July 20

Background info

Glioblastoma (GBM)

Brain tumour that arises from glial cells in the brain

↳ glial cells = supportive cells that provide structure and nourishment to the neurons.

- GBM is most common and deadliest form of primary brain tumours in adults.
- grows rapidly and metastasizes into nearby brain tissues
- World Health Organization ranks GBM grade IV meaning it has a high degree of malignancy

- Exact cause of GBM is still not fully understood
- occurs in both younger and older populations
- Prognosis remains poor
- survival rate in generally low = 1 year

Giloblastoma multiforme

↳ the "multiforme" refers to variety of cell shapes, sizes, features, etc. Making the tumour hetrogeneous

Resource - Cancer.gov

July 26

current treatments for CAR-T cell therapy:

Radiation -

Radiation tipically takes place after surgical efforts of removing tumour. High energy x-rays and others forms of radiation such as external beam therapy which target the tumour cells.

Chemotherapy -

Chemotherapy is often given in combination with radiation therapy. The most commonly used chemo drug for the treatment of GBM is temozolomide.

Optune:

Optune is a FDA medical device, which provides low intensity electric fields to the brain (tumour treating fields).

Temozolomide - TMZ

Is an oral (pills, liquids) chemo drug that is specifically designed to target and damage DNA of rapidly dividing cancer cells. This drug is standard treatment for newly early diagnosed GBM. Temozolomide is effective cause it can cross the blood brain barrier - allowing it to reach and target the tumour cells. And when it does, the drug methylates (adding a chemical group called methyl) the DNA strand. Which damages the DNA leading cancer cells to die or unable to proliferate (grow

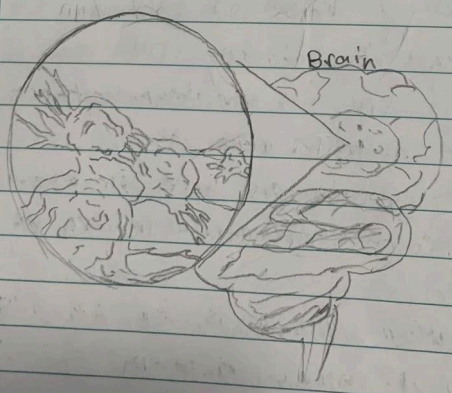
Aug 4

Heterogeneity of Glioblastoma
GBM cells vary from patient to patient — posing a challenge in treating the tumour effectively.

Tumour microenviornment —
the surrounding tissues, blood vessels, and even immune cells can vary among patients.

Molecular subtypes —
GBM can be classified into different molecular subtypes on gene expression (EGFR). These subtypes can influence tumour aggressiveness and prognosis.

Genectic mutations —
GBM holds different genectic mutations, leading to a need specialized treatment and distinct molecular profiles.



Resource —
the-scientist.com
ncbi.nlm.nih.gov

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ih.gov

Aug 5

Glial cells - neuroglia, gia
non-neuronal cells in the nervous system, these cells take up significant portion of cells in the brain and spinal cord.

- Glial cells provide structural support to neurons, and help maintain the structure all over the nervous system

- These cells can regulate the supply of nutrients and energy sources which help the neurons in turn

- Microglia are glial cell that work as immune cells of the nervous system
some of their responsibilities are:
- detecting / removal of damaged cells
- foreign invaders
- debris

- Astrocytes = type of glial cell
- helps regulate levels of neurotransmitters in the synaptic spaces between neurons
- helps with the formation and maintenance of blood-brain barrier - preventing entry of harmful substances
- helps with tissue repair aswell as regeneration after injury to the nervous system

Resource - cancer.gov
ncbi.nlm.nih.gov

Aug

Gliomas are the most common tumours of the center nervous system (CNS)
↳ 30% of all primary brain and CNS malignant tumours
↳ 13,000 deaths and 18,000 cases of primary malignant and CNS tumours happen yearly in us

Glioma consists of 4 different types of forms
1. Astrocytic tumours
   - Astrocytoma grade I
   - Astrocytoma grade II
   - Anaplastic Astrocytoma grade III
   - Glioblastoma grade IV
2. Oligodendrogliomas
3. Ependymomas
4. Mixed Gliomas

↳ 4 types of gliomas are each characterized by distinct cell orgins, growth patterns, and histological features

GBM
- Majority of cases arises in Males
- 60% of cases, the tumour initates de novo meaning that the tumours arise without a clear precursor or identifiable cause
↳ indicates that the tumour froms normal cells that had genectic changes or mutations
- 40% of cases, the tumour forms as a result of transformation or progression of a lower-grade brain tumour into a more mali... form.

