Tumour immunology I

Problem with tumour	Evidence of immune system function
- Tumours result from uncontrolled growth of self-tissue - The immune system is programmed to be self-tolerant	- Lymphoid infiltrates found in tumours + tumours more frequent in immunosuppressed individuals - In vitro T-cells respond to tumour cells

Mechanisms of immune recognition & prevention of cancer

Control of tumorigenic infections

- *Epstein-Barr virus*: **widespread** in human, **latent** infection for life & **normally controlled** by immune system
  - Infection of B-lymphocytes present antigen to CTL >> kill infected B-lymphocytes
  - APCs present antigens to Th >> production of Abs to eliminate extracellular antigens
- *EPV* can cause **lymphoma**, often associated immune suppression
  - NO effective T-cell responses >> infected B-cells are not killed >> **oncogenic gene** translation creates B-cell tumour

Types of virally induced cancers in immunosuppressed individuals

- **Transplantation** & **autoimmune** patients usually take drugs to suppress their immune system >> immunosuppresseds
- **AIDs/transplantation** can develop multiple types of tumours caused by multiple viruses
- **Kidney transplant patient**: 50 – 100 relative risk of developing Kaposi's sarcoma, whereas only 1 – 2 relative risk of developing lung cancer c.f. healthy individuals

cause of immunodeficiency	common tumor types	viruses involved
inherited immunodeficiency	lymphoma	EBV
immunosuppression for organ transplants or due to AIDS	lymphoma cervical cancer skin cancer liver cancer Kaposi's sarcoma	EBV papilloma viruses probably papilloma viruses hepatitis B and C viruses human herpes virus 8
malaria	Burkitt's lymphoma	EBV
autoimmunity	lymphoma	EBV

Relative risk for kidney transplant patient

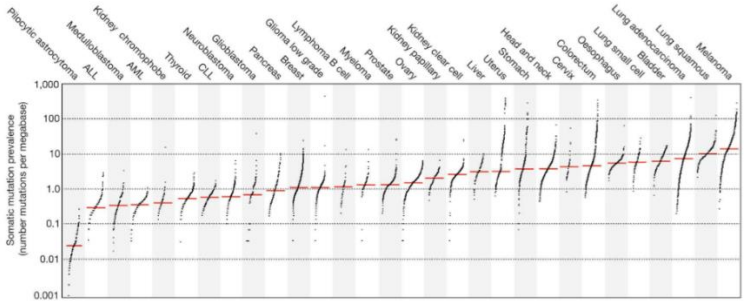
Tumour type	Relative risk
Kaposi' s sarcoma	50-100
Non-Hodgkin lymphoma	25-45
Liver carcinoma	20-35
Skin carcinoma	20-50
Cervical carcinoma	2.5-10
Melanoma	2.5-10
Lung cancer	1-2

Evidence for existence of tumour antigen:

a. Immunise mice with **irradiated** tumour cells (type A) that cannot divide to causing cancer BUT still introduce tumour antigens to the mice
b. Inject viable tumour cells into the mice with type A or type B >> mice is **resistant** to type A tumour but NOT type B

*Types of tumour antigens – **foreign** or **mutated***

1. **Products of mutated genes**: cancers typically carry **many mutations** (1000+) that are often associated with **oncogenes** controlling **cell growth** – Ras, Bcr/Abl, p53 & Myc >> mutated p53 is expressed by 50% of human tumours >> '**altered self**' proteins hence **immunogenic**



# of mutations per **megabase** of DNA:

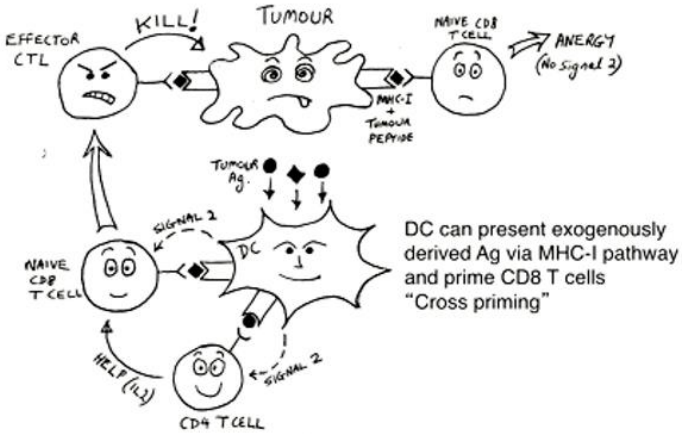
- **Leukemia**: low number of mutations
- **Melanoma**: high number of mutations

