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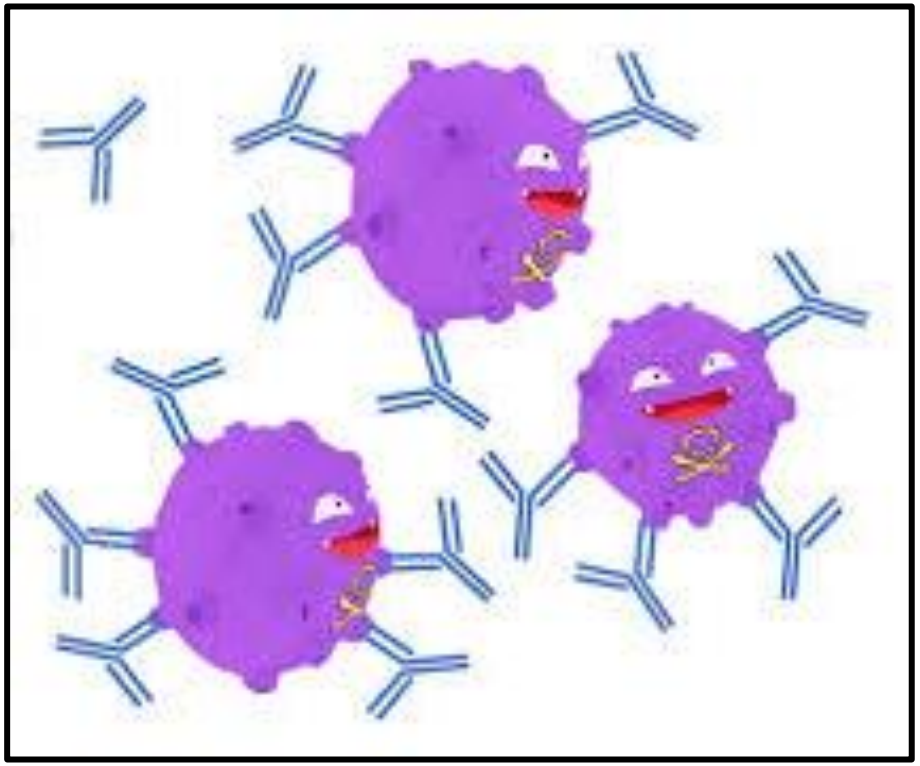
Immunopharmacology



Lecture Content

1. **Therapeutic monoclonal antibodies**
2. **Anti-rejection drugs**
3. **Cancer Immunotherapy**

Dr J Haylor, Medicine
2025

This lecture on immunopharmacology has been divided into 3 bitesize recordings dealing with therapeutic monoclonal antibodies, anti-rejection drugs in organ transplantation and cancer immunotherapy where drugs have been developed to inhibit some of the drivers of lymphocyte proliferation, in contrast to the blanket approach of cytotoxic chemotherapy.

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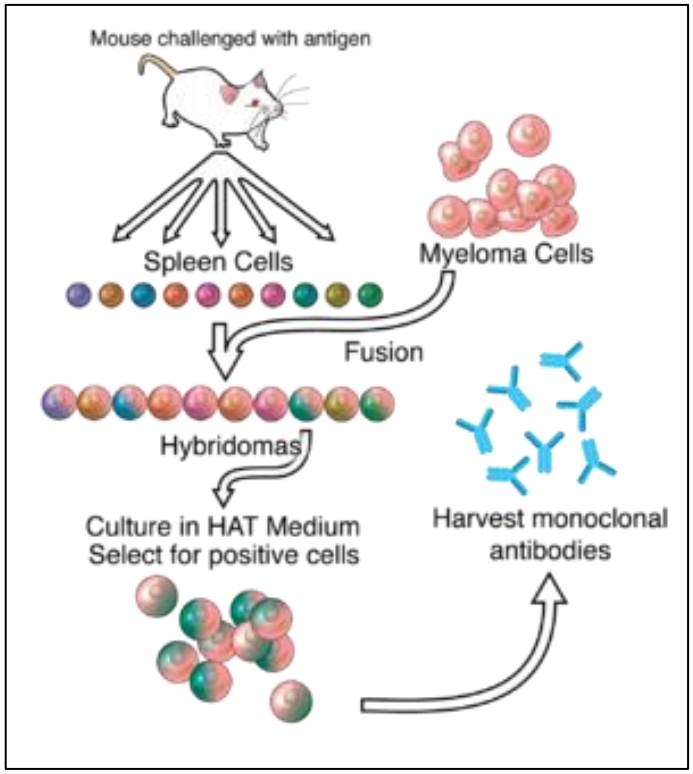
Immunopharmacology

Monoclonal Antibodies



Kohler, Milstein and Kerne received the Nobel prize for the development of technologies to produce monoclonal antibodies (MABs) by fusing myeloma cells with spleen cells derived from immunised mice to produce hybridomas, as shown in the left-hand diagram. Today, MABs are produced by the pharmaceutical industry on an industrial scale for use as therapeutic agents, opening up a whole new series of targets for drugs to influence disease processes particularly in the fields of immunology and cancer.

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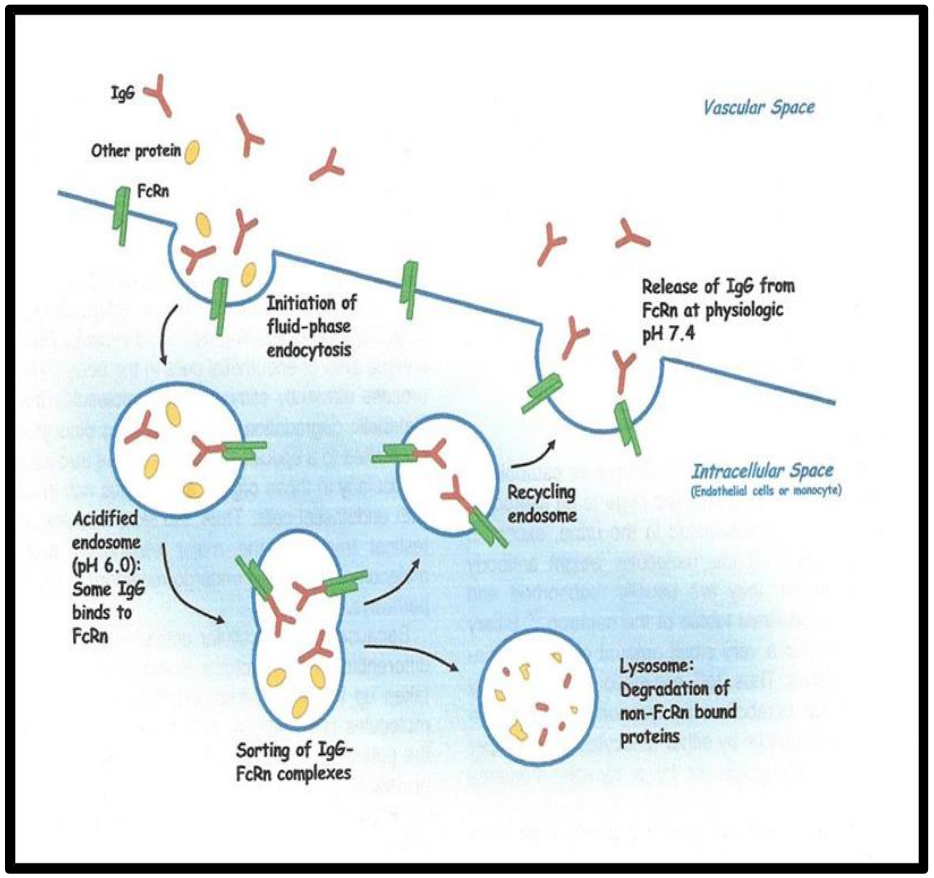
Monoclonal Antibodies: **Pharmacokinetics**



FcRn receptor

pinocytosis prevents lysosymal degradation

Half-life

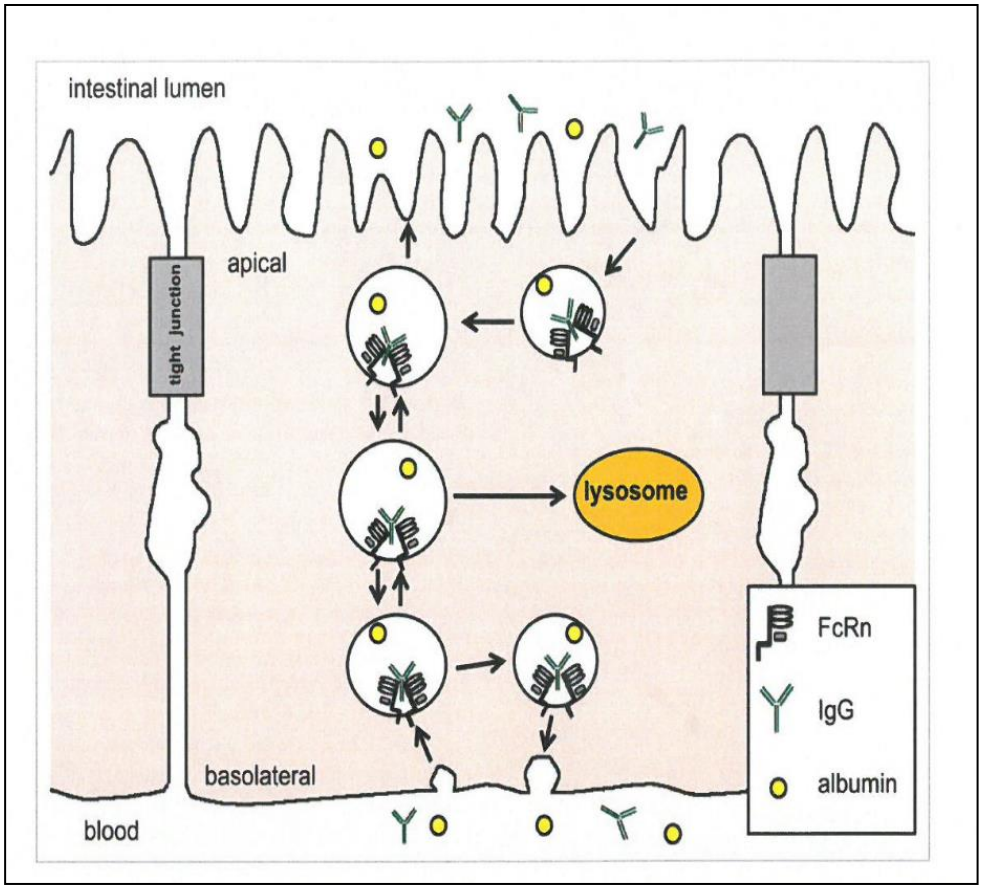
21 days (1-28 days)

Administration Frequency

2-8 weeks

In addition to their selectivity, one additional advantage of monoclonal antibodies over small MW drugs is their long duration of action. This is for two reasons. The first, being IgG immunoglobulins they have a high molecular weight of some 150kD, too high to be filtered by the kidney, double the molecular weight of the plasma protein albumin. Secondly MAB's in the circulation can be bound to endothelial cell membrane FcRN receptors during pinocytosis which confers protection against lysosymal degradation. MABs have a long half-life of average 21 days, giving a long duration of action requiring dosing at only weekly to monthly intervals.

Brambell Receptor



Colostrum
early breast milk
delivers antibodies orally

FcRn receptor

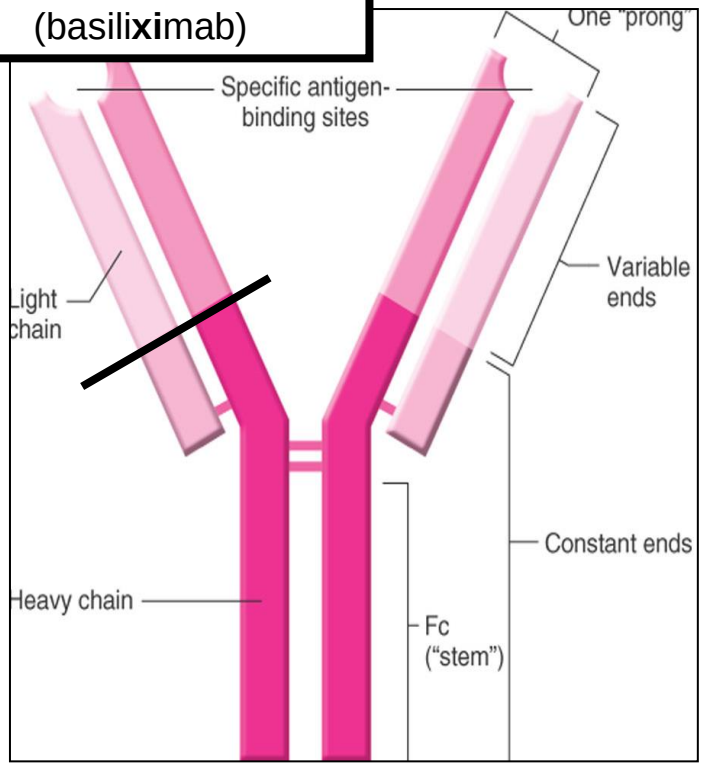
Neonatal Fragment Crystallizable Receptor

The neonatal Fragment Crystallizable Receptor (FcRn) was originally called the 'Brambell receptor' named after the Irish zoologist, Francis Brambell, who identified a luminal antibody receptor in the gut helping to explain how the antibodies present in colostrum, early breast milk, could be absorbed orally by the neonate. This receptor protects the antibody from lysosymal degradation during absorption by pinocytosis but, unfortunately, disappears in the first few weeks of life, so therapeutic antibodies have to be administered either by bolus subcutaneous injection or intravenous infusion.

