Scientific Rationale behind the Integration of Immuno-Therapy with other Anti-Cancer Treatment

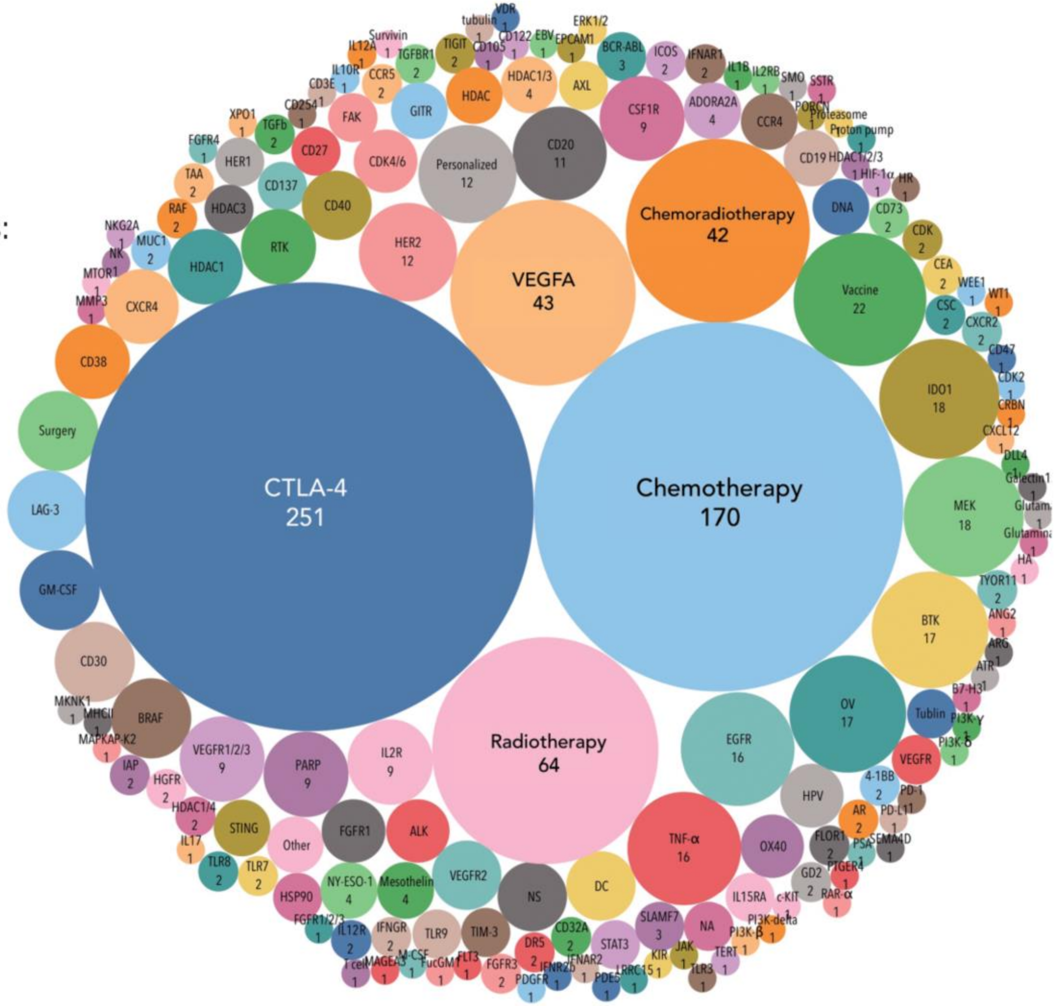
Conflicts of Interest

- Merck – Advisory boards/honoraria/trials
- BMS – trials
- AstraZeneca – Advisory boards, trials
- Roche – Trials
- Pfizer – Trials


T L N
I LOCK U
A C D
H K Y

Numbers of trials using common combo strategies:

1. Anti-CTLA-4 agents: 251
2. Chemotherapies: 170
3. Radiotherapies: 64
4. Anti-VEGFA agents: 43
5. Chemoradiotherapy combos: 42



From: Tang et al: Comprehensive analysis of the clinical immuno-oncology landscape Ann Oncol. 2017;29(1):84-91.

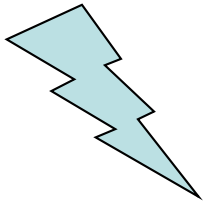
Scientific
Rationale

Competition
Capitalization

Combinations
In Clinic

Legal Contracts

Toxicities



Immune 101

B cells

Abilities to

genetically rearrange (generate diversity)

rapidly grow (respond to infection)

generate memory (immunity)

- Success of immune system
- B lymphoma

Cancer impact:

Ability to engineer monoclonal antibodies led to highly effective diagnostics (IHC), monoclonal abx treatments

T cells

- T cell receptor (Tak Mak 1983)
- T cell receptor gene rearrangement
- positive/negative selection largely in childhood
  - Thymus involutes with age
  - T cell lymphomas/leukemias largely in youth
- Understanding rationale for combinations = understanding the tools of the immune system, and a bit of history...