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↳ these cases originates from pre-existing brain tumours which were of lower grade (less aggressive).

Resource - sciencedirect.com

Current Studies for GBM

In the united states GBM affects annually 17,000 people. It has a poor prognosis with a median survival of 15 months and a 5 year survival rate of less than 5%.

- current treatments such as chemotherapy and radiation have limited effectiveness against GBM due to challenges like early microscopic spread of tumour cells, impaired immune response, and blood-barrier limiting drug delievery

- There has been little clinical progress in GBM treatment. Most phase III clinical trails since 2005 have not shown positive outcomes - except for 1 study
  ↳ immunotherapy, particulary immune checkpoint blockade, has shown success in various advanced cancers but has not been as effective in in GBM.
  ↳ But suggestion from subgroup analyses and preclinical data that an immune-based approach might have activity in GBM

Aug 16

Protien/marker present on the surface of DIPG cells

Activity of CAR-T cells targeting GAD2 in Diffuse Intrinsic Pontine glioma (DIPG) - Brain tumour → pons
↳ data has shown success in using CAR-T cells targeting GAD2 in DIPG, showing the possibility of achieving responses against primary brain tumours through CAR-T cells therapy
MHC-independent activation
↳ unlike regular T-cells, CAR-T cells don't rely on major histocompatibility complex (MHC) presentation for activation. This characteristic allows them to target a broader range of tumour-associated antigens

T cells (CD8+) cytotoxic cells

↳ MHC Class I
- molecules are found on the surface of almost all eukaryotic cells. They present antigens derived from pathogen within the cell → (viral proteins produced by infected cells) to cytotoxic T-cells (CD8+ T-cell). This helps immune system to recognize/eliminate infected or abnormal cells.

↳ MHC Class II
- molecules primarily present on antigen-presenting cells (APCs) such as dendritic cells, macrophages and B-cells. MCH class II molecules present antigen from pathogens that have been engulfed and processed by these APCs to helper T-cells (CD4+ T-cell).

B cells
Memory cells
T helper cells (CD4+)

Resource - ncbi.nlm.nih.gov
Cancer.gov

Aug 28

CAR structure
- antigen-binding domain, Hinge region, transmembrane domain, intracellular domain
- Antigen-binding domain
  - Single-chain variable (scFv) is a type of protein construct derived from a monoclonal antibody
- Transmembrane domain
  - Alpha-Helix from CD4, CD8a or CD28; these are proteins found on the surface of immune cells like T-cells
- Intracellular domain
  - Includes a T-cell receptor CD3 complex that facilitates signal transduction leading to T-cell activation and cytokine production

CAR generations
- First gen - featured CD3 zeta domain and required IL-2 co-stimulation for cytotoxic function
- Second gen - Incorporated co-stimulatory proteins (ex-CD28, 4-1BB, OX40), exhibiting enhansed antitumour response and reduced CAR-T cell death.
- Third gen - Integrated multiple stimulatory pathways for T-cell activation.
- Fourth gen - Designed to produce cytokines that activate antitumour immune responses, aming to further improve treatment and efficency

Sept 5

Success in Hematologic Malignacies

- CAR-T cell treatment has shown significant activity in blood cancers like acute lymphoblastic leukemia (ALL) multiple myeloma (MM) aswell as B-cell lymphomas. The primary target being CD19 and BCMA, highly expressed antigens on cancerous B-cells.

- Compared to antibodies/vacines used to target GBM antigens, CAR-T cell therapy may offer several adventages
  - ↳ improved penetration of the blood-brain barrier and ability to directly kill tumour cells independent of the compromised native immune response in GBM.
    - ↳ by utilizing immune cell trafficking

- A major limitation lies in scarcity of specific tumour antigens and low mutational burden in GBM — limiting target options
- Some potential targets (TAAs) are infrequent or located inside tumour cells — posing challenges in effectively targeting intracellular antigens.