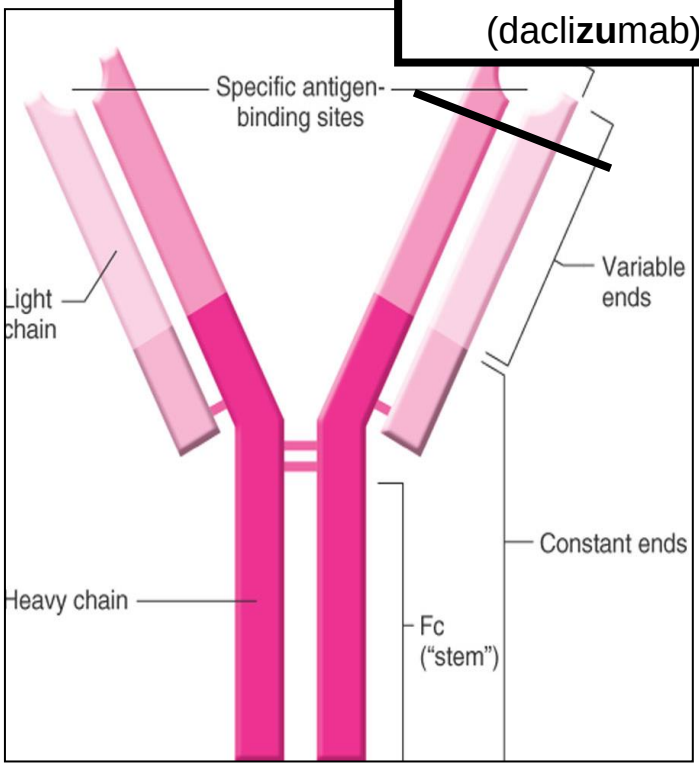
Monoclonal Antibodies **Approved Names**

IL-2 receptor

Mouse – **chimeric** (basili**xi**mab)



Mouse - **humanised** (daclizumab)



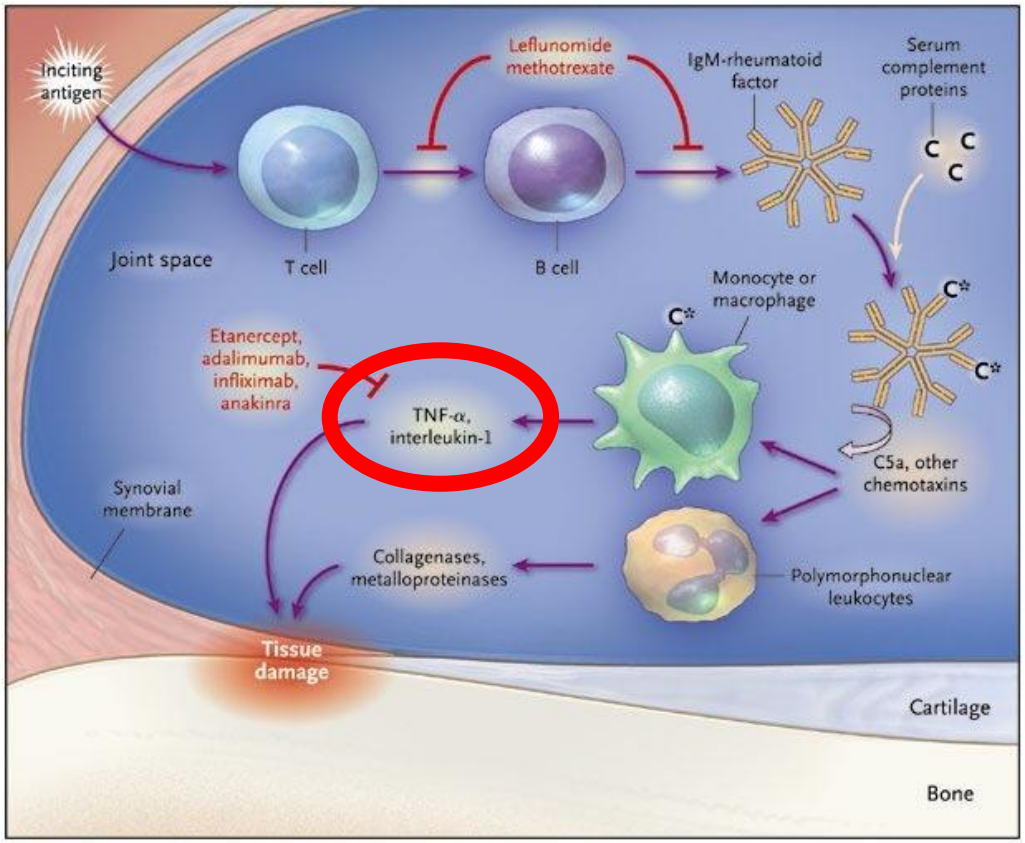
Murine (omab), Chimeric (ximab), Humanised (zumab), Human (umab)

The approved name of all monoclonal antibodies contain the suffix, mab. Two monoclonal antibodies, both IL-2 receptor antagonists, are used to help prevent rejection following organ transplantation. Basiliximab, on the left, is a chimeric antibody, while daclizumab, on the right is a humanised antibody. However, the names of monoclonal antibodies also contain other clues which help to define their origin. Drug names ending in omab, are fully mouse antibodies. Drug names ending in ximab, are chimeric mouse antibodies. Drugs names ending in zumab, are humanised antibodies just containing the variable end of mouse protein. Fully human antibodies end with letters umab and form many of the new therapeutic monoclonal antibodies introduced today.

Tumour Necrosis Factor α

Tumour Necrosis Factor alpha
(212 amino acid homotrimer)

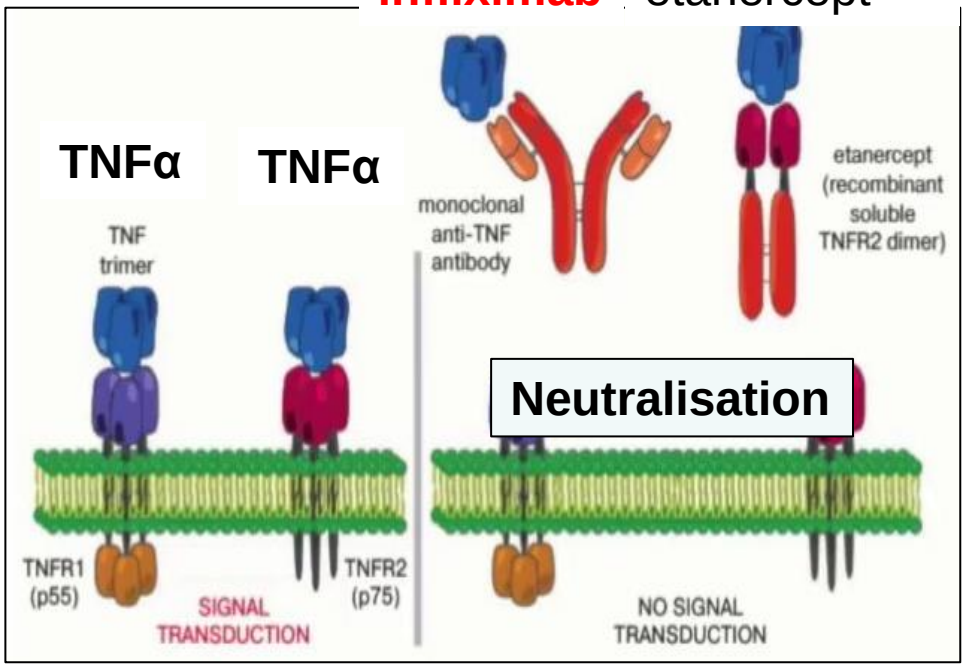
Cytotoxic factor released by activated macrophages stimulates acute phase proteins - mediates endotoxin poisoning and septic shock involved in chronic inflammation



The first cytokine chosen as drug target for treating autoimmune diseases using a monoclonal antibody was the pro-inflammatory, tumour necrosis factor alpha (TNFα) rather than IL-1. Structurally, TNF alpha is a homotrimer. TNF alpha induces tissue damage in chronic inflammation as a cytotoxic factor released from activated macrophages stimulating acute phase proteins and may induce septic shock.

Monoclonal Antibodies: Infliximab

infliximab etanercept



Crohn's disease (inflammatory bowel disorder)
rheumatoid arthritis, psoriasis

IV infusion every 6-8 weeks

Adverse Effects

- Precipitate latent disease (TB, septicaemia hepatitis B)
- Upper respiratory tract /urinary tract infection
- Abdominal pain, heart failure
- Antibody formation

Infliximab binds to the TNF alpha protein. Chemical neutralisation inhibits lymphocyte proliferation, although it is a chimeric antibody still containing some mouse protein. Initially introduced to treat Crohn's Disease, an inflammatory condition of the bowel, it has had a major impact on the symptoms of other autoimmune conditions such as rheumatoid arthritis and is given as an IV infusion every 4-6 weeks. However, an important adverse effect may result from inhibiting some of the beneficial properties of TNF alpha, such as preventing latent infection including that by TB.

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Monoclonal Antibodies **Biosimilars**

Biosimilar drugs and **generic** drugs are very different, mainly because while generic drugs are identical to the original in chemical composition, biosimilar drugs are “highly similar,” but close enough in duplication to accomplish the same therapeutic and clinical result.

Europe prescribe biosimilars by commercial name

(FDA Identified by 4 letter random suffix 3 letters of which must be distinct)

Europe

Infliximab : Remicade

Flixabi (Biogen)

Inflectra (Hospira)

Remsima (Napp)

USA

Infliximab : Remicade

Infliximab-axxq : Avsola (Amgen)

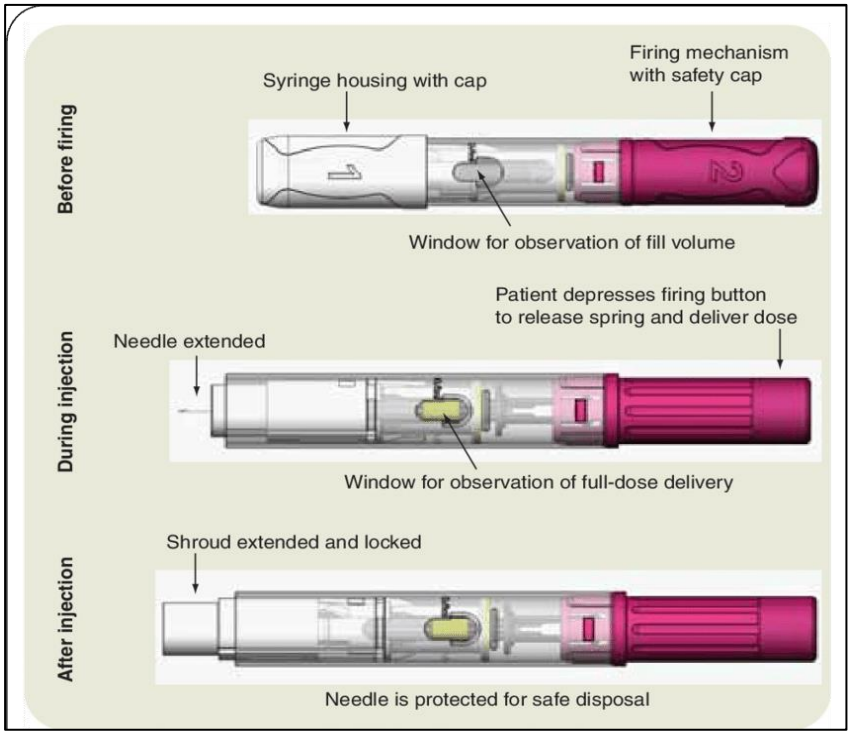
Infliximab-dyyb : Remsima (Celltrion)

Infllximab-abda : Renflexis (Samsung)

Infliximab-qbtx : Ixifi (Pfizer)

Most drugs are prescribed by their generic name. For low MW compounds, drugs with the same generic name contain the same active ingredient, independent of their manufacturer or supplier. Some monoclonal antibodies are now finishing their patent life and may be licensed to be made by a number of different manufacturers. The term biosimilar, not generic, is used to describe the product since there could be minor differences in structure due to different methods of manufacture. Biosimilar monoclonal antibodies importantly have the same clinical efficacy. In the US, the FDA requires the addition of a random 4 letter suffix of which 3 letters must be distinct to identify their commercial origin. However, in Europe, including the UK, a biosimilar monoclonal antibody is prescribed by its commercial name, which means there are a number of different names for essentially the same product. Infliximab has now come off patent and the terminology for biosimilar forms of infliximab is given for both Europe and the US.