Evolution….

Salamanders can regenerate their limbs

Mammals can have adaptive immune system

Fetuses can have scarless wound healing

Adults can fight infections

Immune increased with decreasing stemness

Immune decreases with better tissue regeneration

T cell – tool development

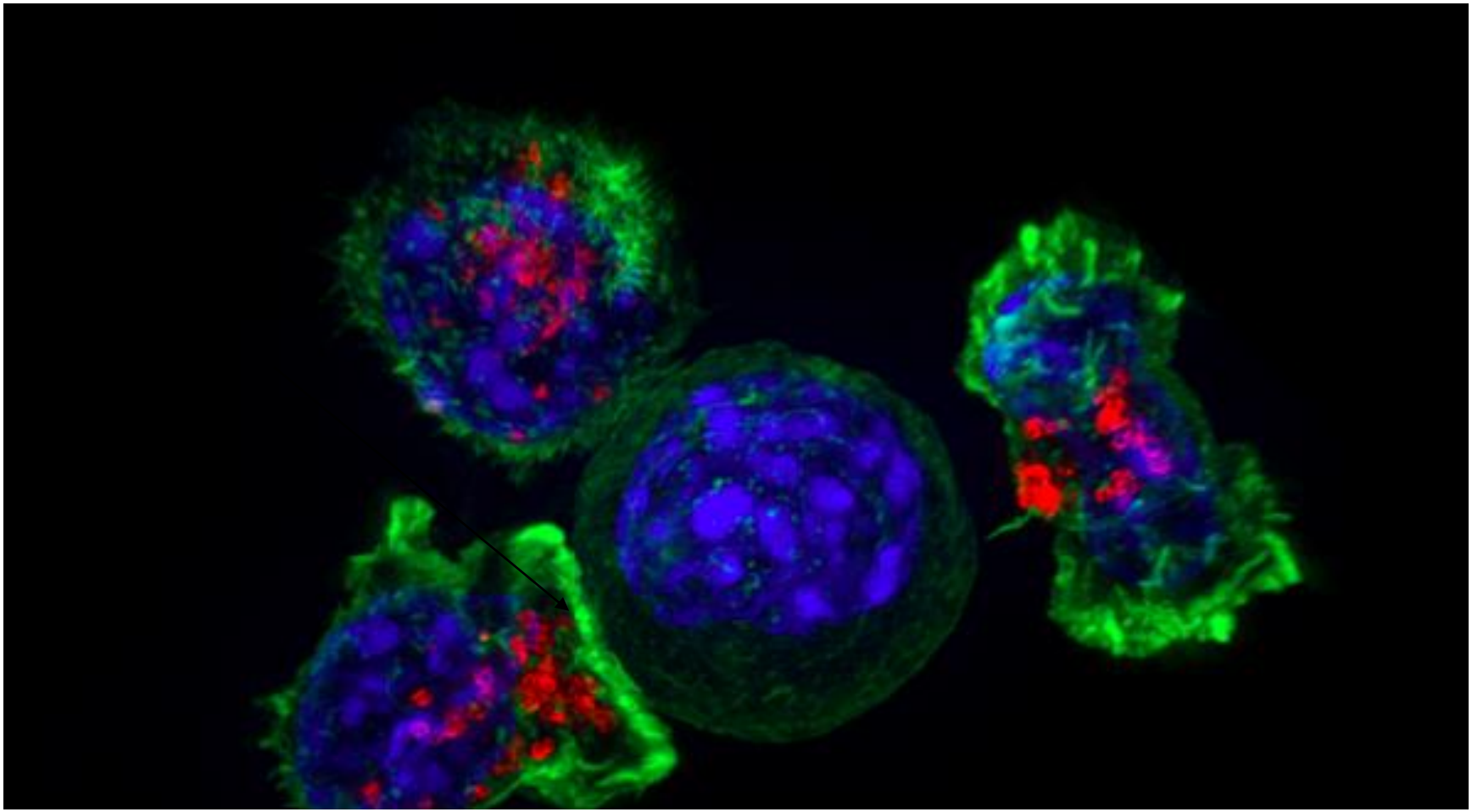
- T cells are “educated” in thymus
  - T cell receptors undergo recombination to generate a diverse range of receptors
  - In thymus, a variety of antigens are presented to the T cell using MHC 1 molecules
  - If the T cell recognizes MHC, then it can survive. If it recognizes MHC + self-antigen, then it’s killed.
    - Avoid development of self identifying T-cells
- “Naïve T cells”
- We all have a T cell reservoir – it decreases with age (profoundly at 65)

