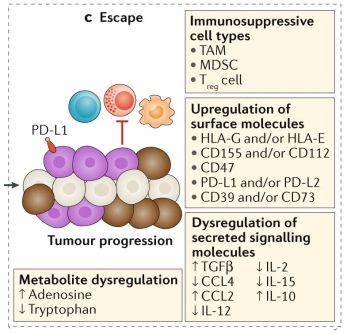
Cancer immunoediting - Escape

Unrestrained tumor growth of the less immunogenic tumor clones, clinically apparent disease



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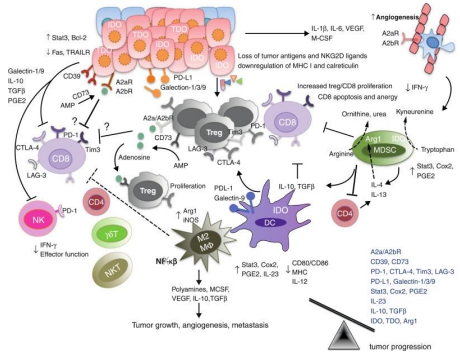
Cancer immunoediting - Escape

Multiple mechanisms… a mess!



Mittal et al, Current Opinion in Immunology, 2014

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Cancer immunoediting - Escape

Tumor cell-intrinsic mechanisms…

Reduced immunogenicity	Increased resistance/survival	Immunosuppressive molecules	Immunosuppressive cytokines
• Tumor antigen loss • ↓ MHC-I • ↓ Calreticulin (↓ ICD)	• ↑ STAT3 • ↑ BCl-2 • ↓ FAS/TRAILR	• ↑ PD-L1 • ↑ CD39/CD73 • ↑ Adenosine receptor • ↑ IDO	• ↑ TGF-β • ↑ IL-10

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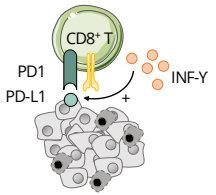
Cancer immunoediting - Escape

Immune cell-mediated suppressive mechanisms

IFN-Y secreted by CD8+ T cells stimulates PD-L1 expression by cancer cells



Immunosuppressive cell recruitment

Regulatory T cells

Myeloid-derived suppressive cells

Tumor associated macrophages (M2)

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Cancer immunoediting - Escape

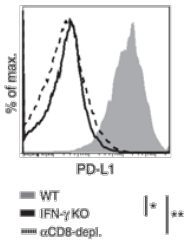
IFN-Y secreted by CD8+ T cells stimulates PD-L1 expression by tumor cells

Control or IFN-Y KO or CD8+ T cell depletion

Tumor cell innoculation

Tumor cell retrieval

PD-L1 expression analysis (Flow cytometry)



Reduced PD-L1 expression in tumor cells in the absence of IFN-Y or CD8+ T cells (IFN-Y-producing cells)

Spranger et al, Sc Transl Med, 2013

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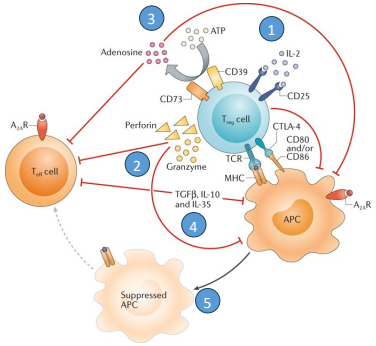
Cancer immunoediting - Escape

Immunosuppressive functions of regulatory T cells (Tregs)



1. **IL-2**: Tregs consume IL-2 → ↓ effector helper T cell activity
2. **Granzyme/Perforin**: effector T cell killing
3. **ATP to Adenosine conversion** (by CD39/CD73): ↓ ATP availability, inhibition of effector T cell activity
4. **Immunosuppressive cytokine** (TGF-β, IL-10, IL-35) production : inhibition of effector T cell activity
5. **CTLA-4 expression**: inhibition of APC-mediated activation of effector T cells

Togashi et al, Nature Reviews Clinical Oncology, 2019

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Cancer immunoediting - Escape

Myeloid-derived suppressive cells (MDSCs)

- MDSCs: heterogenous population
- Similarities with monocytes and neutrophils
- Two classes of MDSCs:

Granulocyte Monocyte Progenitors (GMP)

MDSCs Granulocytic/polymorphonuclear **PMN-MDSCs**

MDSCs Monocytic **M-MDSCs**

Phenotype & Morphology	« Neutrophil-like »	« Monocyte-like »

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Cancer immunoediting - Escape