Adalimumab



Adalimumab (2002)

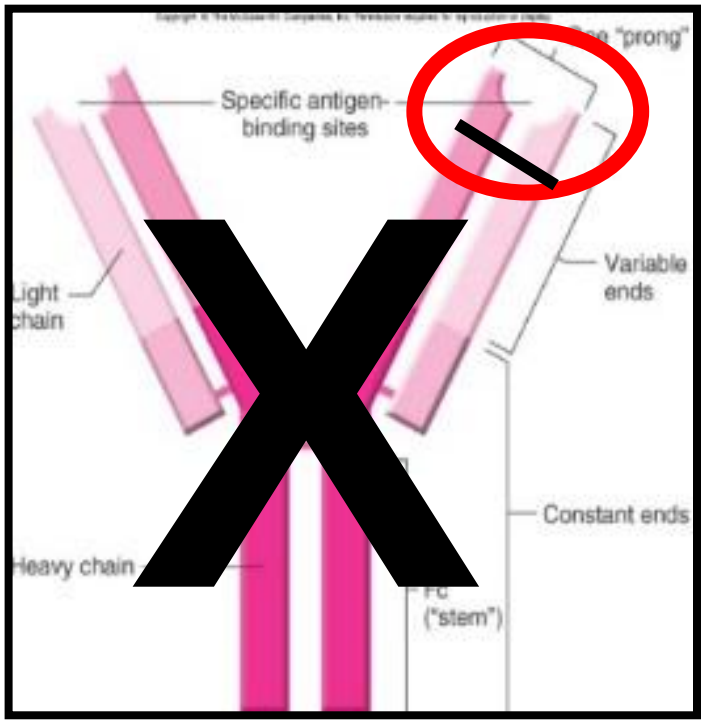
HUman **M**onoclonal antibody In **R**heumatoid **A**rthritis (HUMIRA)

- 1st fully human TNF alpha antibody
- inhibits lymphocyte proliferation
- downregulates inflammatory reactions associated with autoimmune disease

Subcutaneous injection
40mg every 2 weeks
(single dose autoinjector)
If no response increase to weekly

The first fully human monoclonal antibody to neutralise TNF alpha was adalimumab. A second major advantage of adalimumab is that it can be delivered once every 2 weeks by SC injection via an autoinjector, so can be used by a patient at home. This represented a major advantage over infliximab which was given by slow IV infusion over 2 hours under medical supervision. Adalimumab was the top drug in terms of sales in 2020 for Abbvie, grossing some $20 billion. A biosimilar form of infliximab called Remsima (Celltrion) is now available in single dose autoinjectors also to be administered subcutaneously every 2 weeks for inflammatory bowel disease.

Certolizumab Pegol



PEGylated Fab fragment of a humanised monoclonal antibody (binds to TNF alpha)

Polyethylene glycol required to increase MW and prolong half-life

A third approach to neutralising TNF alpha is with certolizumab. This drug isn't actually a MAB but contains just the small variable portion of the light chain part of the antibody (circled in red) which neutralises the cytokine. However, since it now has a low molecular weight, to be retained in the circulation the MW of certolizumab had to be increased combination with polyethylene glycol, a common method of enhancing the systemic duration of action of proteins.

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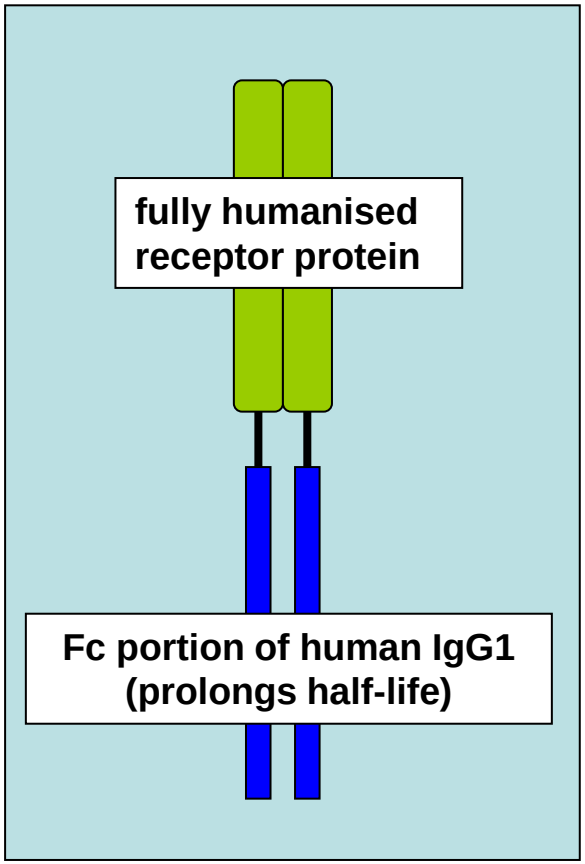
Fusion Proteins



Etanercept

TNF alpha type II receptor

neutralises TNF alpha

(rheumatoid arthritis)

Aflibercept

VEGF receptor type ½

Neutralises VEGF isoforms

(cancers, macula degeneration)

An alternative approach to monoclonal antibodies, was to develop fusion proteins which contain the heavy chain portion of the IgG immunoglobulin (shown in blue), helping to maintain a long duration of action. In addition, the light chain portion of IgG is replaced by a human receptor protein. Etanercept contains the TNF alpha type II receptor and neutralises TNF alpha while aflibercept contains the VEGF type 1 & type 2 receptor therefore neutralising VEGF, an angiogenic growth factor.

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Therapeutic MABs for Phase 1

Soluble Proteins

Neutralising

- TNFα - **infliximab, adalimumab**
- IL-5 - **mepolizumab** (year 1)
- IL-6 – **toclizumab**
- VEGF-A - **bevacizumab**

Receptor Antagonist

- HER2 - **trastuzumab**
- IL-2 - **basiliximab**

Surface Proteins

Lymphocyte Inhibitors

- CD20 – **rituximab**
- integrin – **vedolizumab**

Cancer Immunotherapy

- CD28 – **theralizumab**
- CTLA4 - **ipilimumab**
- PD1 – **nivolumab**

* CD19 – **tisagenlecleucel**

Viral Inhibitors

RSV - **palivizumab**
Covid-19 – **casirivimab + imdevimab**

Monoclonal antibodies are now commonly used therapeutics. This slide contains the major monoclonal antibodies discussed in pharmacology, primarily in year 2. This includes neutralising and receptor antagonists against soluble proteins such as cytokines and growth factors together with MABs against surface proteins on lymphocytes and cancer cells.

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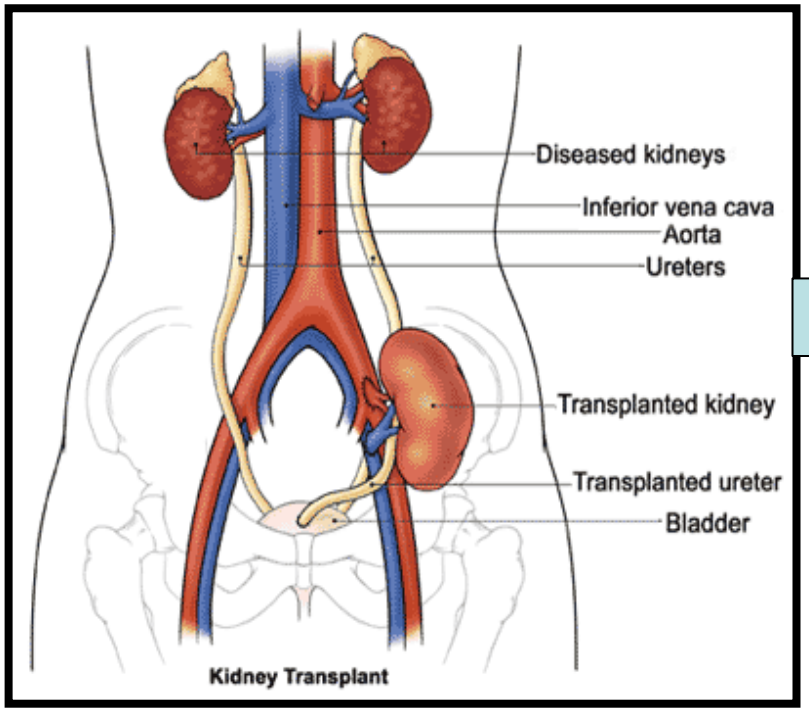
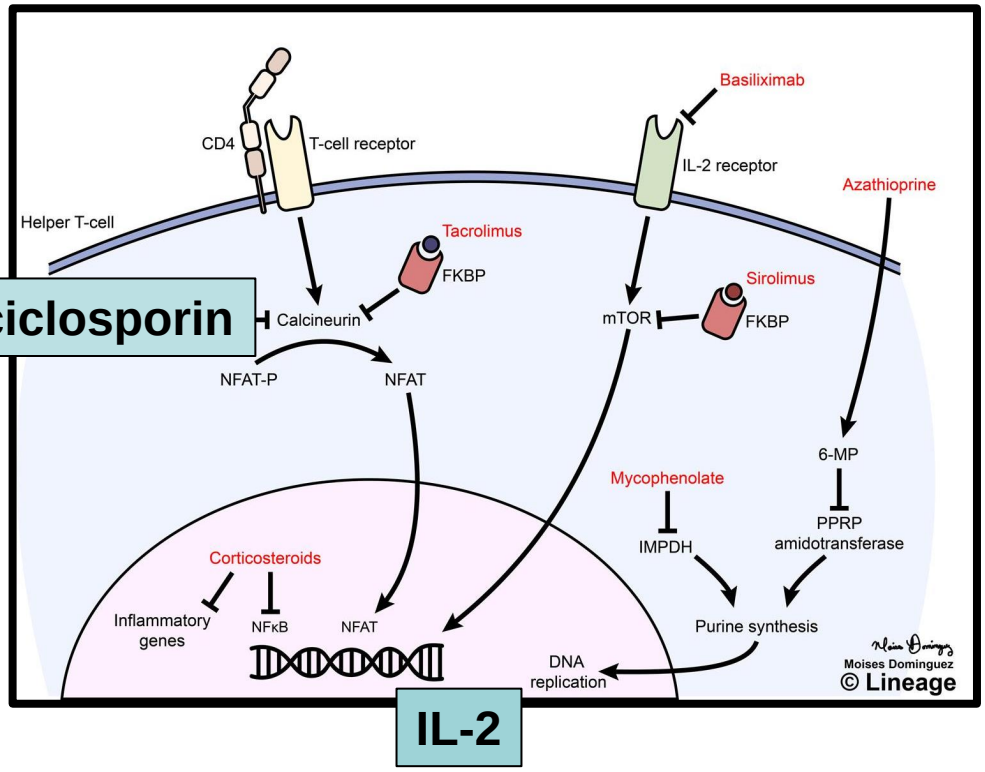
Immunopharmacology