CD8 T –cell

- CD8+ T-cells circulate, reside in lymph nodes, reside along GI tract

- If a somatic cell
  - presents a peptide on MHC
  - there is costimulatory molecule
  - T cell receptor has this specificity, and can activate, then:

T cell getting ready to attack tumour cell –



Immune system Control

- Immune system is good, and T cells need to be diverse, but for real success of a powerful tool:
- Need to be able to turn off/control if chronic infection (tolerance), if need to grow/repair (Placental/Fetal growth/wound)
- Need to be able to direct where to go

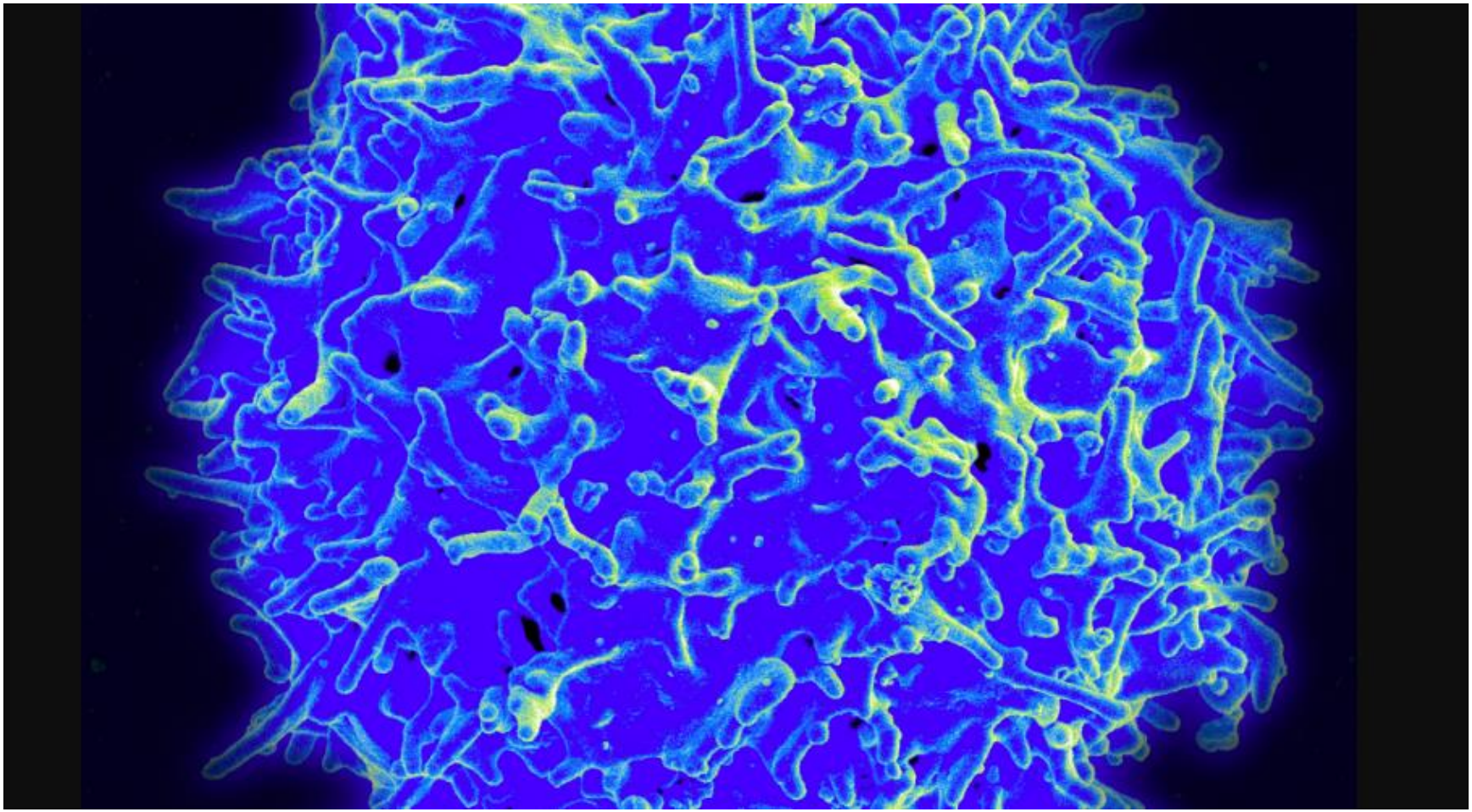
Cancers select for avoiding immune system…

- Cancers evolve to use these mechanisms of control to avoid immune system
  - “Hey, I’m just a chronic infection”
  - “Hey, I’m just wound healing/placental growth”
  - “Hey, don’t come over here. Stay in your lane T-cell”
  - “Hey, I’m just a regular Joe cell”
- Treatment tries to block the cancers from using these programs