2. **Products of oncogene virus**: **oncogenic virus-derived proteins** >> e.g. **E6** & **E7** **proteins** from *HPV16* common to HPV-induced tumours *AND* **EBNA-1** **protein** from *EBV* common to EBV-induced **lymphomas** >> '**foreign**' proteins hence **VERY immunogenic**
3. **Altered glycolipid/glycoprotein antigens**: **abnormal forms/levels** >> e.g. **MUC-1** **glycoprotein** is **altered form** expressed by breast carcinoma *AND* **GM2** & **GM3** **glycolipids** are **overexpressed** in melanoma >> **immunogenic** to immune cells that are able to recognise **glycolipid/glycoprotein – NKT** cells

*Types of tumour antigens – **unmutated***

4. **Aberrantly expressed antigens**: **cancers overexpress antigens that are normally silent in adult tissue** >> e.g. **MAGE**, **MART** & **NY-ESO1** **antigens** that are expressed during development but not in **adult** melanocyte tissue, however re-expressed in **adult** tissue during melanoma >> these **antigens** often expressed in **testes** – collectively named C/T (cancer/testes) **antigens**
5. **Tissue specific differentiation antigens**: **cancers overexpress molecules that are normally present on cells of origin** >> e.g. **CD20** & **CD30** are expressed on **mature** & **activated** B-cells respectively BUT also on B-cell cancers >> **treatment**: rituximab (anti-CD20) is used for B-cell **leukemia/lymphoma** in combination with chemotherapy – double survival rate

Adaptive immunity to tumours:



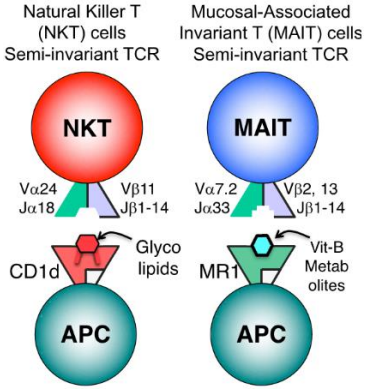
T-cells:

a. **CTL** is the **main player** in anti-tumour response & directly recognise tumour antigens presented on MHC-I > without signal 2, **CTL** becomes **anergic**
b. Tumour cells **shed** antigens that are picked up by **DCs** >> 1. **Cross-presenting** to **CTL** via MHC-1 + providing signal 2 2. Provide to **Th** via MHC-2 >> releasing IL2 & IFN-gamma cytokines + can also release **TNF & LT tumoricidal cytokines** >> help **CTL**
c. **Effector CTL** lyse tumour cells

B-cells: 1. **Activation of NK cells**: Abs bind to tumour antigen >> Fc region of Ab recognised by FcR – **CD16** on NK cells >> **ADCC** (antibody dependent cellular cytotoxicity) 2. **Activation of complement cascade** 3. **Ab blockage of key receptors**

NK cells:

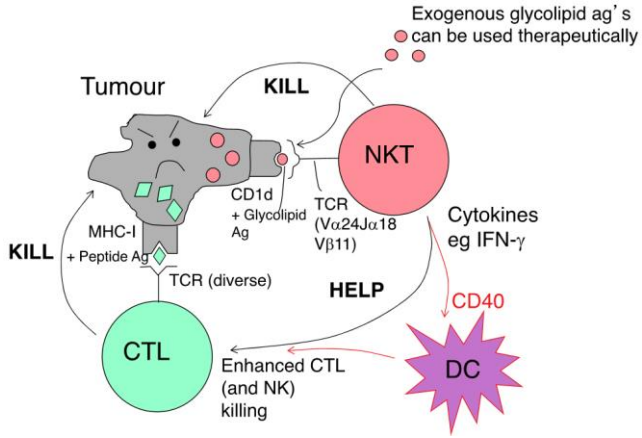
- **Stimulatory** receptors: FcR (CD16), CD2 & NKG2**D**
  - NKG2**D** can recognise stressed cells that express stress ligands – MIC-A, RAE-1 & ULBP >> activate NK cells in the presence of MHC-1
- **Inhibitory** receptors: MHC-1 binding receptors – KIR (killer cell Ig-like receptors), NKG2**A** (human) & Ly49 (mice)