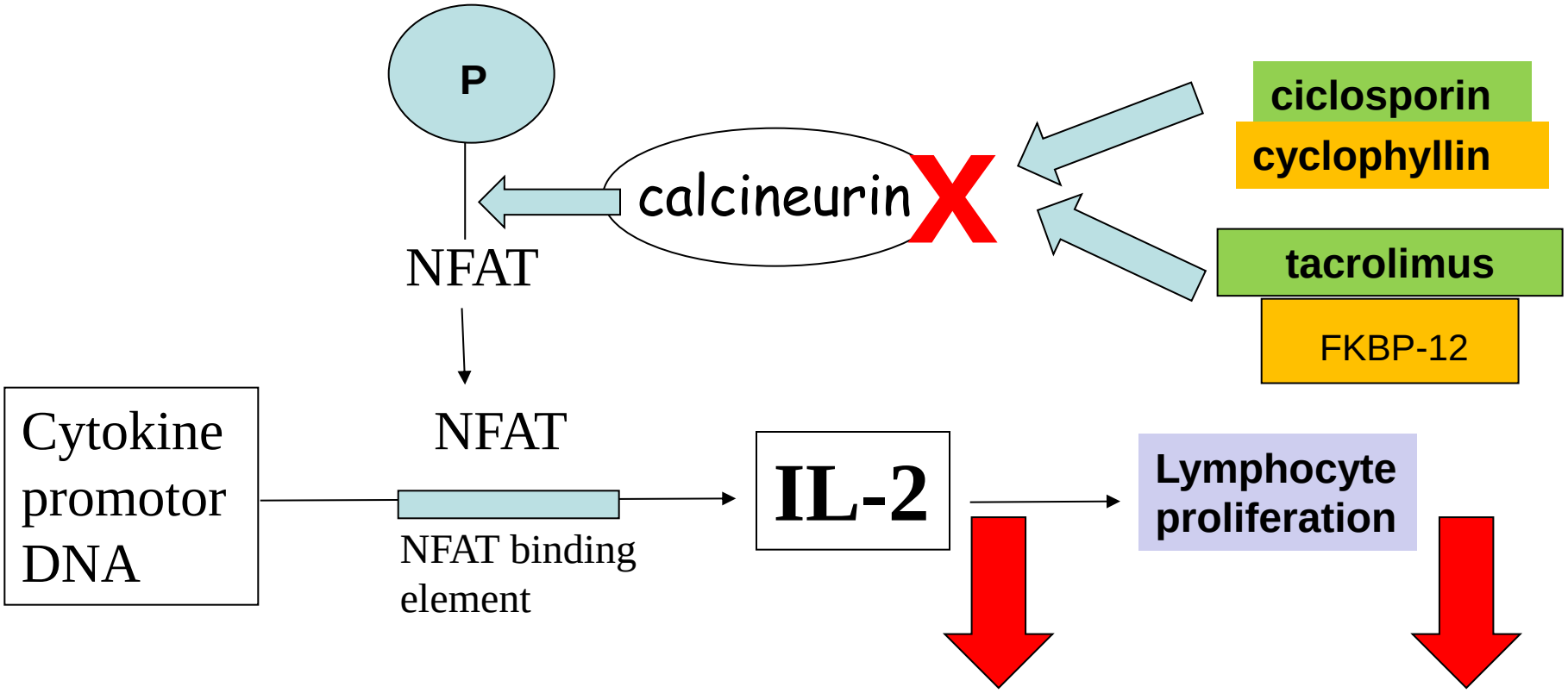
Anti-Rejection Drugs





Renal replacement therapy for patients with chronic kidney disease involves either dialysis (hemodialysis or peritoneal dialysis) or a kidney transplant, where the transplanted organ is normally placed in the groin, the diseased organs being left *in situ.* The first successful human organ transplant was performed in Boston in 1954 of a kidney transplanted between twins without therefore the normal problems of organ rejection due to activation of the immune system in response to foreign tissue. Initially, immunosuppression for kidney transplants was provided using azathioprine and a corticosteroid but, it was the discovery of ciclosporin with its ability to reduce the production of the cytokine, interleukin 2 (IL-2), followed by tacrolimus, which allowed kidney transplantation to become a routine hospital procedure.

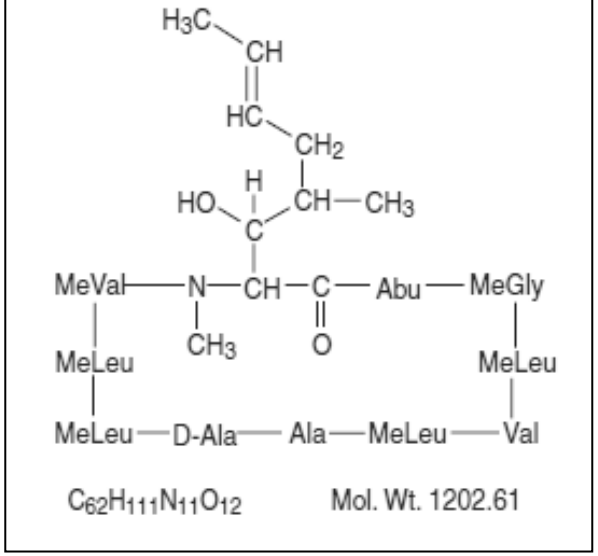
1. Inhibition of IL-2 Synthesis



Ciclosporin combines with an intracellular binding protein, cyclophyllin to inhibit calcineurin, a phosphatase enzyme. Its successor, tacrolimus, combines with a different binding protein FKBP-12 to inhibit the same enzyme. Inhibition of calcineurin reduces the activation of NF-AT (nuclear factor for T cell activation), preventing binding to the gene promotor for IL-2 transcription. Inhibition of IL-2 synthesis reduces the clonal expansion of T-helper lymphocytes involved in the immune response to the foreign organ. This is the first of 3 major mechanisms by which immunosuppressants inhibit the rejection of transplanted organs.

Ciclosporin

fungal cyclic decapeptide
orally active
low bioavailability
excreted by bile



Enzyme inhibitor

Forms complex with a binding protein cyclophilin to inhibit calcineurin

Prevents acute rejection –

1yr graft survival 95%

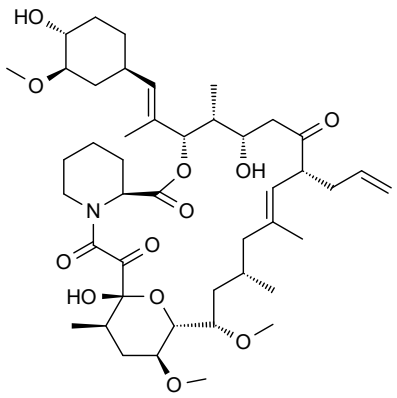
- Viable treatment - upturn in transplant numbers
- **NO** bone marrow suppression

Adverse Effects

nephrotoxic, hepatotoxic, diabetes, neuropathy, tremor, gingival hypertrophy, hypertrichosis

Ciclosporin is a 10 amino acid cyclic fungal peptide that, unusually for a peptide, is orally active, albeit with a low bioavailability and is excreted by the bile into the faeces. For kidney transplants,1 year survival data improved to 95%, showing the effectiveness of ciclosporin in preventing acute organ rejection, elevating transplant numbers. Ciclosporin works by inhibiting lymphocyte proliferation but very importantly and, unlike cytotoxic chemotherapy, without suppressing the bone marrow. Ciclosporin may also be used to inhibit lymphocyte proliferation in a variety of autoimmune disorders.

Tacrolimus



Tacrolimus

Derived from soil bacteria *Streptomyces tsukubaensis* chemically related to macrolide antibiotics

Unlike ciclosporin NOT a peptide

Mechanism

calcineurin inhibitor, better adverse effect profile than ciclosporin

Therapeutic Use

kidney grafts, no further improvement in 1-year survival, no effect on 5 year survival rates

Adverse Effects

immunosuppression

INFECTION, TUMOURS

NHS Digital 2017

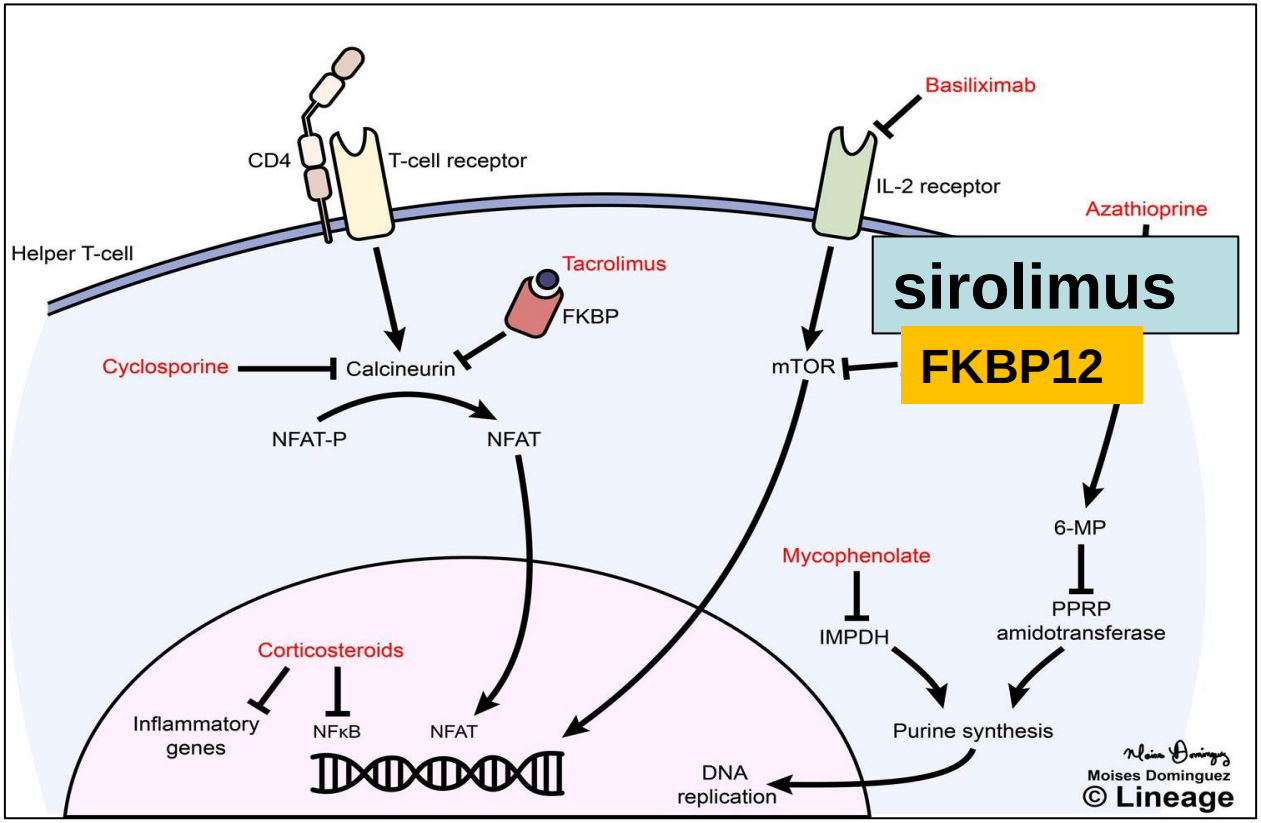
tacrolimus in top 20 drug therapies on which NHS spent most money

However, for organ rejection, ciclosporin has now been largely replaced by tacrolimus. Tacrolimus, derived from a Streptomyces soil bacteria, is not a peptide but structurally is called a macrolide (simply means big molecule) similar to the macrolide antibiotics like erythromycin. It has improved oral bioavailability, although as with any immunosuppressant it may increase the susceptibility to infection and tumour development by inhibiting the body's immune surveillance systems. In kidney transplantation, tacrolimus produces similar benefit to ciclosporin on 1-year survival times suppressing acute rejection but with fewer adverse effects. However, due to the development of chronic allograft nephropathy, treatment with tacrolimus does not improve survival at either 5 years or 10 years post transplant.

2. Inhibition of IL-2 Response

Provides steroid free immunosuppression Inhibits the cellular response of proliferative cytokines such as IL-2.

- Synergistic with tacrolimus.
- Inhibits wound healing – delay treatment for 1 month after surgery.
- Polymer-coated stents in angioplasty to reduce restenosis



Modern anti-rejection drug therapy may consist of using two other agents with different mechanisms of action in combination with tacrolimus. Sirolimus inhibits the response of the T-lymphocyte to IL-2, working well in combination with the calcineurin inhibitors which reduce IL-2 synthesis. Sirolimus, like tacrolimus, first requires combination with the intracellular binding protein FKBP-12 after which it inhibits mTOR. The term mTOR stands for the molecular target of rapamycin, the older name for sirolimus. mTOR is a protein kinase involved in the regulation of cell growth, proliferation and survival. Due to the inhibition of wound healing, treatment with sirolimus must be delayed for 4 weeks post-transplant. Sirolimus is also used to coat stents used in angioplasty for example following a heart attack to prevent restenosis.

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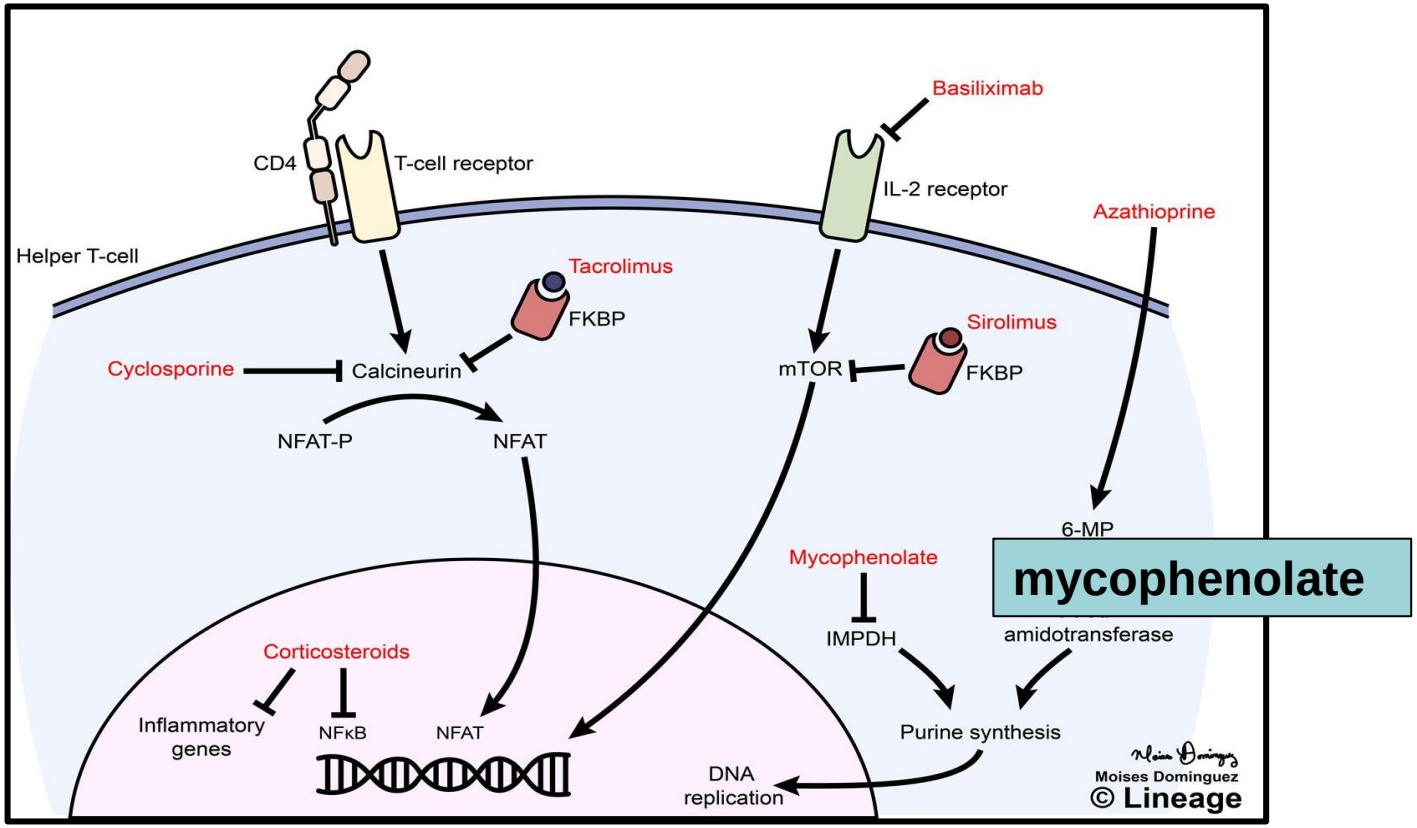
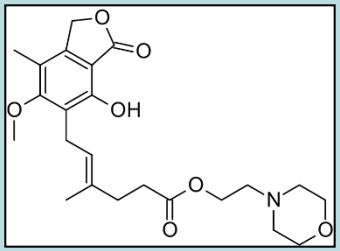
3. Inhibition of lymphocyte base synthesis