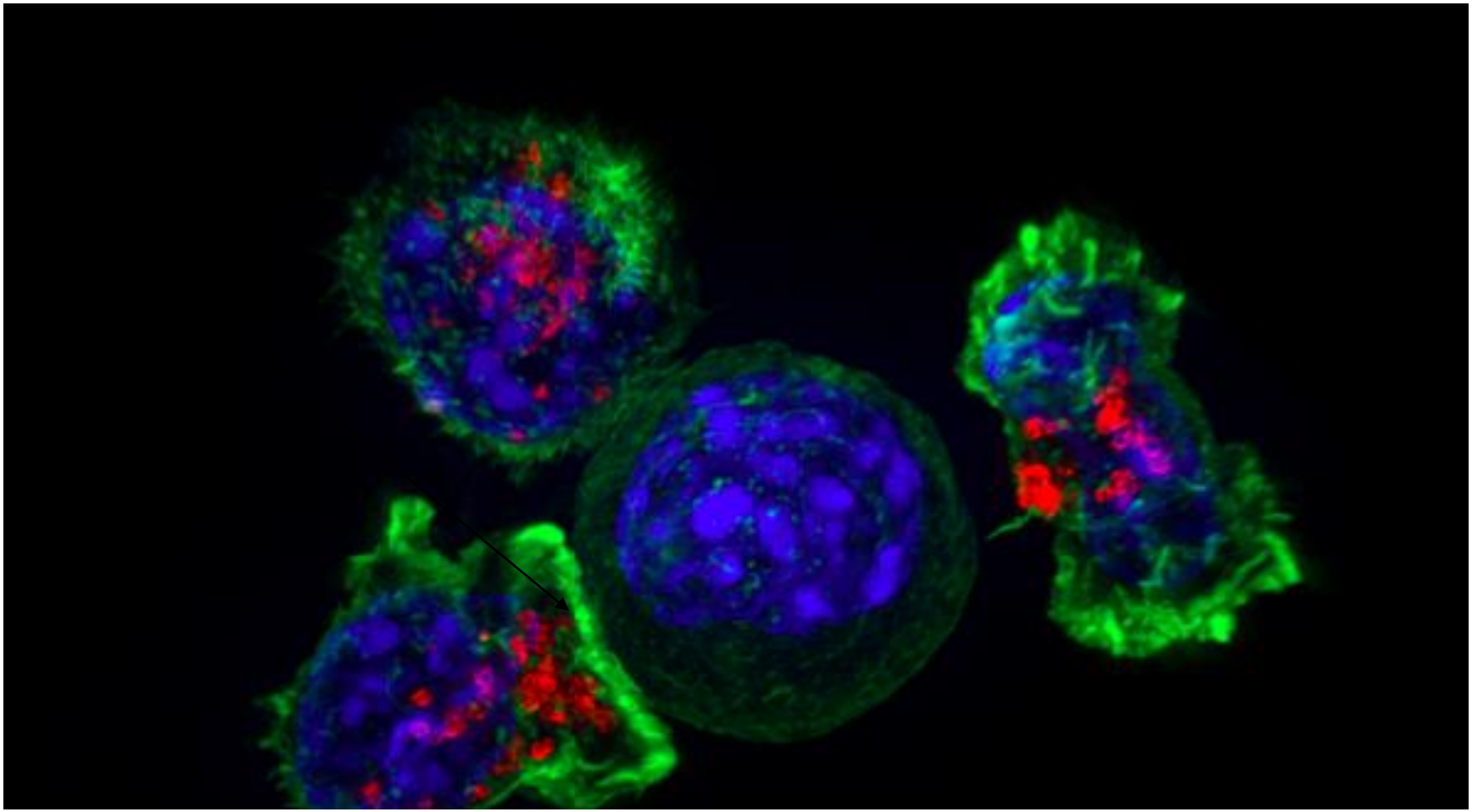
Treatment goals:

- Increase the effectiveness of the immune system
- Decrease the effectiveness of the tumour defenses

T-cell (NIH image gallery)



T cell getting ready to attack tumour cell – but it needs to be there!



How do T-cells and Tumour cells come in contact?

- Chemokines lead to positive attraction (CXCL, CXCR, CCL etc.)
  - Typically move from low concentration to high concentration

The body is big, and the what makes a T cells want to go somewhere – how can we make it?

1. More T cells circulating/Active from Tumour Draining Lymph Node (CTLA-4 agonist, other checkpoint agonists)
2. Increase signals from cancer/stromal cells to recruit

Attract more T cells…

"Hey, there is a lot of DNA in the cytoplasm – maybe it's a virus – activate IFN pathways and get some T cells in Here!" ---- *Somatic cell infected by virus.*

"Hey, we've got some cells dying over here – this looks like an infective pattern – lets send off some Danger signals (Danger Associated Molecular Patterns) – better get some inflammatory cells and immune cells over here!" -- *Cancer cell attacked by chemotherapy or radiation and dying.*

“Hey, there is a lot of DNA in the cytoplasm – maybe it’s a virus – activate IFN pathways and get some T cells in Here!”