Unconventional T-cells:

- **NKT** cells: **semi-invariant** TCR with **fixed** Vα24 & Jα18 α-chain >> recognise **glycolipids** presented via MHC-class 1 like molecule **CD1d**
- **MAIT** cells: **semi-invariant** TCR with **fixed** Vα7.2 & Jα33 α-chain >> recognise **VitB metabolites** presented via MHC-class 1 like molecule **MR1** >> up to 40% MAIT cells in some tissue

NOTE: **CD1d** & **MR1** are non-polymorphic Ag-presenting molecules >> everyone has **same CD1d** & **MR1** >> recognised by all people



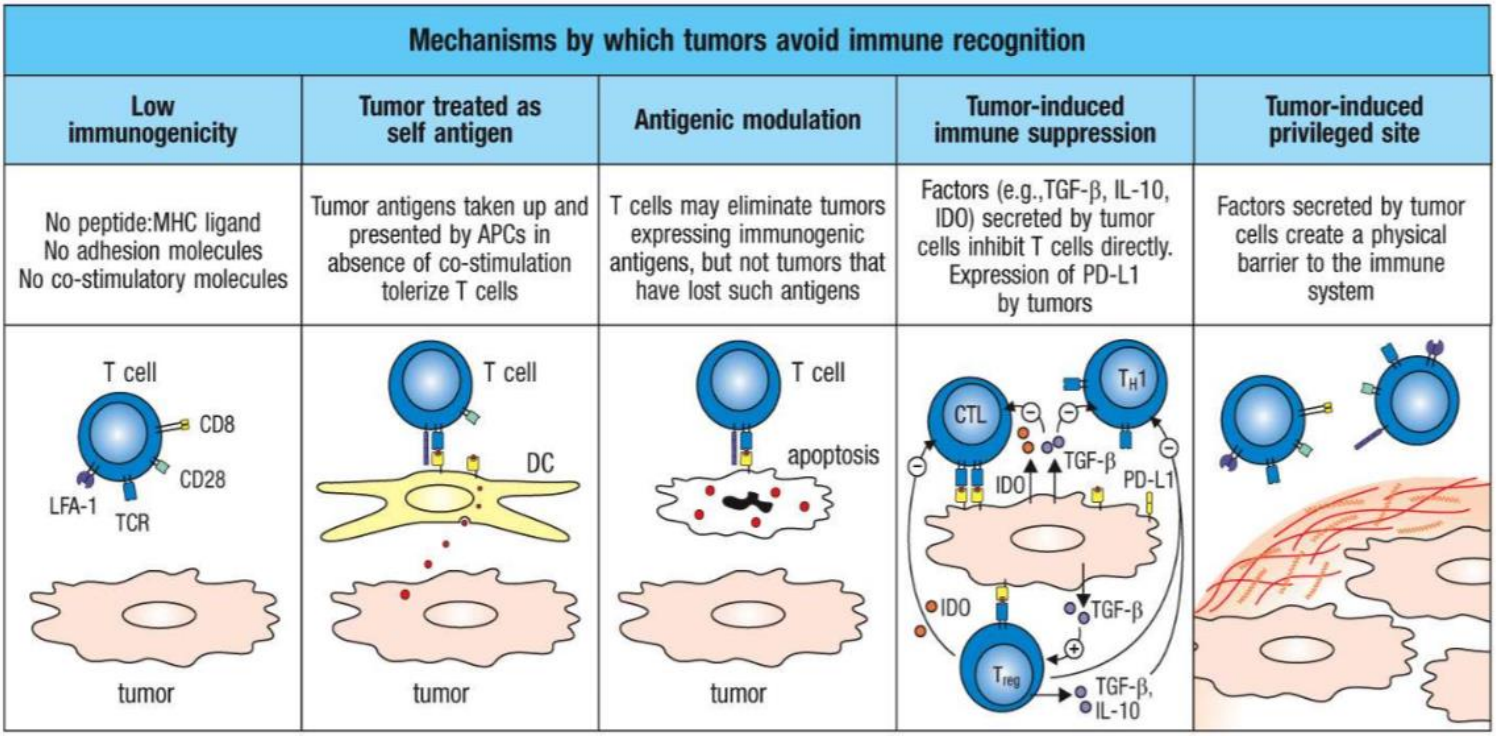
NKT cells:

- Express **$\alpha\beta$ TCR** & **NK1.1** molecules >> recognise **glycolipids** presented via **CD1d** >> **exogenous** **glycolipids** added to **enhance** patient's responses of NKT cells
- Express **CD40** – enhance DC maturation
- Potent **cytokine** production – IFNγ, TNF, IL-4 & IL-17 >> provide helps for **NK** & **CTL** in vivo
- Kill tumours in a **perforin-dependent** manner in vitro

NOTE: in vivo, NTK cells have been shown to enhance **tumour protection**

Diagnosis tool: NKT contributes to the **surveillance** & represents one of the most important **prognosis factors** to determine if the patient is going to progress or remain stable with **chronic lymphocytic leukemia**

Mechanisms of tumour escape



Antigenic modulation:

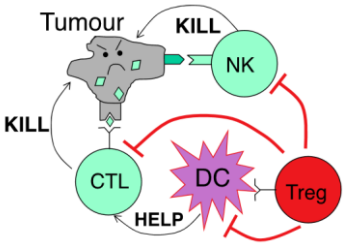
- Tumours tend to **genetically unstable**: **lose antigens by mutation** OR **generate escape mutants that evade recognition** >> may kill 99.9% cancer cells BUT one loses its antigen hence **survived from immunotherapy** *AND* **reproliferate**
- Tumours lose **MHC-I allele**: very **common** with **colon cancer** – more than 50% colon cancer tumours lose one or more MHC-I allele >> if lose **all** MHC-1 molecule, may become NK targets >> if lose only **some** MHC-1, may evade CTL & NK

Tumour-induced immunosuppression:

- Tumours produce **immunosuppressive factors**:

1. **TGF-beta**: **first identified in the culture supernatant of a tumour** *AND* **suppresses cell-mediated immunity**
2. **IL-10**: **produced by a range of tumours** including melanoma &B lymphoma *AND* **reduces DC activity** & **suppresses antigen presentation**
3. **PD-L1**: **produced by a range of tumours** including melanoma, renal & ovarian *AND* binds to **PD1** on T/B/NK cells to **inhibit their action** & associated with **poor outcome**

- Recruit **T-reg cells**:



- **CD25+**, **CD4+** & **FoxP3+** >> function via the production of **TGF-beta** & **IL-10** that suppress immune response for **DC**, **CTL** & **NK** cells
- **Treg** often found at **high frequency** in tumour infiltrates >> elimination of Treg can promote **spontaneous** tumour rejection

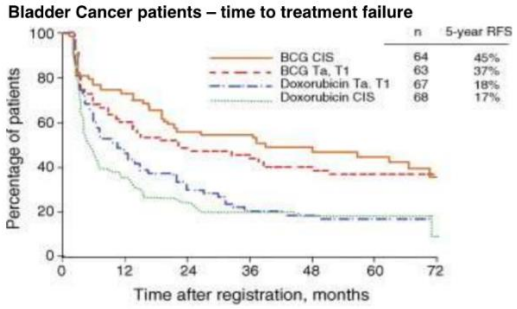
Three Es of tumorigenesis

- **Elimination**: when a tumour arises in a tissue, a **number of immune cells** can recognise & **eliminate** them
- **Equilibrium**: variant tumour arises that are **more resistant** to being killed *AND* over time, a **variety of different variants** develop >> people may stay in equilibrium stage **without realising** it & can be transferred from organ **donor** to **recipient**
- **Escape**: one variant may **escape the killing mechanism** or **recruit Tregs to protect it** >> spread unchallenged