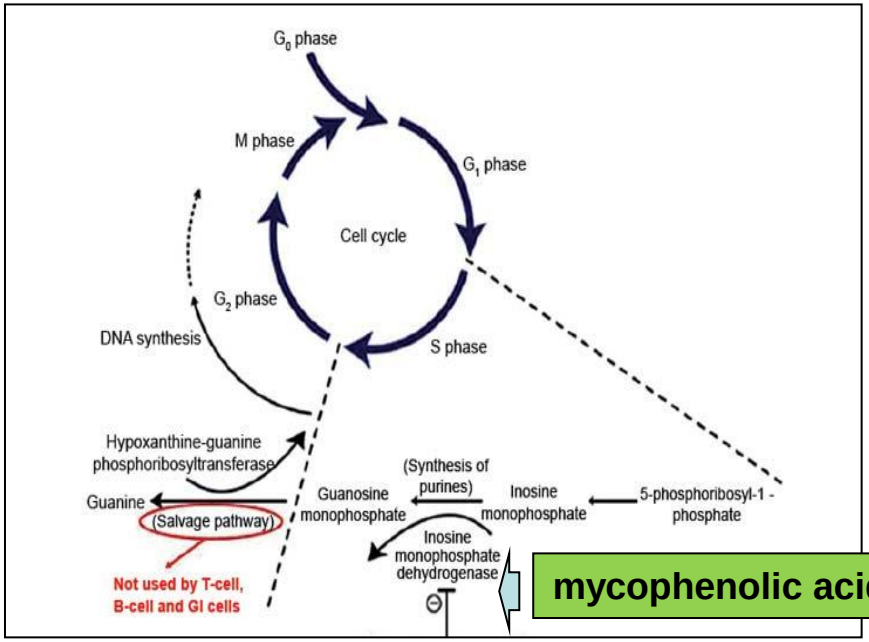


Before the introduction of ciclosporin, anti-rejection drug therapy included a steroid together with azathioprine, a cytotoxic agent which inhibits purine base synthesis. The third anti-rejection drug, mycophenolate, like azathioprine, inhibits the synthesis of purines which contribute to the base pairs in DNA, thus inhibiting DNA replication. However, unlike azathioprine, it is selective for purine base synthesis in the lymphocyte.

Mycophenolate Mofetil



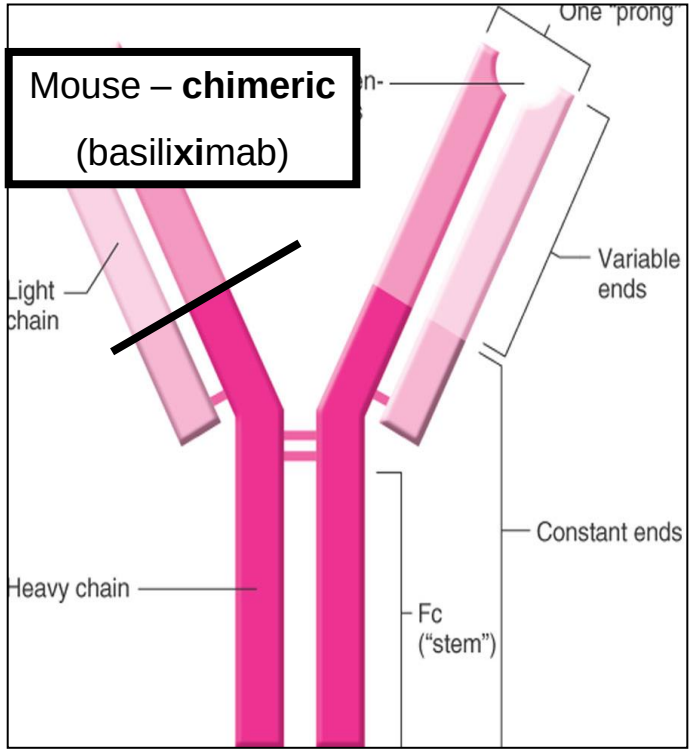
- Derived from Penicillinum Stonoiferum
- Prodrug converted to mycophenolic acid
- Inhibits **inosine monophosphate dehydrogenase** (IMDH) an enzyme involved in purine synthesis
- T & B lymphocytes highly dependent on this pathway for proliferation (don't have a salvage pathway)

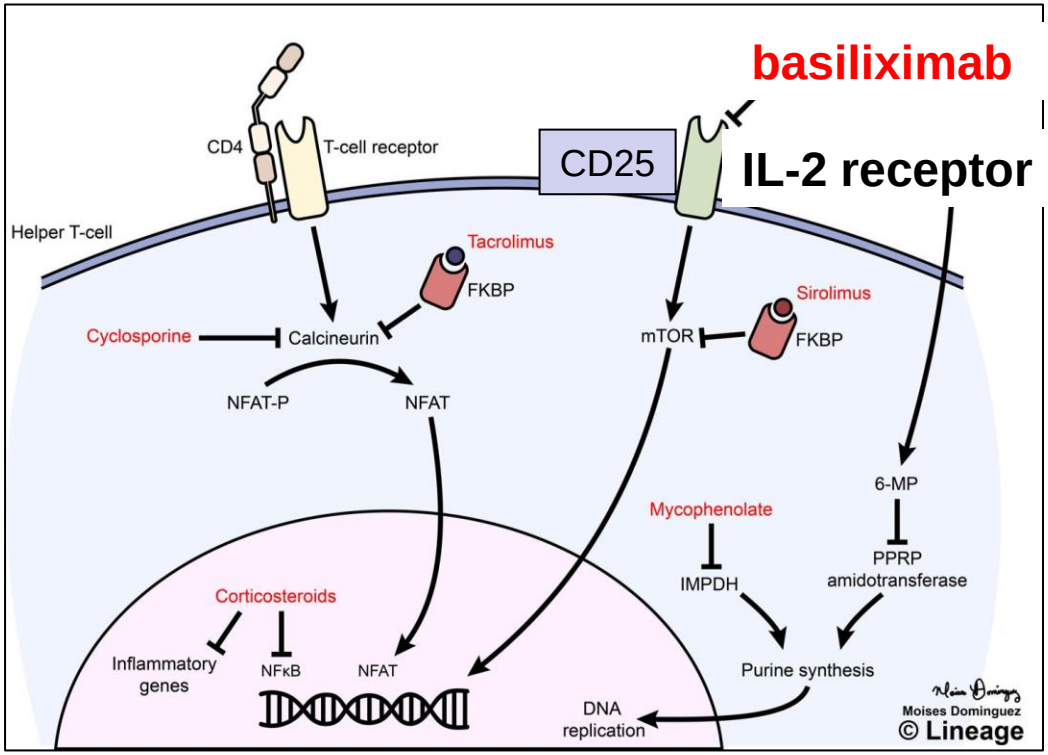


Inhibits cell cycle in S phase
Replaces azathioprine in immunosuppression
Combination with tacrolimus and sirolimus

Mycophenolate mofetil is a prodrug which inhibits the enzyme inosine monophosphate dehydrogenase, IMDH, the rate limiting step in guanine base synthesis. Unlike other cell types, lymphocytes do not have a salvage pathway to re-cycle such bases and therefore rely on their de novo synthesis. As a result, mycophenolate works well in combination with tacrolimus and sirolimus, each inhibiting a different cellular pathway. Mycophenolate could therefore also be classified as a lymphocyte selective, antiproliferative agent, inhibiting the cell cycle in S phase.

Basiliximab





Although ciclosporin was the first drug demonstrated to inhibit the synthesis of the cytokine IL-2, the development of commercial methods for monoclonal antibody production allowed the introduction of a therapeutic monoclonal antibody basiliximab, which is an antagonist of the IL-2 receptor, to prevent acute organ rejection. Basiliximab is a chimeric monoclonal antibody against CD25, a surface protein which is part of the cell membrane receptor for IL-2.

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Important Anti-rejection Drugs

Ciclosporin

Tacrolimus

Sirolimus

Mycophenolate

Basiliximab

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Immunopharmacology

Cancer Immunotherapy

Cancer Patients

Mysterious remission in patients who contract a bacterial infection

(Can immune system kill tumours when made hypervigilant by infection?)

Molecular Targets

1. **Direct immunostimulation (CD28)**
2. **Inhibition of checkpoint proteins (CTLA4/PD1)**
3. **Inhibition of tumour driver (CD19)**

The system of immune surveillance is in continual operation under healthy conditions correcting replication errors, preventing the development of cancerous tissue. Observations from cancer patients who have contracted a bacterial infection have previously shown mysterious examples of remission, giving credence to the idea that the immune system can kill tumours when made hypervigilant by infection. Approaches to cancer immunotherapy include the development of monoclonal antibodies with exciting new targets against surface proteins in 3 specific ways including direct immunostimulation (through CD28), inhibition of checkpoint proteins (CTLA4/PD-1) and inhibition of the tumour driver (CD19).

1. Direct Immunostimulation

Theralizumab (TGN 1412)

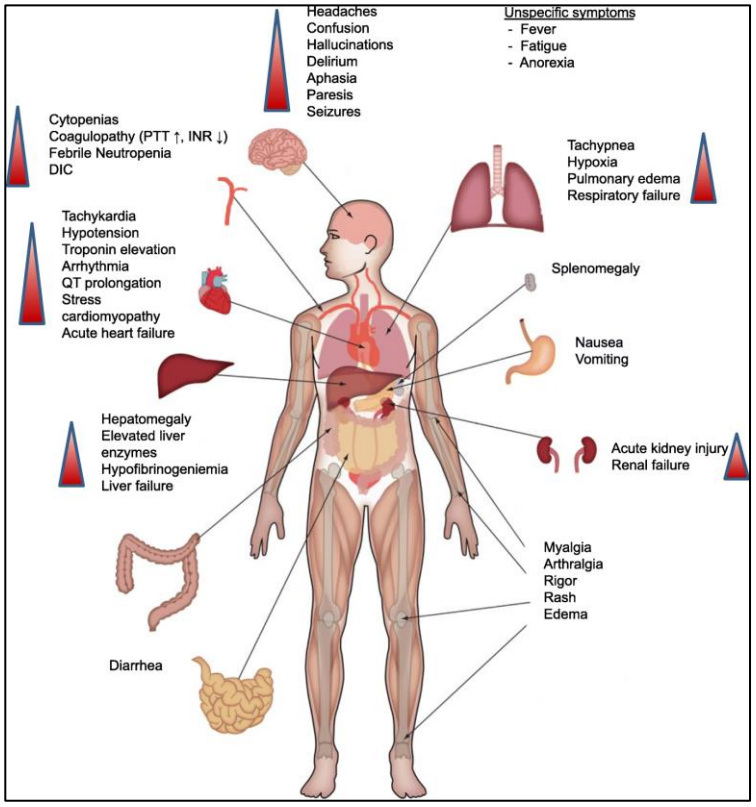
Elephant Man : First in man drug trial goes wrong

- **Phase I trial - 6 volunteers injected with theralizumab became very ill (multiorgan failure) at Parexel's research unit at Northwick Park**
- **life support required – angioedema, swollen skin and mucous membranes**
- **theralizumab is a T-cell superagonist which induced a ' cytokine release syndrome '**

Questions?