- Some drugs activate “Stimulator of Interferon Genes” – STING
- Likely through increased cytosolic DNA
- Rationale for combination trials (underway)
  - PARP-inhibitors
  - CDK4/6 inhibitors

Hey, we've got some cells dying over here – they are sending off some Danger signals (Danger Associated Molecular Patterns) – better get some inflammatory cells and immune cells over here!
Dendritic cells, start showing these guys to the T-cells!

- Part of "Immunogenic cell death"
- Known response to certain chemotherapy agents (but some very drug specific anomalies – i.e. cisplatin and oxaliplatin)
- Known response to radiation
- Rationale for combination chemotherapy and immune therapy
- Rationale for combination radiation (chemoradiation) and immune therapy

Immunogenic Cell Death Video

https://www.youtube.com/watch?v=A0nuQpiU5N4

“Immunogenic Cell Death”

- Radiation, and SOME chemotherapy
- Causes changes in cell, dying cells release “FIND ME and EAT ME”
- Recruits T-cells/inflammatory response

Laboratory Evidence

- Certain chemotherapies don’t work if immune system is ablated
- Work better if chemotherapy combined with PD-1 therapy
- Vaccination experiments

Clinical Implications

- Chemo/radiation cause influx of immune cells (if cells undergoing ICD)
- PD1/PDL1 inhibitors increase anti tumour lymphocyte responses and augment Immunogenic cell death
- Unclear how long these conditions occur after chemo
- Rationale for treatment within 6 wks of chemorads
- Unlikely any meaningful difference if PD1 is given the day or two after chemotherapy
- Response is dependent (in part) on the chemotherapy or radiation actually working – don’t dose reduce needlessly for tolerance.

T-cells get there…

"Ok, I've released some signals to try to recruit some T cells. I hope someone hears the signal!"

"Ok, nice job. Maybe you should make sure the T-cells are active and circulating from the lymph node with some CTLA-4. Also, we should try to make the path as easy as possible, and there will be other cells trying to keep them away with other signals/physical barriers! Let's deal with those!"

Wound healing, Vasculature, and VEGF

- In wound healing, normal to move from hemostasis, to inflammatory, to healing/scarring
- As VEGF increases, and tissue/vasculature is regrowing, would like to be tolerant
- Tumour vasculature is ++tortuous, and Interstitial pressure is high (tumour are hard)
  - T cells “I’ll try to move there, but it’s hard! And when I get there, it’s going to be in a regenerative state with immunosuppression because of all this VEGF!”

Wound healing/vasculature/VEGF

- Anti-VEGF agents
  - Increase CD8 T-cell infiltration, reduce “anti-T-cell” infiltration
- Rationale for combination therapies:
  - Bevacizumab/Atezolizumab (NSCLC, Renal)
  - Pembrolizumab/Axitinib (Renal cell)
  - Pembrolizumab/Lenvatinib (Endometrial)
  - Multiple other combinations