Tumour immunology II

Immunotherapy

Origin of immunotherapy:



- William Coley noticed **cancer remission** after **bacterial** **infection** >> inject **bacterial extracts** into tumours >> **sporadically** induced tumour **rejection** >> caused an **adjuvant** like effect that initiated/enhanced **immune response**
- A similar form **BCG** (Bacillus Chalmette Guerin) is used to treat bladder cancer >> enhance immunity via **TLR** >> patients have a **better survival rate**

Tumour immunotherapy:

Preventative immunotherapy – anti-viral vaccines

- **Many tumours are virally induced** >> vaccine prevents infection of those viruses >> e.g. **Gardasil** – HPV vaccine

Antibody based immunotherapy

- **Monoclonal Ab** generated against tumour antigens to be injected to **kill** tumours
- Either **alone** or **coupled to reagents** that kill target: 1. mAb **alone** – block important growth receptors OR activate FcR of NK cells 2. mAb-**toxin** conjugate – **Ricin** gets **internalised** by the tumour & kills it 3. mAb-**radioisotope** conjugate – deliver local radiation near the tumour >> **advantage**: kill the **escape mutant neighbouring tumour**
- **Challenges**: 1. **Specificity** of Ab >> not bind to non-tumour cells 2. Penetration of **large tumour** – mAb therapy is most effective for liquid tumours 3. **Instability of tumours** – constantly modulate its antigens 4. Limited knowledge of **dominant** tumour antigens
- **Examples**:
  - **Rituximab** (anti-CD20): kill **CD20** expressed by non-hodgkin's B lymphoma & B leukemias >> twice survival over 8 yrs c.f. control group
  - **Trastuzumab**/*Herceptin* (anti-HER2): kill human **epidermal growth factor receptor 2** expressed by **25%** breast cancer associated with **poorer** **prognosis**, as those breast cancer replies on HER2 to receive growth signal allowing them to grow >> cell cycle arrest: blocks EGF driven tumour growth
  - **Brentuximab**/*Adcetris* (anti-CD30): kill CD30 expressed by **many** lymphoma cells >> Antibody-drug conjugate – component **Vedotin** targets **microtubule formation** killing dividing cells >> **94%** of 102 patients with hodgkin's B lymphoma had tumours shrink *AND* **73%** objective response – partial/complete remission

Cytokine based immunotherapy

- **Many** cytokines have been used for tumour therapy

IL12	Melanoma	**Increase** **NK** & **CTL** activities
IL2	Melanoma, colon & renal cancer	**Increase** **NK** & **T** activities
IFN-alpha	Melanoma, lymphoma & renal cancer	**Increase** **NK** activities & **MHC-I**
TNF	Melanoma, malignant ascites & sarcoma	**Increase** **macrophage** & **lymphocytes** activities

- **Problems**: 1. **Toxic** when used systematically 2. **Limited success rate** – IL2 gives 10% success 3. **Short half-life**

Immunisation with tumour cells/antigens

- **Approach**: immunise with 1. Killed tumour cells 2. Purified tumour Ag + adjuvants like **BCG** 3. Tumour Ag loaded on patient's DCs
- **Results**: 1. **Prophylactic** – works in animal model **prior to tumour exposure** 2. **Therapeutic** – less effective with **established tumours** 3. Ongoing in many clinical trials with variable success 4. If delivery or better adjuvants known, it may enhance its effects
- **Problems**: 1. Dominant tumour Ag may be **underrepresented** in whole tumour population 2. Dominant tumour Ag may be **patient specific** – MHC-restricted Ag presentation e.g. **MAGE1** only in **HLA-A1** patients 3. **Antigen modulation** – outgrowth of escape mutants 4. Risk of **autoimmunity** due to non-tumour Ag in preparation – immunised with certain self cells that may cause autoimmunity e.g. **vitiligo**, destruction of normal melanocytes