Sequential dosing, dose, relevance of animal testing

TGN 1412 From discovery to disaster
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2964774/



Early trials on direct immunostimulation using the monoclonal antibody, theralizumab, produced disastrous results. Healthy volunteers given theralizumab immediately developed a cytokine storm, increasing vascular permeability generating an *elephant man* type of condition producing cerebral oedema leading to multi-organ failure. The results also raised important questions as to how human clinical drug trials are undertaken.

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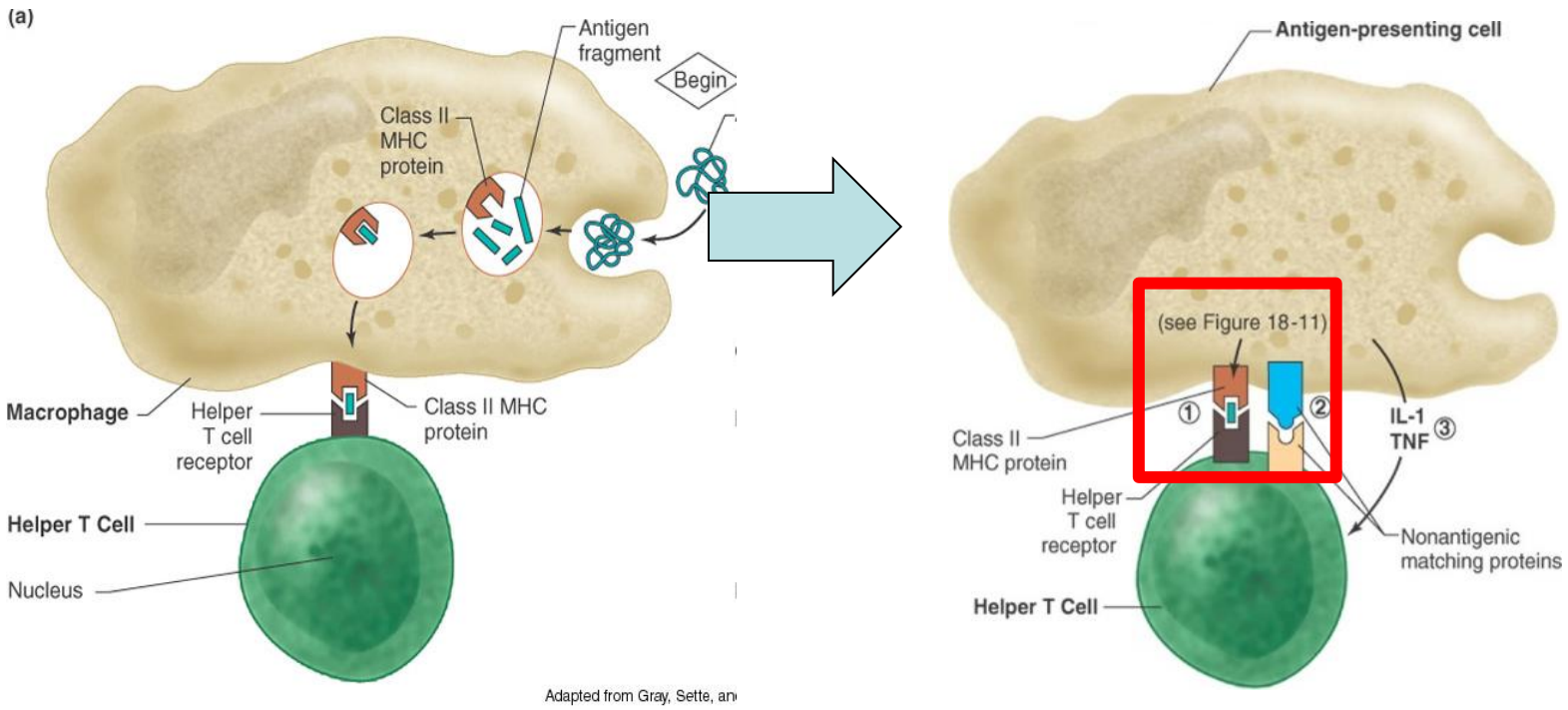
Helper T-Cell Receptor



To understand how theralizumab worked, consider the interaction between antigen presenting cells (APC) such as the macrophage and Helper T-cells in 2 steps. Firstly, antigen presenting cells take up and degrade antigens which combine with major histocompatibility proteins to express antigen fragments on the cell surface (MHC proteins). The MHC protein then combines with the T cell receptor to activate the helper T cell.

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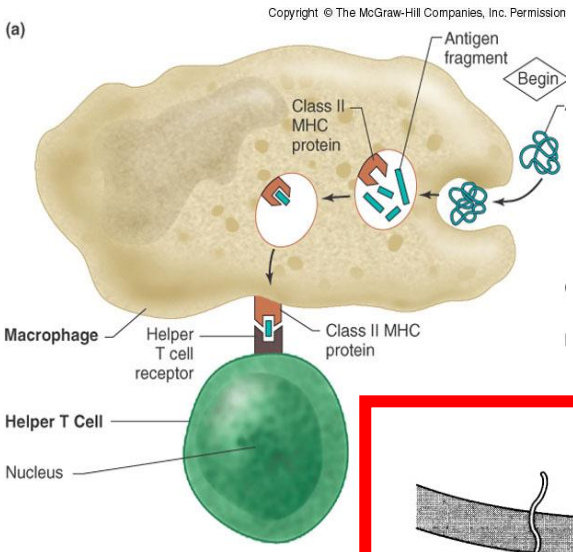
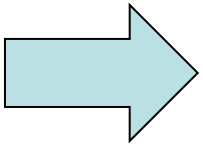
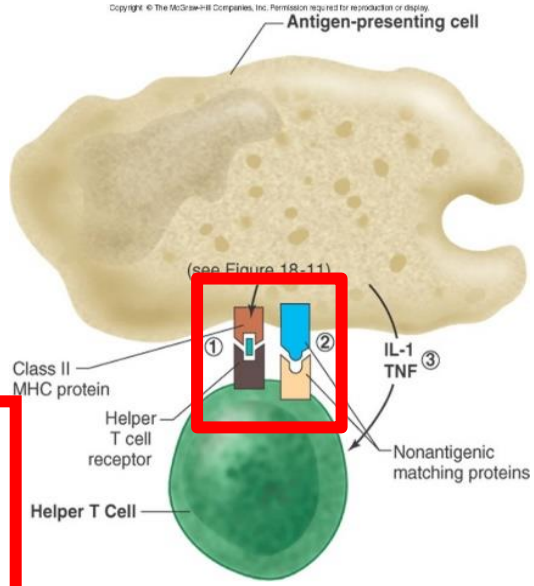
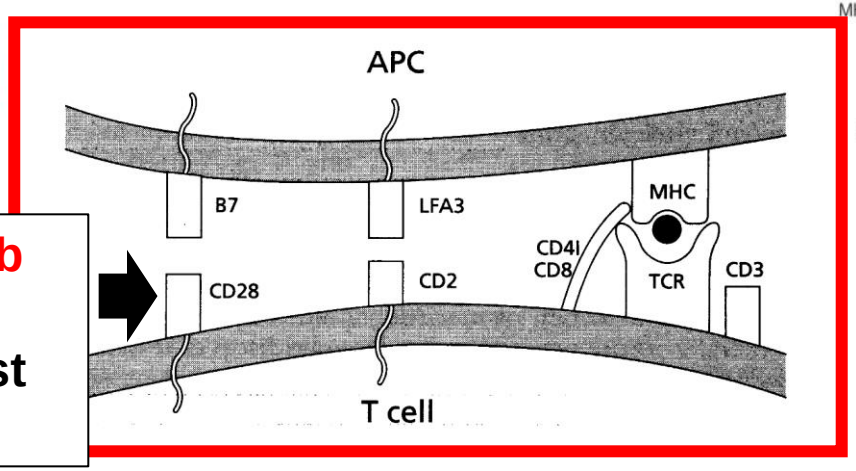
CD28 Agonist: Immunostimulation







theralizumab
CD28 superagonist antibody



Binding of the antigen presenting cell and helper T-cell brings other nonantigenic matching proteins into play, such as the combination of B7 on the antigen presenting cell with CD28 on the T helper cell to produce cell activation. The company TeGenro developed a monoclonal antibody, theralizumab which was a CD28 superagonist, which activated CD28 directly to stimulate helper T-cell expansion. The direct activation of CD28 induced a cytokine storm suggesting an alternative approach was required.

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2. Inhibition of Checkpoint Proteins

Tumours suppress the immune response
(T-cells gather at edge of tumour and stop)
Therapy – stimulate the immune system to detect and fight tumours
How : **Switch off checkpoint proteins**

Histological evidence from solid tumours suggested that the T-lymphocytes which could prevent tumour growth may actually stop at the edge of a solid tumour. The inference being that tumours produce specific inhibitors, known as checkpoint proteins, which prevent the ingress of immune cells into the tumour. An appropriate pharmacological approach would therefore be to try and switch off checkpoint proteins.

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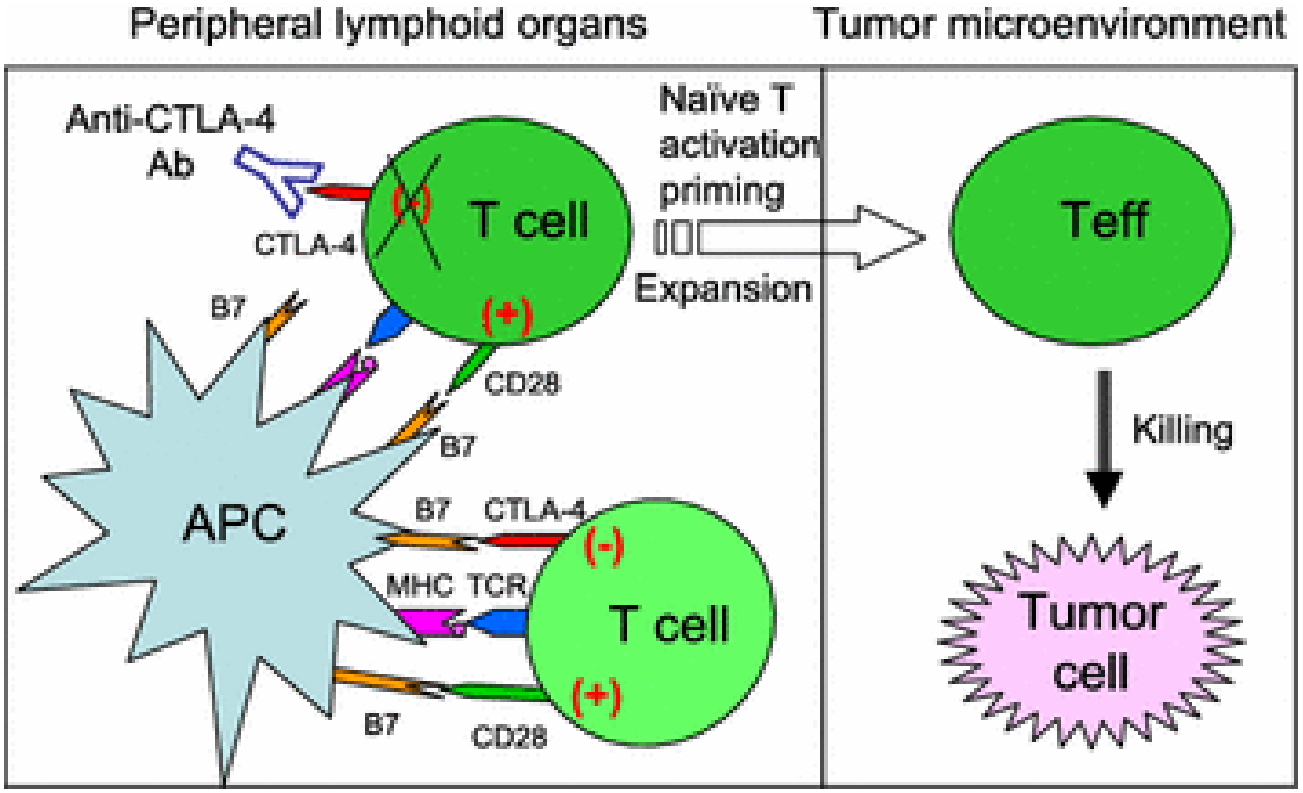
Cytotoxic T-lymphocyte Associated Protein (CTLA4)



The first checkpoint protein to be used as a drug target was CTLA-4 (cytotoxic T-Lymphocyte associated protein 4). T-lymphocytes (shown in green) are activated to proliferate initially by the generation of a MHC protein (shown in purple) expressed on the surface of an APC cell (shown in light blue) which binds to the T-cell receptor (shown in dark blue). The nonantigenic matching protein B7 on the antigen presenting cell may be stimulated by combination with CD28 but inhibited by combination with CTLA-4. While direct activation of CD28 with theralizumab induced a cytokine storm, it was hoped that by preventing the inhibitory effect of CTLA-4, with a monoclonal antibody, a more measured activation of the immune system might be produced to inhibit tumour development.