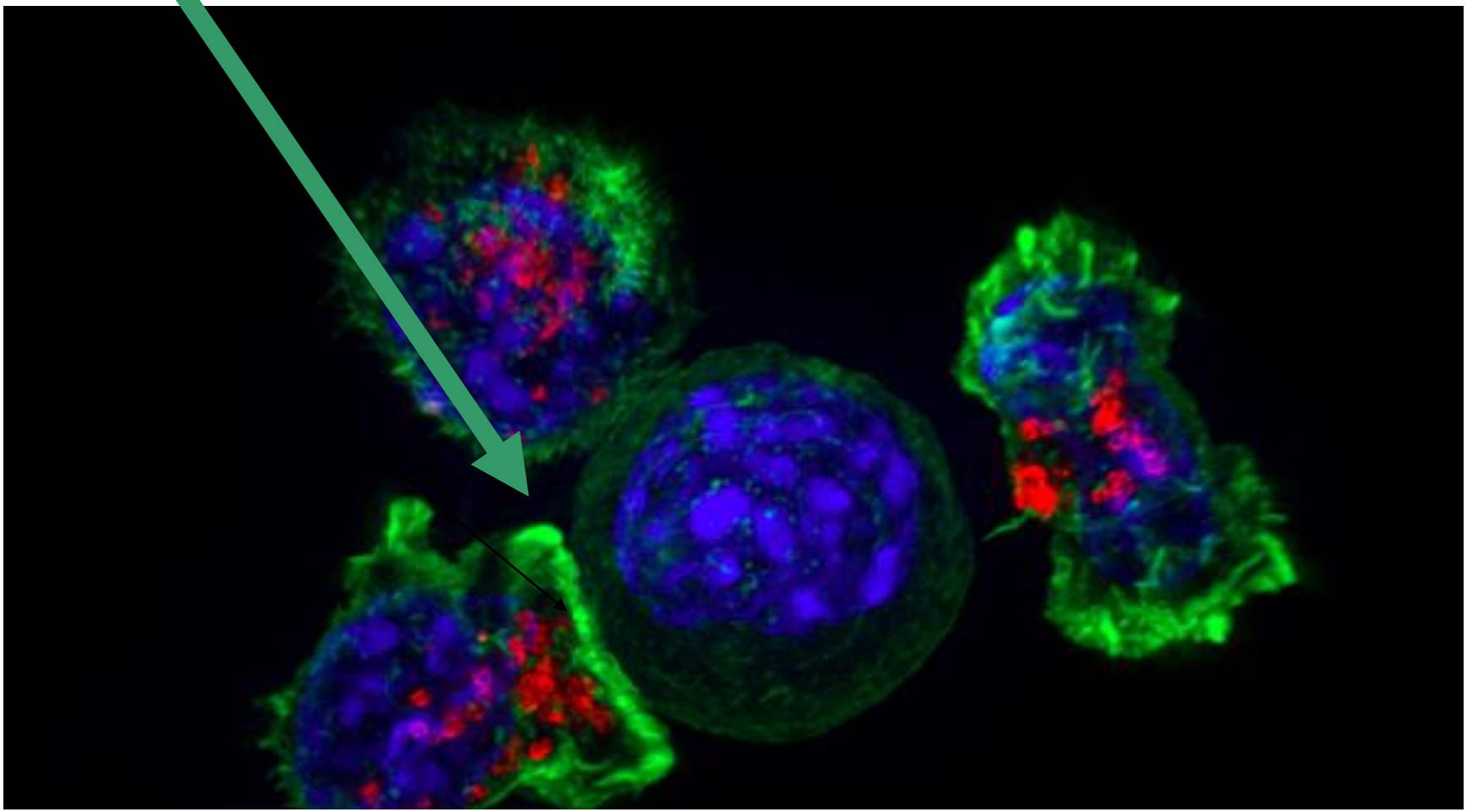
Clinical Implications

- Combination(s) of multiple drugs in future (VEGF+CTLA4+PD1+chemo etc.)
- We need to know (but don’t) who responds to maximal circulation and PD-1, and who needs anti-angiogenic therapy!
- Effect (if using with TKI) is likely dependent on maximizing effective VEGF suppression – i.e. if using axitinib, dose appropriately with escalations/reductions

So far

- T cells getting activated from TDLN and other reservoirs – CTLA-4
- T cells getting signal to come to tumour and/or presented antigen – chemo/radiation/PARP-I/CDK inhibitors etc.
- T cells not being ‘suppressed’ by angiogenic bullies, and being able to move through tissue – VEGF inhibitors

T cell getting ready to attack tumour cell



T cell is in vicinity, how does cancer cell avoid it now?

- Turn on/upregulate Programmed Death Ligand

  "Nothing to see here. I'm not a cancer cell, I'm a self cell with just a little infection, Shoo Shoo."

- Treat with anti-PD1/PDL1 axis therapies

What else?

"*Hey T-cell, I'm just a regular somatic cell. See my proteins! None of them are too bad or antigenic. No need to kill me*" – suspicious cancer cell disguising itself as normal

"*Hmmm, how do I know you aren't a bad cell with just pretty normal looking proteins?*" – Tcell – big and dumb

How do cancer cell avoid showing their true stripes?

- Present peptides that are so similar to self that T cell doesn't recognize as foreign

  Cancers with low "antigenic" burden, not mutated.

- Not present antigen at all

  lose MHC

- Don't give co-stimulatory signal

  Lose B2Microglobulin

Antigen burden:

- Number of neoantigens
  - In general more mutations = more potential neoantigens
- Immunogenicity of neoantigens
  - Frame shift mutations/indels – more antigenic
    - You can reed this. Yoc anr eedt his. Copy copy number number variations variations not not
  - Point mutations/Termination – less antigenic
- Particularly immunogenic
  - Viral antigens (HBV, MerkelCV,HPV)
  - Endogenous Retroviruses (our caveman genome)

Dealing with low antigen diversity/increasing immunogenicity of tumour

- Epigenetic modification

  Increase endogenous retrovirus protein expression or other ‘antigenic’ proteins

- Increase number of mutations

  Increase genomic instability (PARP/Platinums) in the hopes that one of the mutations will lead to an increased change of immunogenicity (and you won’t also increase a mutation that’s bad…)

?Neoantigen load

- highTMB (melanoma) has 100's predicted neoantigens
- Only 2-5 are capable of inducing an immunogenic response
  - number is higher with healthy patient T cells- unclear why
- Rationale for combination neoantigen vaccines

“Ok, so you’ve got an immunogenic peptide. I’m going to just not present it to you. I’m going to downregulate my MHC or mutate it so that you won’t ever see it”

“Ok tough guy, if you’ve just repressed your MHC we will encourage it – chemotherapy, epigenetic modifiers etc. may increase MHC expression”

“And what if I just lose MHC altogether, or Beta two microglobulin”

“well then, I guess my regular T cells with T cell receptors won’t work very well. But maybe if I have a T cell that has a chimeric antigen receptor I can still find and kill you…”

Other rationales for combinations that don't involve synergy/antigens etc.