Resource - Cancer.gov

Sep 9

Targets for CAR-T cell in GBM

- EGFRvIII as a target (EGFR)
  - ↳ epidermal growth factor receptor ^ is amplified in over 50% of GBMs, with a mutated variant - EGFRvIII found in 30% of the cases.
  - ↳ This mutated variant contributes to tumour growth and is associated with a negative prognosis
  - ↳ EGFRvIII has been explored as a target for CAR-T cell therapy. Clinical trails targeting EGFRvIII with third-generation CAR-T cells showed adverse events related to lymphodepleting chemotherapy and some severe neurological side effects
  - ↳ Reproducible reponse has been difficult, with a median progression free survival of only 1 month and a median survival of 6.9 months after treatment

IL13Rα2 as a target

- ↳ is a variant receptor primarily expressed on GBM tumour cells, has been targeted for CAR-T cell therapy. This variant lacks the downstream signaling pathway present in the normal receptor and drives tumour progression
- ↳ Clinical trails have shown safety in patients with reccurrent GBM.
- ↳ While one patient intially responded well, most patients did not experience significant benifits
- ↳ some patients showed decreased tumour reccurrence in the periphery, but overall the therapy did not provide a curative effect.

A

HER2 as a target
- Human growth factor receptor 2 is overexpressed in up to 80% of GBMs
- however expression on healthy cells
tissues raises concerns about potential
on-target-off-tumour effects
- clinical trials using CAR-T cell to target
HER2 in GBM patients showed acceptable
safety profiles, with adverse events
primarily related to lymphodepletion. Some
patients displayed possible benefits, with
a median survival of 24.5 months from
diagnosis and indication of no progression
in some cases

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Sept 21

Challenges with CAR-T cell therapy in GBM
- Antigen escape
↳ tumours develop mutated or truncated variants of targeted antigens (eg. CD19, EGFRvIII; IL13Rα2) avoiding recognition by CAR-T cells
↳ recurrences often feature tumour cells lacking the targeted antigens due to selective pressure from CAR-T cells
- Molecular heterogeneity
↳ GBM tumours exhibit diverse spatial and temporal expression of antigens
↳ variability in antigen expression is influenced by factors like immune infiltration, hypoxia and metabolic changes within the tumour microenvironment
↳ different antigen may respond inversely to these factors - like EGFR expression might relate inversely to IL13Rα2 and HER2
- Impact of tumour environment on immune responses
↳ unlike liquid tumours, the microenvironment of solid tumours like GBM affects immune cells and their response to therapy
↳ Glioblastoma organoids, reflecting tumour complexity are aiding in exploring this complexity for CAR-T cell studies.
- Rapid intratumoural changes
↳ GBMs intratumoural heterogenity poses challenges: only a subset of tumour cells can be targeted, and targeted cells may rapidly change molecular makeup, reducing available

Sept 22

targets for CAR-T cells.

Promising antigens for CAR-T cell therapy

- B7-H3
  - overexpressed in GBM with minimal expres in normal tissues
  - shown promise in preclinical studies by eliminating tumour cells in vitro and controlling tumour growth in murine models

- EphA2
  - found in up to 90% of GBM samples without expression in healthy brain tissue
  - showed efficacy in vitro and in murine models, including tumour cell lysis and promo survival without adverse effects

- CD70
  - mostly seen in mesenchymal GBM cell subtypes and negatively linked to survival
  - CAR-T cell targeting CD70 induces tumour cell death, increases cytokine expression, an improves survival in murine models without significant immune supression.

- NKG2D Ligands
  - GBM expresses this target
  - NKG2D-targeted CAR-T cells exhibited cytotoxi against GBM cell lines in vetro and showed complete tumour elemination in murine models

- GD2
  - shown inc
  reduced tu
  significant

CSPG4
- expresse
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- CAR-T ce
effects
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- Derive
to tumo
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effects
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Sep 22

- GD2
  - shown increased survival in murine models and reduced tumour size in some GBM patients without significant adverse effects

CSPG4
- expressed in 67% of GBM specimens, associated with tumour progression and therapy resistance
- CAR-T cells targeting CSPG4 exhibit cytotoxic effects against tumour cells, prolonged survival in murine models, and can be induced by INF-α secretion from microglia

Chlorotoxin (CLTX)
- Derived from scorpion venom, selectively binds to tumours without normal brain tissue interaction
- CLTX-engineered CAR-T cells show broad treatment effects in GBM models, inducing tumour regression, and are being tested in clinical trials.