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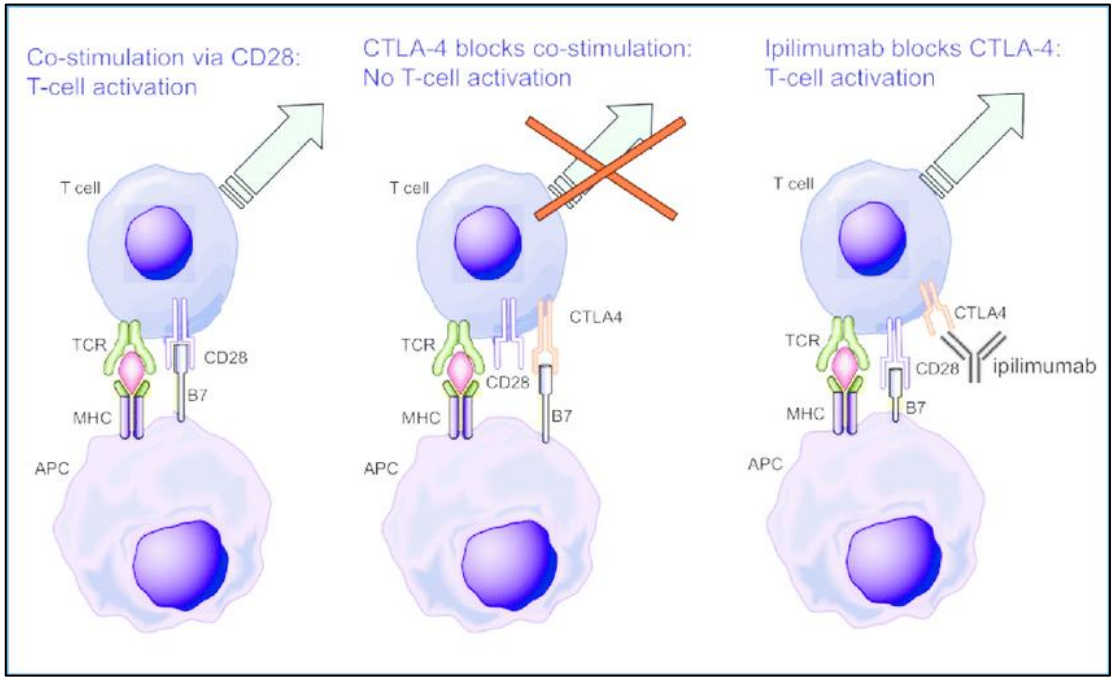
Ipilimumab (CTLA inhibitor)



Malignant melanoma

Second commonest cancer in young adults

(10,000 new cases a year, 2,000 deaths),

fastest increasing cancer in UK.

Inherent resistance to cytotoxic drugs

Ipilimumab

monoclonal antibody against CTLA-4

Prevents the inhibitory effect of the co-stimulatory pathway from activating T-cells.

Malignant melanoma - ipilimumab gave 4 months survival gain for 20% patients

**** however, some recipients still alive after 10 years***

Ipilimumab was the first CTLA-4 inhibitory antibody to be trialled for use in malignant melanoma, the second commonest cancer in young adults, a cancer which is inherently resistant to cytotoxic chemotherapy. Ipilimumab reduces the inhibitory effect of co-stimulation, activating T-cells at the tumour surface, allowing them to penetrate into the tumour mass. Ipilimumab only produced a small 20% survival gain over a 4 month period in patients with malignant melanoma who were unresponsive to other treatments. However, although only a small survival gain was obtained in the trial, some trial participants were still alive some 10 years later!! The lengthy survival of a small number of patients suggested that combination therapy with other checkpoint inhibitors might prove beneficial.

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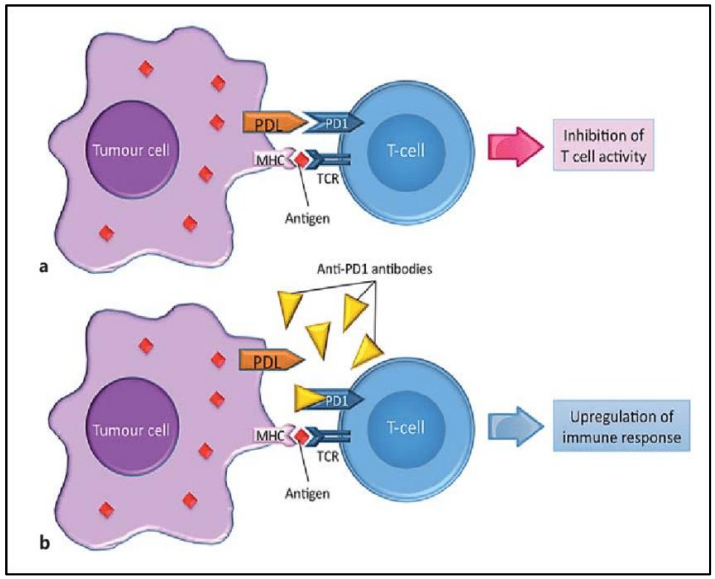
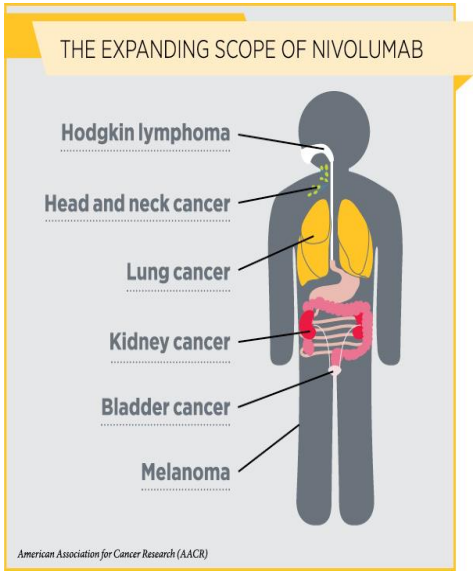
Programmed Death receptor **PD-1 Inhibitor : Nivolumab**



Combination Therapy Ipilimumab with programmed death (PD-1) inhibitor

nivolumab

melanoma survival increase from 20 to 60%



PD-1 blocks T-cells once they have infiltrated the tumour and its environment

Once T-cells have infiltrated the tumour and its environment other co-stimulator pathways may come into play. In addition to CTLA-4, a second inhibitory cell surface protein on T cells is PD1, programmed death protein 1. Tumour cells have a cell surface programmed death ligand (PDL) which combines with PD1 inhibiting T cell activation. Nivolumab is an inhibitory monoclonal antibody which binds to PD-1, thus removing its inhibitory effect. Nivolumab, when employed in combination therapy with ipilimumab, increased the survival of patients with malignant melanoma from 20 to 60%.

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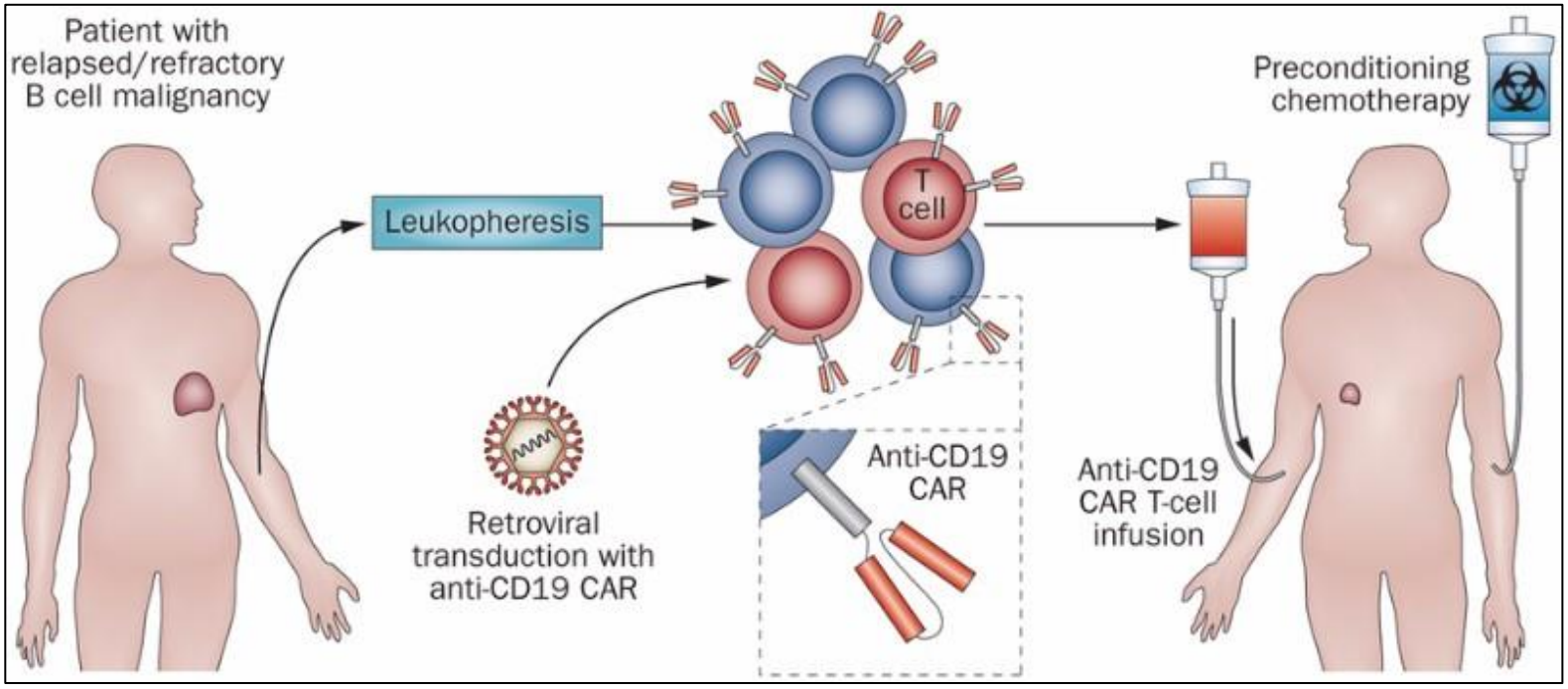
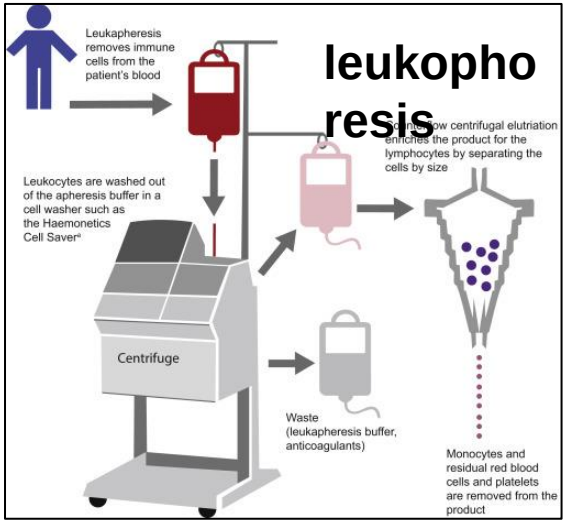
3. Inhibition of CD19 : CAR-T Cell Therapy



Stem cell transplants are an important feature for the treatment of non-solid tumours. However, a new form of cell transfer therapy called CAR-T therapies are have been developed, introducing the concepts of a living drug and personalised medicine. For patients with a B-Lymphocyte malignancy, who are refractory to other treatments, their blood is subjected to the process of leukophoresis, separating out the T-lymphocytes. These T-cells are then genetically modified to introduce a receptor to CD19, an important B-cell surface protein. Following preconditioning chemotherapy, the modified T cells are reintroduced, to multiply in the patient's bone marrow.

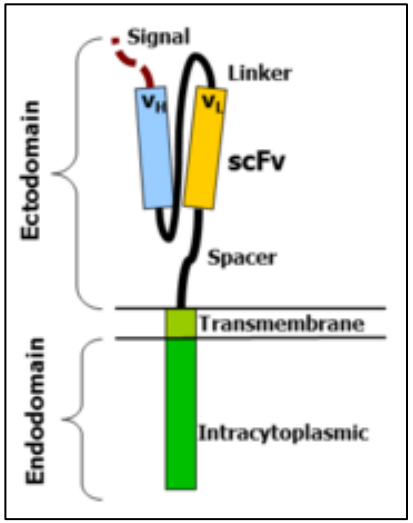
Chimeric Antigen Receptor (CAR)



CAR-T

- **T-lymphocytes are genetically modified to produce a CAR protein, an artificial receptor designed to target a specific protein.**
- **All current CAR-T trials target CD19.**
- **The CAR-T-cells are activated & proliferate in vitro**
- **CAR-T cells becomes cytotoxic when binding to CD19, inhibiting tumour B-cells driving resistant forms of acute lymphoblastic leukaemia.**

Ideally the CAR-T cells should be engineered not to target proteins expressed by healthy cells.