- Cells that don't respond to immunotherapy might respond to chemotherapy/radiation/surgery
  - Avoid the initial "dip"/crossing of curves
  - ?avoid "Hyperprogression"

- Likelihood of resistance is proportional to tumour burden – combination(s) reduce likelihood of resistance generation
  - Rationale for both combination strategies of chemo and multi-site radiation

- Tumours in body may have high Tumour infiltrating lymphocytes that can be more easily activated when they are still in patient
  - Rationale for neoadjuvant approaches

How do tumours stop the T-cells from getting there?

- Recruit suppressive cells through chemokines/don’t release T cell chemokines

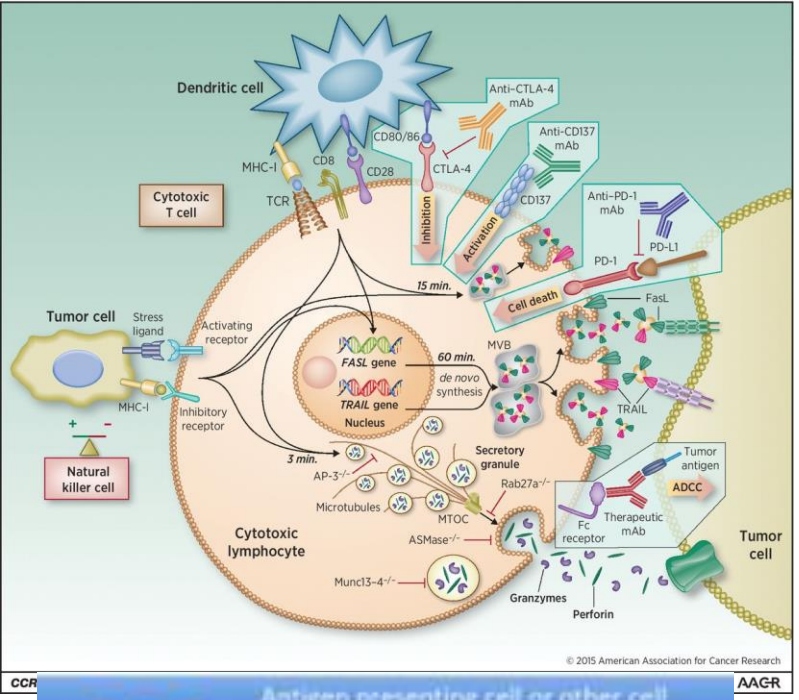
- Make vasculature and interstitium difficult to navigate through (abnormal vasculature, high interstitial pressures)

Cytosolic fragments of DNA lead to immune response

“Hey, is there a virus? Something is wrong, we better activate the STING pathway to get an immune response!”