Strategies and considerations

- Targeting Multiple antigens
  ↳ combining multiple antigens in CAR-T de
  design can reduce antigen escape and
  improve efficacy, as seen in some preclinic
  models

- prime-and-kill circuit
  ↳ using synthetic receptors to prime
  CAR-T cells specifically in the tumour environ
  may circumvent antigen loss and limit o
  tumour effects

- Targeting Cancer stem cells
  ↳ GBM stem cells, critical for tumour recu
  ce, are being explored as potential targets
  for CAR-T therapy to prevent regrowth after
  treatment

- Adjuvant therapies
  ↳ radiation, chemo, oncolytic viruses and focu
  ultrasound are being investigated to induce
  antigen expression, enhance immune respo
  can improve CAR-T therapy

- Addressing immune exhaustion
  ↳ strategies like checkpoint inhibitors, cytok
  expression, and temporary CAR downregulation a
  explored to counter immune exhaustion, improving th

BRD4 inhibition boosts the therapeutic effects of epidermal growth factor receptor-targeted chimeric antigen receptor T-cells in glioblastoma:

- There is high potential for immunotherapy approaches in treating brain tumours including GBM.
- These approaches involve modifying T-cells, using tumour neo-antigen vaccines, oncolytic viruses and immune checkpoint inhibitors (ICIs). The focus is particularly on CAR-T cells targeting specific antigens like IL13RA2, EGFRvIII and HER2
- Using CAR-T cells targeting IL13RA2 antigen resulted in a complete regression of metastic GBM in one study on patient
- Immune responses mediated by CAR-T cells might be compromised due to increased expression of immunosuppressive molecules like PD-1 and IDO1 in the TME of GBM patients after EGFRvIII CART infusion.
- Super-enhancers are clusters of active enhancers regulating vital cancer related genes. BRD4 is a protein involved in activating these enhancers
- Blocking BRD4 impacts the expression of immune related genes and the release of cytokines. This inhibition potentially alters immune response against GBM cells
- Intial studies showed success in EGFR targeted CAR-T cells; however overtime tumour cells developed resistence

NOV 12

- using a compound like JQ1 to inhibit BRD4 activity disrupts the activation of enhancers induced by CAR-T cells. This disruption affects genes associated with immunosuppression.

Resource - molecular-therapy.com

Symptoms of GBM
- symptoms produced by a brain tumour largely depend on its location rather than its its specific pathological properties
- In GBM symptoms are due to both physical mass affecting the surrounding tissue and their direct infiltration of brain tissue
- common symptoms:
  - persistant headaches
  - nausea
  - vomiting
  - seizures

Neurological deficits due to tumour location
↳ Cerebellum lobe
  - weakness
  - tiredness
  - balance
  - cordination
↳ sensory cortex
  - numbness
  - difficulty identifying objects through tou

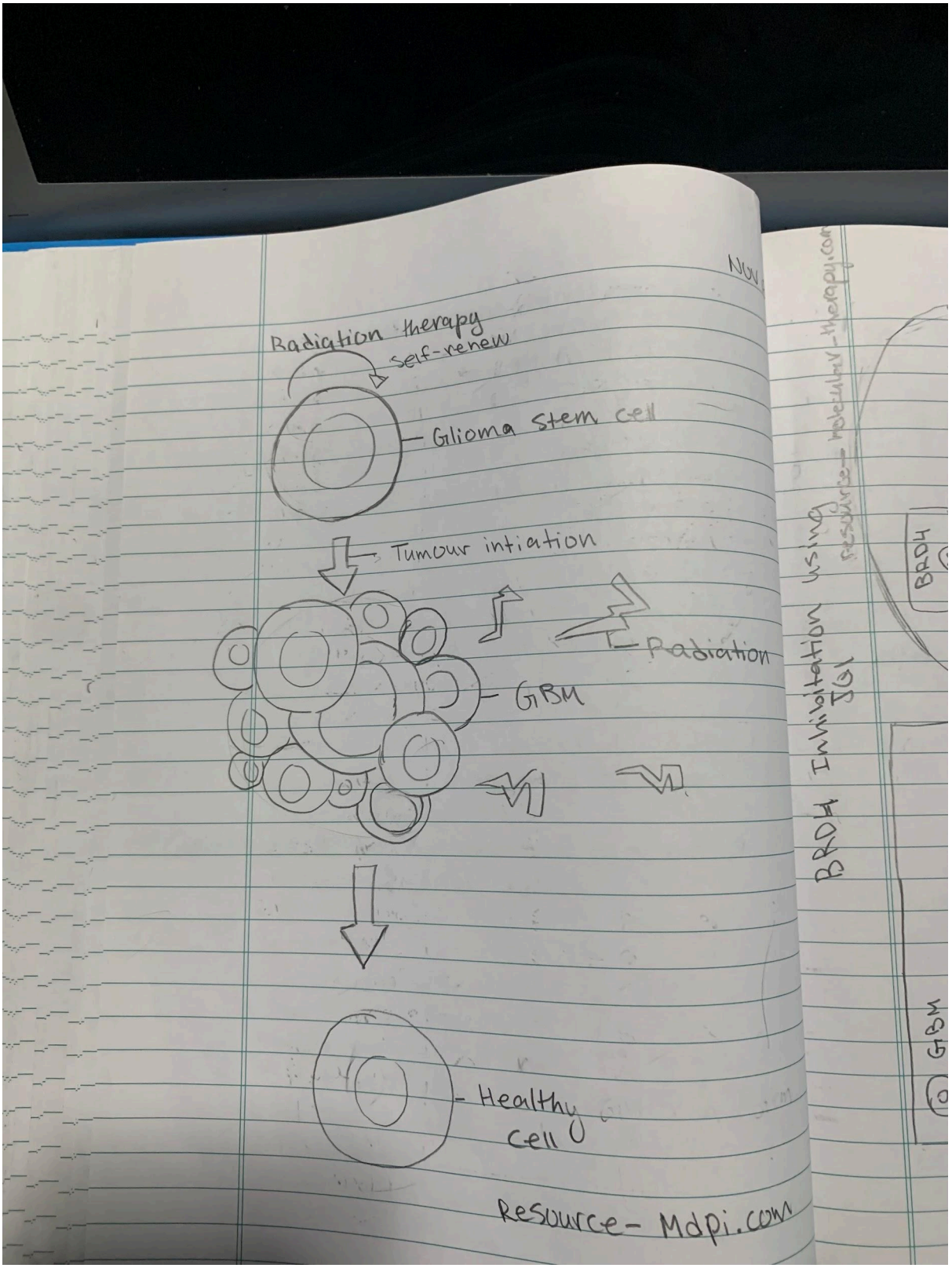
↳ language re
  - hinder spee
  - language
  - hearing
  - behavior
↳ frontal lo
  - reasoning
  - personalit
  - judgment
  - memory
  - planing
  - decision
  - mood
↳ occipital
  - partial b
  - comple