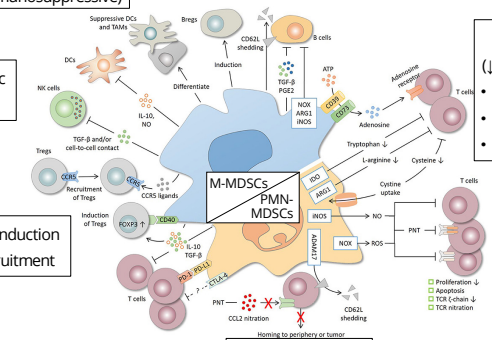
↑ TAM (immunosuppressive)

B cell inhibition

NK and Dendritic Cell inhibition

T cell suppression (↓ proliferation/activation, apoptosis)
- ↓ ATP levels (CD39/CD73)
- ↓ amino-acids (L-Arg, Cys, Trp)
- Oxidative stress

↑ Treg induction & recruitment



↓ T cell recruitment

Yang *et al*, Front. Immunol., 2020

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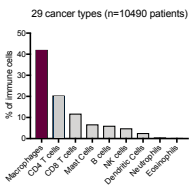
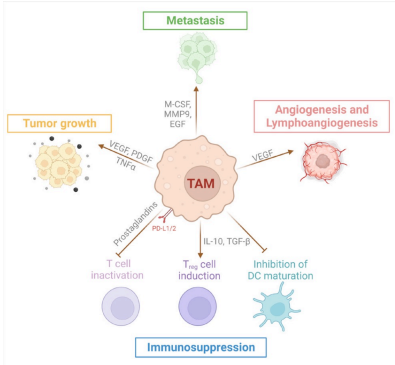
Cancer immunoediting - Escape

Tumor-Associated Macrophages (TAM)

The most abundant tumor-infiltrating immune cells



Thorsson *et al*. Immunity, 2018



Monnier et al, JITC, 2022

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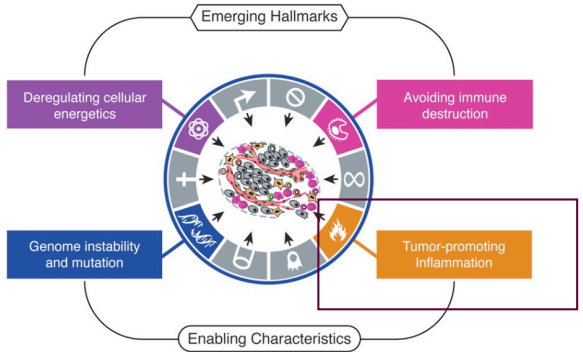
Inflammation and cancer



Pro-inflammatory molecules → immune cell recruitment and activation → Anti-tumor immunity

Contradictory?

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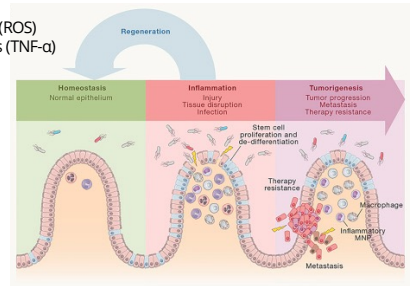
Inflammation and cancer

Reactive Oxygen Species (ROS)
Pro-Inflammatory molecules (TNF-α)
↓
↑ mutation rate
↓
Tumor initiation



Pro-Inflammatory molecules (TNF-α, IL-6, IL-1β)
↓
Transcription factors (NFκB, STAT3, AP-1)
↓
Stemness → ↑ CSCs

Survival, proliferation, angiogenesis, invasion → **Tumor promotion**

Controlled pro-inflammatory molecule release and inflammation required to stimulate anti-tumor immunity while limiting tumor promotion

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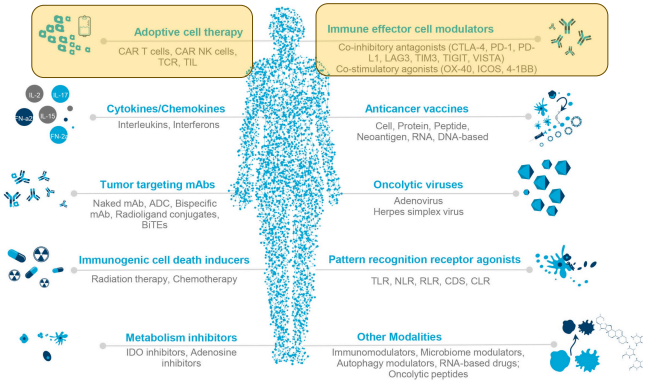
Cancer immunotherapies