T-lymphocytes isolated from an individual patient's blood by leukophoresis are sent to a drug company laboratory, in this case Novartis in the US for viral infection with an artificial chimeric antigen receptor raised against CD19. In fact, all early CAR-T trials were designed to target CD19. The T-cells proliferate in vitro and when infused back into the patient, bind to CD19 on the malignant B cells. Crucially the patient's own T-cell population must be markedly reduced by cytotoxic chemotherapy prior to the T cell infusion. That is, if effective cytotoxic chemotherapy had not previously been developed, CAR-T cell therapy could not be successfully employed. Facilities to genetically modify T-cells are now available in the UK. Cytotoxic chemotherapy will be fully explored in semester 2 within GI module.

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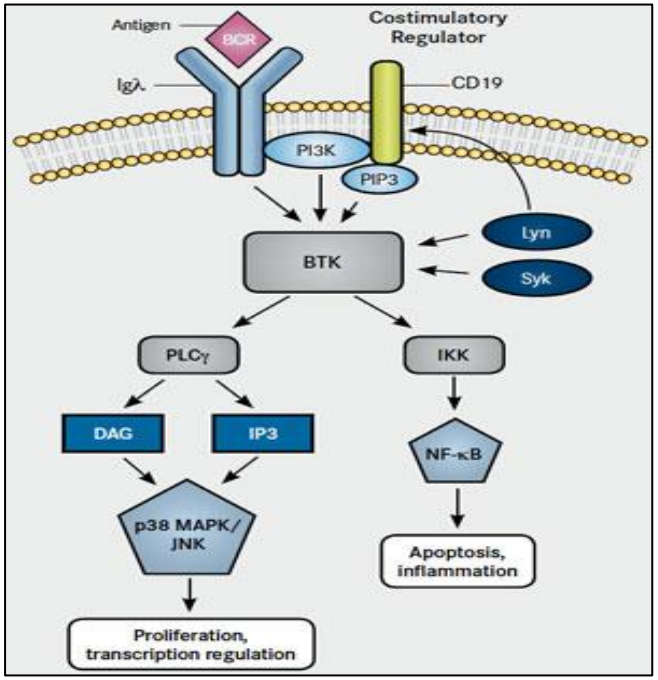
CD19 : B-lymphocyte antigen



B-lymphocyte antigen CD19, also known as CD19 molecule

Transmembrane protein that in humans is encoded by the gene *CD19*. CD19 is expressed in all B lineage cells (except for plasma cells) and in follicular dendritic cells.

CD19 plays two major roles in human B cells.

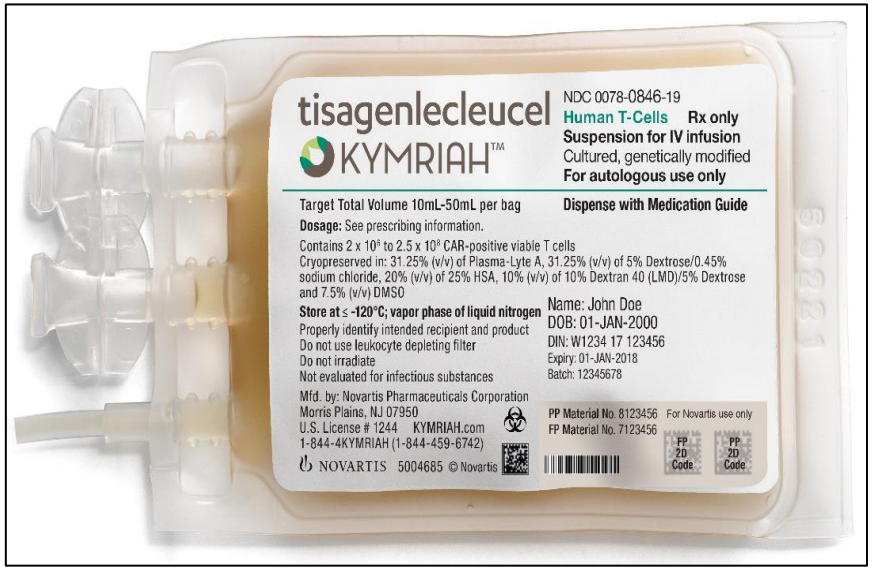
(a) It acts as an adaptor protein to recruit cytoplasmic signalling proteins to the membrane

(b) It works within the CD19/CD21 complex to decrease the threshold for B cell receptor signalling pathways.

Due to its presence on all B cells, it is a biomarker for B lymphocyte development, lymphoma diagnosis and can be utilized as a target for leukaemia immunotherapies.

CAR-T cell therapy introduces a new major drug target CD19. The cell membrane receptor CD19 is expressed by all B-lymphocytes. CD19 is a co-stimulator for antigen activation decreasing the threshold for B-cell signalling and proliferation. Following the success of the checkpoint inhibitors, the big Pharm companies are now attempting to re-wire the immune system using other cellular targets and molecular approaches to enhance the response of the immune system to a specific tumour antigen.

CD19 : Tisagenlecleucel



Kymriah (Novartis)
One time treatment (£282,000/patient)
Acute lymphoblastic leukemia
15-20 children
(London, Manchester, Newcastle)
Advanced disease where other treatments (including stem cell therapy) have failed
80% success in advanced disease

Adverse Effect - cytokine release syndrome (50% patients)

*** *Juno Therapeutics* trials – cerebral oedema (5 deaths)**

The first CAR-T cell therapy against CD19 to be funded by the NHS in the UK was from Novartis called Kymriah, used to treat forms of resistant acute lymphoblastic leukaemia. This involves children who have failed to respond to other treatments including stem cell therapy. The approved name for this 'drug' is tisagenlecleucel. It is an intravenous infusion of the patients own modified T-cells. Initially funded for 15-20 children in the UK., this is a one-time therapy designed for each individual patient at a reported cost of some £300,000. Remarkably, success rates were very high at 83% after 3 months. However, half of the patients also suffered from a strong cytokine release syndrome. In CAR-T trials by another company Juno Pharmaceuticals, outside the UK, 5 deaths occurred from cerebral oedema and the trials had to be stopped.

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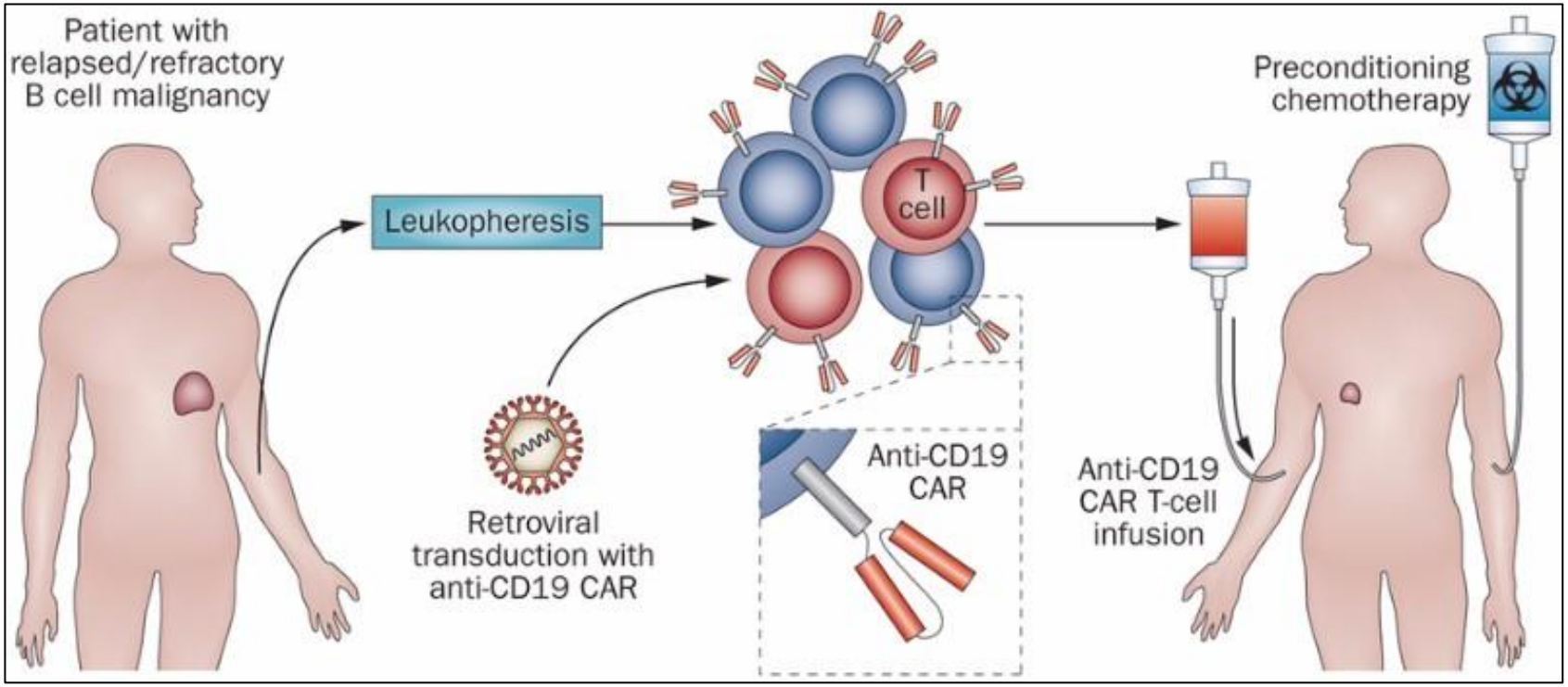
Lymphodepletion : Pre-conditioning Chemotherapy



In the days prior to infusion of CAR T cells, the patient undergoes chemotherapy to deplete endogenous lymphocytes, which allows for the engraftment and expansion of CAR T cells. Lymphodepletion "makes room" for the CAR T cells and reduces immunosuppressive cells that may threaten CAR T-cell expansion. Lymphodepletion also releases endogenous intracellular inflammatory cytokines, which promote CAR T-cell activity once the cells are infused. Preconditioning regimens vary by protocol and by individual patient but allow the concept of Living Drug, manufactured in the patient's own bone marrow to develop.

CAR-T therapy : Adverse Effects

Cytokine Release Syndrome (CRS)

can be mild flu-like symptoms (malaise, fatigue, myalgia, nausea, anorexia) which may resolve by 3 weeks post infusion.

- Severe - High fever, tachycardia, hypotension, hypoxia and organ failure requiring vasopressors, ventilator support & ICU care.
- *Treatment with IL-6 antibody (tocilizumab) + corticosteroids (dampen anti-tumour effect).*

Neurotoxicity

CAR-T cells found in spinal fluid, normally reversible but can produce cerebral oedema which can be fatal

Macrophage Activation Syndrome (MAS) – elevated triglycerides, bleeding treat with tocilizumab.

B-Cell Aplasia

(on-target, off tumour toxicity) is expected and can last for years - immunoglobulin replacement and antimicrobial cover required.

The major problem with CAR-T therapy is the induction of a cytokine release syndrome. This can simply produce mild flu-like symptoms which may resolve within 3 weeks of infusion. Alternatively, it may produce a high fever and organ failure requiring artificial ventilation and intensive care support. Drug treatment with tocilizumab, an IL-6 antagonist, together with corticosteroids may help, but will dampen the anti-tumour effect. Cerebral oedema maybe fatal while activation of macrophages could result in bleeding. One expected adverse effect is normal B-cell aplasia, increasing the susceptibility to infection.

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Future Develoments

CART cells reach peak levels after 1-2 weeks

Development to memory cells detected out to 4 years so far

1. **Combination therapy -** with check point inhibitors
2. **Drug Switch** -– introduce drug switch ie rapamycin (Cellectis) to allow control of cytokine release syndrome (Go-CAR-T)
3. **Allogeneic Infusion** - possibility of developing allogeneic infusions for off the shelf treatment
4. **Solid tumours** – future development use natural killer receptor (NK2) binds to 8 ligands present on 80% of solid and haematological malignancies

CAR-T therapy was envisaged as a one time treatment, where peak T-cells levels are reached within 2 weeks of IV infusion with memory cells being detected out as far as 4 years at the present time. Future developments will probably start with combination therapy using checkpoint point inhibitors. The adverse effect profile presents a major problem which might be reduced by the introduction of drug switches such as sirolimus, which may offer greater control. At present, the T-cell infusion is autologous ie using the patient's own T-cells, so each IV bag is different and only used in one patient. The possibility of developing an off-the-shelf allogeneic infusion is being examined, as are alternative targets away from CD19 such as to natural killer cell receptors which may bind to some 80% of solid tumours, as well as haematological malignancies.

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Cancer Immunotherapy

1. Direct immunostimulation
(CD28 theralizumab- problems)
2. Inhibition of checkpoint proteins
(CTLA4 ipilimumab/PD1 nivolumab)
3. Inhibition of tumour driver
(CD19 tisagenlecleucel)

In summary, at the present time the two major methods of stimulating the immune system to inhibit tumour growth, are the inhibition of either checkpoint proteins or inhibition of tumour drivers such as the B-cell CD19. Drugs names important in this area are ipilimumab and nivolumab the the allogenic CD19 T cell infusion of tisagenlecleucel (Kymriah, Novartis).