Resourse

NOV 12

- Language related regions / Temporal lobe
  - hinder speech
  - language comprehension
  - hearing
  - behavior
- Frontal lobe
  - reasoning
  - personality
  - judgment
  - memory
  - planing
  - decision making
  - mood
- Occipital
  - partial blindness
  - complete blindness

Resourse - "Glioblastoma (year of the zebra)
by Osmosis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BRD4 Inhibitation using JQ1

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Meeting with Chris

Immune cells often fight tumour cells
↳ evade immune cells
↳ loose growth regulation
↳ immune cells can recognise any protien cells

turn immuno-suppressive

microenvironment / physical barrier
↳ tumour
↳ microtumours
bone pain — multiple myloma
larger tumour "vaporized"
↳ but bigger have more difficulty
in effective
exhausted T-cell's
- check point inhibitor
↳ anti-PD-1, anti PD-L1

antigen presenting on class 2

pro inflamtory
quick to inflamtion - dea cells bect

Strategies

Enet immune-cells (pattern receptors)
↳ repersent molecules that are bad anti
↓ inflamotory
engineer kill switch
↳ limit singling peptide
- peptide MCH's
↳ enginered T-cells
- adaptive immune cells

- T-cells and B-cells take awhile for T-cells to expand
- Damage cells / APC's that signal
  ↳ antigen and Danger response

- immune cells (T-cells) recognize insuline cells as
- enhance danger signal
  ↳ bone marrow
  * Armed CAR-T cell
  ↳ atoerine - IL-2 cytockine, IL-7 - controls
  UOF C
  ↳ BCL BCMa against MM
  ↳ T-cell are artificaly en
- autoimmunity X
  ↳ kill switch
  - engineer CAR-T cells
  - come EGFRR key tiktknuzikane
  - Regulate CAR that controls T
    ↳ deal with exhaustion
  ↳ turn off for week, turn of 2 weeks

regulte CAR-T cells with drugs
- viper CAR
  ↳ griopivar - HIPtice C
  prodivance a protien
  ↳ polly protien viral protien

↳ expensive

allogenic - to another
autoplagus - to self
↳ 15% T-cell cross react
↳ MCH expression
- remove MCH - killer cell kill if MCH
- down regulte

- CTR - Microfetchers?