Current drug development pipeline : over 4700 therapeutic agents



Franklin et al, JITC, 2022

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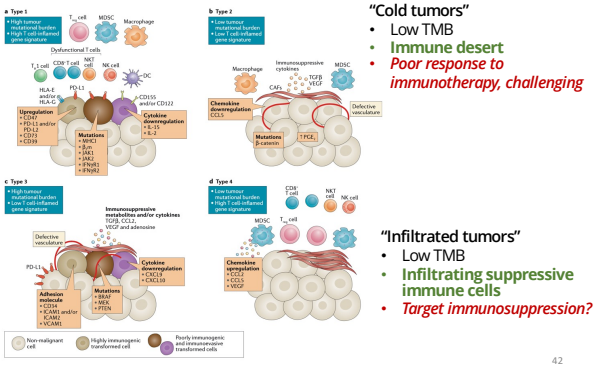
Cancer immunotherapies

Classification of the immune component of the tumor microenvironment

"Hot tumors"
- High TMB
- Immune cells
- **Suppressed but present immunity**
- *Responsive to immune checkpoint inhibitors*



"Cold tumors"
- Low TMB
- **Immune desert**
- *Poor response to immunotherapy, challenging*

"Mutated tumors"
- Moderate to high TMB
- **Excluded immune cells**
- *Strategies to recruit immune cells and allow their entry?*

"Infiltrated tumors"
- Low TMB
- **Infiltrating suppressive immune cells**
- *Target immunosuppression?*

O'Donnell, Teng & Smyth, Nat Rev Clin Oncol, 2019

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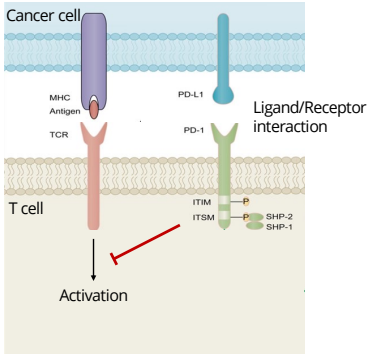
Cancer immunotherapies

T cell inhibition : the PD-L1/PD-1 axis, an immune checkpoint



- Programmed cell death ligand 1 (PD-L1) expression by cancer cells
- Programmed cell death 1 (PD1) receptor expression by T cells
- Ligand/Receptor interaction
- Inhibition of T cell activation
- **Suppression of antitumor T cell activity**

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Cancer immunotherapies

Nobel Prize in Physiology and Medicine 2018

James P. Allison

Tasuku Honjo

"for their discovery of cancer therapy by inhibition of negative immune regulation."

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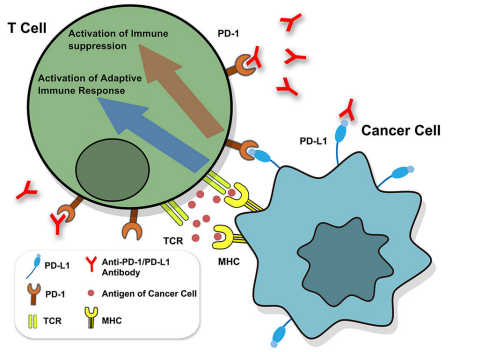
Cancer immunotherapies

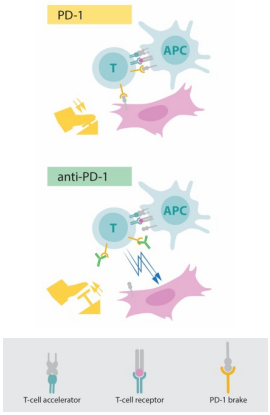
Immune checkpoint inhibitors: anti-PD-1 and anti-PD-L1 antibodies



Antibodies targeting PD-1 and PD-L1 reverse T cell inhibition

Zhang et al, Front Pharmacol, 2020

Cancer immunotherapies



↑ PD-L1 expression in cancer cells and APCs

↑ PD-1 expression in T cells

PD-1/PD-L1 interactions = T cell dysfunctions

- ↓ T cell activation upon tumor antigen presentation by APCs
- ↓ T cell effector functions towards cancer cells