Immunisation with immunogenically enhanced, viable tumour cells

- **Alternative approach:** isolate tumour cells & transfect with **immunogenic** molecules
- **Examples**:
  - **B7** (CD80) transfected-tumour cells – able to activate tumour specific naïve T-cells to generate **effector CTL** that 1. Firstly, reject B+ **transfected** tumour cells 2. Then, reject the **original** B- tumour cells
  - **Cytokine GMCSF** transfected tumour cells – **GMCSF** is a powerful cytokine of recruiting DCs to present tumour Ag to immune system >> more effective than B7 transfected tumour cells
  - **Other cytokines** transfected tumour cells tested – enhance cell mediated immunity BUT limited success

Adoptive cell therapy

- **Method**: 1. Isolate **tumour infiltrating lymphocytes** (TIL) from tumour 2. Expand in culture with IL-2 & TCR stimulation 3. **Select tumour specific CTL** on IFN-gamma ELISA after reacting CTL with tumour antigen (more IFN-gamma produce if CTL is specific) *AND* **enrich tumour specific CTL** 4. Precondition – chemotherapy to reduce the endogenous immune cells allowing space for the transferred T-cells to expand 5. Transfer back to the patients
- **Results**: **dramatic improvement** – 50% partial/complete response with metastatic melanoma in the absence of radiations

Adoptive CAR T-cell therapy

- **Method**: 1. T-cells infected with retrovirus containing CAR (chimeric antigen receptor) genes to express **modified tumour-Ag specific Ab receptor** that is fused to **intracytoplasmic T-cell signalling domain** (**CD3zeta**) & **costimulatory domains** (**CD28**) 2. Expand in vitro >> transfer back to the patients
- **Results**:
  - **Serial killer**, capable of **potent tumour destruction** with possible lethal side effects (2 patients died due to toxicity) >> effective in both **established** & **metastatic** tumour
  - **Clinical trials**: 1. most effective on CD19 antigen of B cell with acute lymphocytic leukemia – **90%** remission with **chemotherapy-resistant** patients >> less effective with solid cancers

Checkpoint blockade inhibitors

- **Method**:

1. **Anti-CTLA-4**: **Ipilumumab**/*Yervoy*, CTLA-4 upregulated after T-cell **activation** & binds **B7.1/7.2** more avidly than CD28 to deliver inhibitory signals to activated T-cells >> Anti-CTLA-4 is the **frontline therapy** for **metastatic** melanoma
2. **Anti-PD1**: **Nivolumab**/**Pembrolizumab**, PD-1 (55kDa member of Ig superfamily) upregulated after T-cell **activation** & binds **PD-L1/2** of tumour cells to receive inhibitory signals >> **approved** treatment for melanoma

- **Results**:
  - Much better c.f. **conventional chemotherapy**
  - After 4 doses of Anti-CTLA-4 within the first year, it gives **20%** long-term survival in melanoma
  - Combination of Anti-PD1 & Anti-CTLA-4 is more effective than Anti-CTLA-4 alone for melanoma
- **Side effects**:
  - Combination of Anti-PD1 & Anti-CTLA-4 gives **91%** adverse effects with **54%** experiencing severe adverse effects

Tumour immunology III

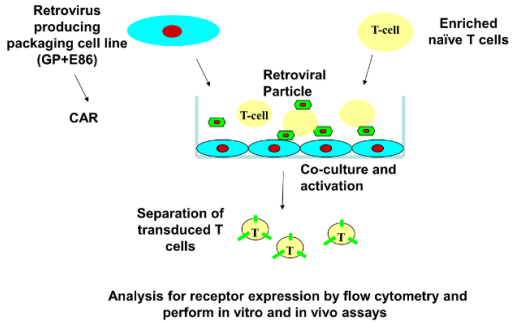
History of adoptive immunotherapy

- Demonstrated that the transfer of **LN cells** or **solenocytes** could mediate some responses against sarcoma >> however, those responses are ONLY achieved against **early** & **small tumour** *AND* those cells are **non-specific**
- Steven Rosenberg observed **tumour regression** with **infection** near tumour >> **intratumoral** injection of **BCG** is effective
- In 1980's, the discovery of IL-2 allowed adoptive immunotherapy investigation using cultured lymphocytes:
  - **Lymphokine activated killer** (LAK) cells & IL-2 could inhibit **3-day** lung metastases of methylcholanthrene (MCA) induced tumours >> LAK cells are taken from blood – **not specific**
  - **Tumour infiltrating lymphocyte** (TIL) & IL-2 could eradicate **established** lung/liver metastases of MC38 colon adenocarcinoma
- 1985 – 2004: adoptive transfer of cultured lymphocyte + IL2 gives **30% response** & **7% complete responses** on melanoma
- 2004 – 2006: **response** rate increased to **50%** by prior **non-myeloablative conditioning** on melanoma
- 2006 – 2017: **response** rate increased to **70%** with **deeper precondition** – **chemotherapy** + **whole body radiation** on melanoma