Strategies to enhance CAR-T therapy Dec 5

Locoregional administration
↳ intratumoural or intracavity injection
↳ targeted delivery
- increases precision and efficiency
↳ When delievered into or in close proximity it offers more advantages than systemic delivery
↳ reduces on-target-off tumour effects
↳ Optimized cytokine release (IL-2)
- increased CAR-T activity

Combination therapies
Check-point inhibitors
↳ combates immunosuppresive cells such as PD-L1

Oncolytic viruses
↳ contribute to inflammatory response
↳ proliferate inside tumour → easier activation for CAR-T cells

Enzymes
↳ enhance CAR-T infiltration
↳ clearer a "path" more more effect attack

Antibodies
↳ "nutralices" and blocks immunosuppresive cells, allowing for CAR-T cells to be more efficient

Nanoparticles
↳ enhance CAR-T delievery by infiltarting BBB
↳ offers protective encapsulation - shielding

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CAR-T cells from degradation
↳ provides sustained release (CAR-T ce
↳ minimizes off-target effects

Multi-targeting
↳ By using multiple antigens as targe
the strategy diminishes the likelihoo
of glioblastoma cells developing resista
and mutated variants
↳ solves issues of antigen escape
and tumour hetrogeneity

Engineering CAR constructs to inclu
or secrete active cytokines
↳ cytokines such interlukins play
a crucial role in regulating immune re
↳ promotes prolonged survival and
persistance, contributing to a more susta
anti-tumour response

Disrupting immunosuppressive immune
checkpoint molecules
↳ CAR-T cells can be enginered to
secrete antibodies against PD-L1
↳ genetic modification
- CRISPR/Cas9-mediated knockout of genes
associated with checkpoint molecules (PD-1/
↳ incorperating PD-1 ectodomain linked to
transmembrane and cytoplasmic domains
of CD28, aim to convert immunosuppressive
to co-stimulatory

Side effects
Cytokine re
↳ result of
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Dec 19

Side effects / risks

Cytokine release syndrome (CRS)
↳ result of rapid and massive activation of immune cells - T cells, following CAR-T infusion
↳ When CAR-T cells recognize and engage with target antigens on cancer cells, they become highly activated and release large amount of cytokines into the blood stream.
↳ In CRS, the excessive release of interleukins such as IL-6, IL-2 etc lead to a heightened immune response, causing flu-like symptoms = fever, fatigue, muscle aches, but can escalate to more sever manifestations including hypotension (low blood pressure), vascular leakage and multiorgan dysfunction
↳ Well immune responses are normal part of fighting off infections - the uncontrolled and excessive cytokine release in CRS can lead to systemic inflamation and even tissue damage and death
↳ Strategies include anti-cytokine therapies like Tocilizumab, an antibody that blocks IL-6 receptors
↳ there is also a dysregulation of the normal blance between pro-inflammatory and anti-inflammatory cytokines.
↳ Excessive pro-inflammatory cytokines contribute to symtomes such as fever, hypotension and organ damage
↳ anti-inflammatory signals may be released to counteract the overwhelming inflamation.

Neurotoxicity (ICANS)
↳ administering CAR-T cells directly into the brain amplifies the risk of neurotoxicity — often concurrent with CNS endothelial cell activation is impacted.
↳ this activation increases increases blood-brain barrier permeability, causing cytokine influx, seizures and cerebral edema
↳ CAR-T therapy can also induce immune effector-cell associated neurotoxicity (ICANS) — causing confusion and seizures
↳ Anakinra, an IL-1 receiptor blocking antibody is shown as a treatment for the associated side effects

On-target off-tumour
↳ CAR-T cells attack both cancer and healthy cells that express the antigen
↳ causing harm to healthy cells
↳ ex. HER2 a target overexpressed in 80% of cases of GBM also has a wide expression on healthy cells of the gastrointestinal tract, lungs and ovaries, among others

Off-target off-tumour
↳ It's the unintended attacks on and causing damage to healthy tissues

Dec 20

Safety measures / product contamination

↳ Depends on adherence to protocols

↳ Product contamination → complications like sepsis. The CAR-T cell product potentially carrying blood-borne pathogens shows the importance of strict adherence to handling and preparation guideline.

Resource — meeting with Kris

Feb 16

Molecular docking
~~MD~~ bioinformatics technique
used for drug delivery
- predicts the preferred/optimal complex/orientation of a molecule when docked to target

- I could find the different bind sites of on EGFR and its importancy to GBM pathogenicity

Aim - Identify potential targets for CAR design with EGFR structure
↳ also look into varients (EGFR)
- through the molecular interactions
I'll find the binding affinity between EGFR sites and the designed CAR constructs - using molecular docking to stimulate their interactions
↳ meeting with Mani