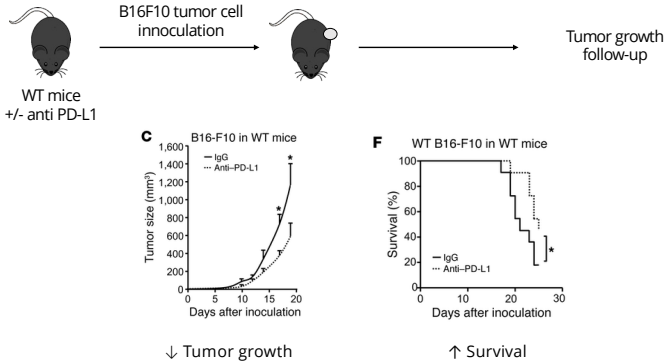
Antibodies against PD-1/PD-L1

Restoration of T cell effector functions

https://www.nobelprize.org/prizes/medicine/2018/press-release/

Cancer immunotherapies

Preclinical models : anti-PD-L1 for melanoma treatment



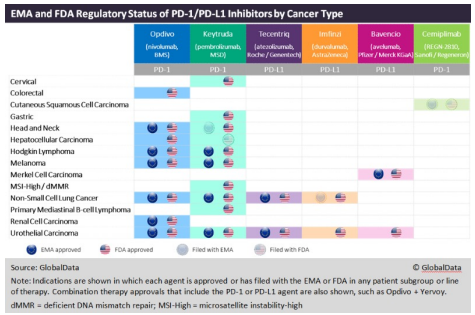
↓ Tumor growth

↑ Survival

Lin et al, JCI, 2018

Cancer immunotherapies

From clinical trials to cancer treatment



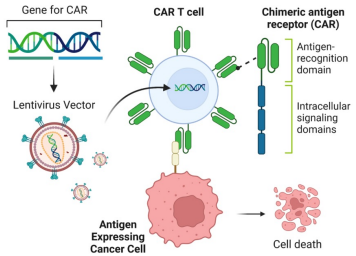
Following FDA/EMA approvals of anti-PD1 for melanoma treatment in 2014/2015, other indications (lung, bladder cancers…)

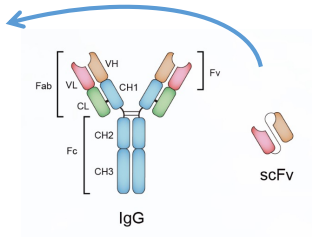
https://www.pharmaceutical-technology.com/comment/sanofi-regenerons-cemiplimab-will-sixth-marketed-pd-1-pd-l1-inhibitor/

Cancer immunotherapies

Adoptive Immunotherapy : targeting tumour antigens

CAR-T Cells
Chimeric
Antigen
Receptor





https://bpsbioscience.com/custom-car-t-cell-development

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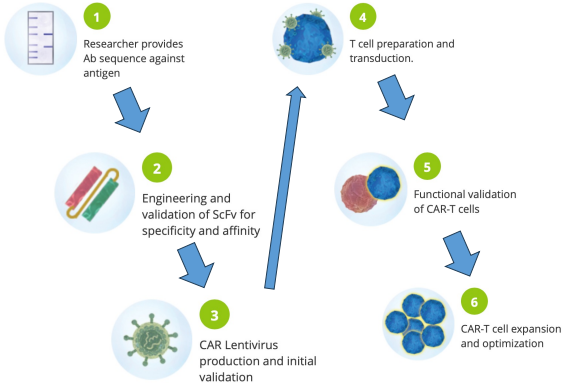
Cancer immunotherapies



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Cancer immunotherapies

Promising therapy

Approval for :

Approved in France (HAS)

FDA-Approved CAR T-Cell Therapies

Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

But...

Two major adverse effects:

- Cytokine-Release Syndrome
  = "On-target" effect
  High fever + drop in blood pressure → can be fatal if Macrophage Activation Syndrome controlled by glucocorticoids / anti-IL6
- Immune effector cell–associated neurotoxicity syndrome = ICANS

New strategies to modify the CAR

Very high cost > 100,000 € /y

https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

Rafiq et al., Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat. Rev Clin Oncol, 2020, 17:147-67.

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Cancer immunotherapies *CAR-T Cells evolution strategies*

Improve CAR-T Cell access to tumours / limit their local inhibition

Increase of tumour homing and penetration :
- *Expression of chemokine receptors*
- *Expression of heparanase*

Overcoming immunosuppression in the TME:
- *Secretion of anti-PD-1 /Down-regulation of PD-1*

Control of CAR-T Cell activity

ON/OFF switch by external drug to promote:
- *Inhibition of transduction with Dasatinib (TKI)*
- *CAR proteasomal degradation with specific antiprotease*

CAR-T Cell Suicide strategies

Inihibition of cytokines

Control of CAR-T Cell specificity

Combination of several CAR to target several antigens:
- *Against tumour cells*
- *Deactivation upon interaction with healthy cells*

Rafiq et al., Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat. Rev Clin Oncol, 2020, 17:147-67.

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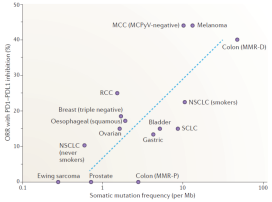
Cancer immunotherapies, future perspectives

- Biomarkers of efficiency
  - PD-1/PD-L1 expression in tumor cells and circulating tumor-derived vesicles
  - TMB implementation in clinics
  - …

Combination therapies to sensitize tumors to checkpoint inhibitors:

- Chemotherapies + checkpoint inhibitors
- Checkpoint inhibitor combinations (anti-PD-1/PD-L1 + anti-CTLA-4)
- Pattern Recognition Receptor agonists + checkpoint inhibitors



Yarchoan et al, Nat Rev Cancer, 2017