Adoptive immunotherapy using gene-engineered cells

- Normally, cannot **isolate enough** endogenous tumour-specific lymphocytes >> genetical modification to generate large number of CAR-T-cells
  - First generation **CAR**: **Ab receptor** + **T-cell signalling domain** >> not effective
  - Second generation **CAR**: **Ab receptor** + **T-cell signalling domain** + **costimulatory domains**
  - Second generation **CAR**: **Ab receptor** + **T-cell signalling domain** + **2 costimulatory domains** >> little activity

Retroviruses as gene vehicles:



1. Co-culture **retrovirus-producing packaging cell line** & **naïve T-cells** >> activate T-cells with IL-2 & TCR stimulation
2. Separation of transduced T-cells that expresses **CAR**
3. Analysis for receptor expression by
   a. *Flow cytometry*
   b. *In vitro & vivo assays*
   c. *Live cell microscopy*

- T-cell flashes **green** upon **calcium influx** caused by **recognition**
- Tumour cell flashes **red** upon **apoptosis** when **perforin pores form**

CAR T-cell trial on Acute Myeloid Leukaemia:

- 5-year survival < 10%, new cases USA is 12,000 per year & located in **marrow** & **blood**
- **CAR** T-cells: humanised scFv Ab with affinity 4.19x10^6 M
- **Targeting** the **Lewis-Y** tumour-associated antigen: **Lewis-Y** is a **fucosylated carbohydrate** on a variety of **proteins** & **lipids** >> expression associated with **poor prognosis** >> overexpression on approximately 60% carcinoma (good target for solid cancer) *AND* 10% of AML & myelomas >> expression to **varying degrees** on some cells in normal tissues
- **Clinical protocol**:
  - **Phase I** clinical trial on 5 AML patients, **selection based on** their **Lewis-Y expression** – received 5x10^8 – 1.3x10^9 **T-cells**, including **10%** cells labelled with **indium-111**, with preconditioning by chemotherapy drug **fludarabine**
  - **Primary** objective – safety *AND* **Secondary** objective – cell persistence + trafficking, serum cytokines & anti-cancer activity
- **Results**:
  - Well **tolerised** – either **NO** or **minor** adverse effects
  - **Biological responses** observed in AML patients – due to the **persistence of T-cells** reflected from **qPCR measurements**
  - **Traffic** to diseases sites – reflected from **indium-111** & **qPCR** demonstrating that CAR T-cells travel to the site of metastatic tumour

Checkpoint blockade therapy

- Effective in **VERY immunogenic** cancers – **high level** of T-cells >> melanoma (objective response – **28%**), non-small cell lung cancer (NSCLC) (OR – **18%**) & renal cancer (OR – **27%**)
- Not effective in **prostate** & **colon** cancers – **low level** of T-cells

Combination therapy

Anti-CTLA-4 & anti-PD-1:

- **Anti-CTLA-4** & **anti-PD-1** work in different ways >> Enhanced immune activity c.f. either therapy alone >> **immune related toxicities** increased
  - **Anti-CTLA-4** increases the **priming** of T-cells in the **lymph nodes**
  - **Anti-PD-1** increases the **activation** of T-cells at the **tumour sites** >> high level of T-cells required to be present at the tumour site for effective responses

CAR T-cells & anti-PD-1:

- CAR T-cells & anti-PD-1 **enhance** regression of tumour c.f. either therapy alone
- For patients who are resistant to **CAR T-cells**, injection of **anti-PD-1** can **reactivate** those T-cells again
- Except **inhibitory Rs**, T-cells also have stimulatory Rs like 4-1BB or OX40 >> can be targeted by CAR T-cells