STING – stimulator of interferon genes

Rationale for combination with : PARP-Inhibitors; CDK4/6 inhibitors


Dendritic cell
Anti-CTLA-4 mAb
CD80/86
CTLA-4
MHC-I
CD8
CD28
TCR
Cytotoxic T cell
Inhibition
Activation
Anti-CD137 mAb
CD137
Anti-PD-1 mAb
PD-L1
PD-1
15 min.
Cell death
FasL
Tumor cell
Stress ligand
Activating receptor
MHC-I
Inhibitory receptor
FASL gene
TRAIL gene
Nucleus
60 min.
de novo synthesis
MVB
TRAIL
3 min.
Secretory granule
AP-3-/-
Microtubules
MTOC
Rab27a-/-
ASMase-/-
Munc13-4-/-
Tumor antigen
ADCC
Fc receptor
Therapeutic mAb
Granzymes
Perforin
Tumor cell
Natural killer cell
Cytotoxic lymphocyte
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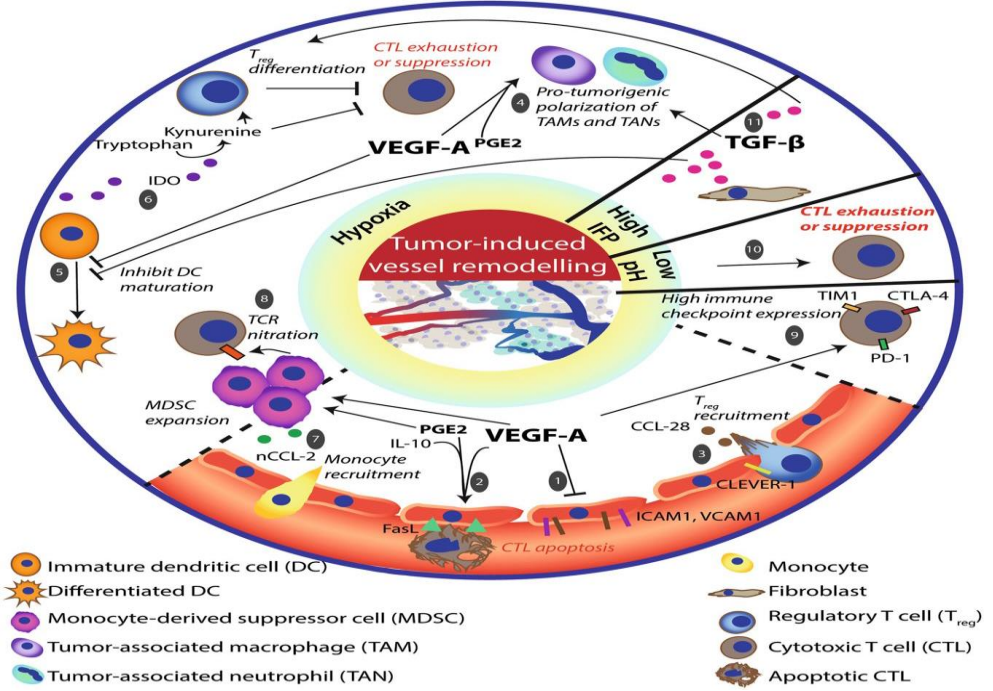
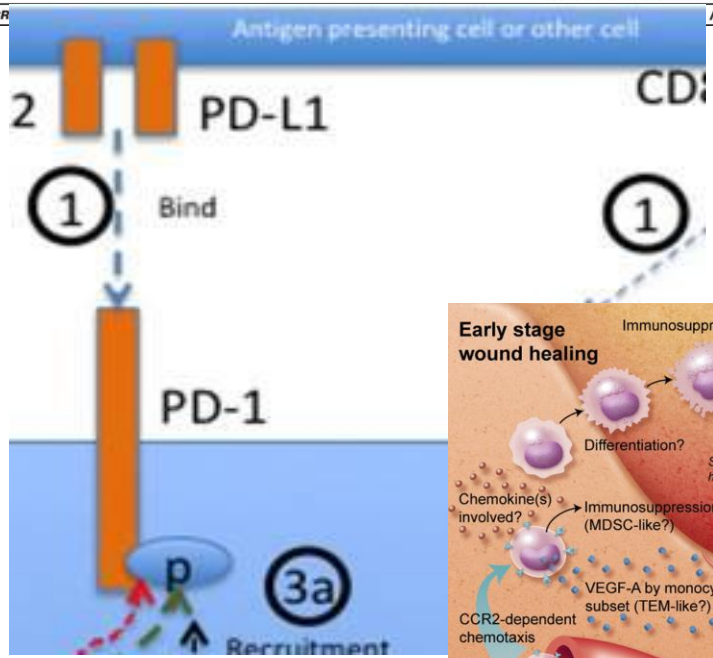
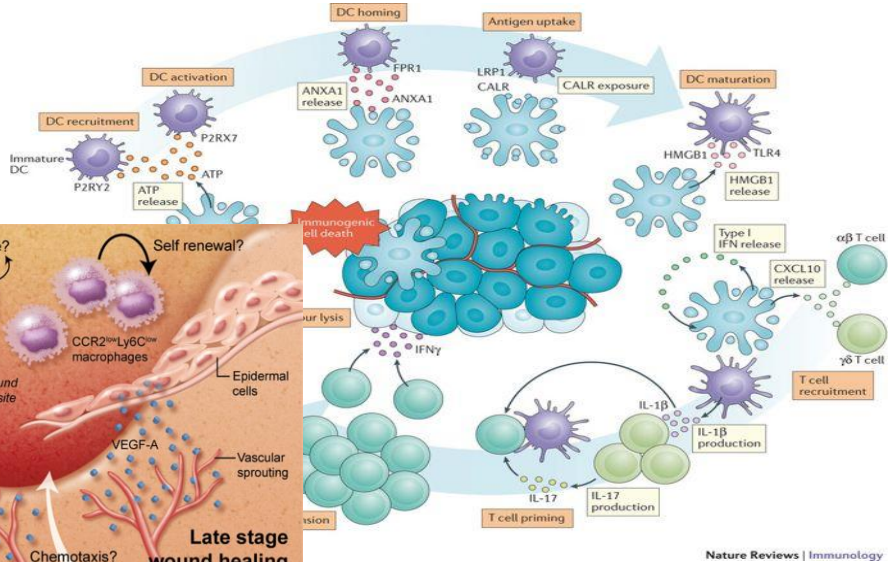
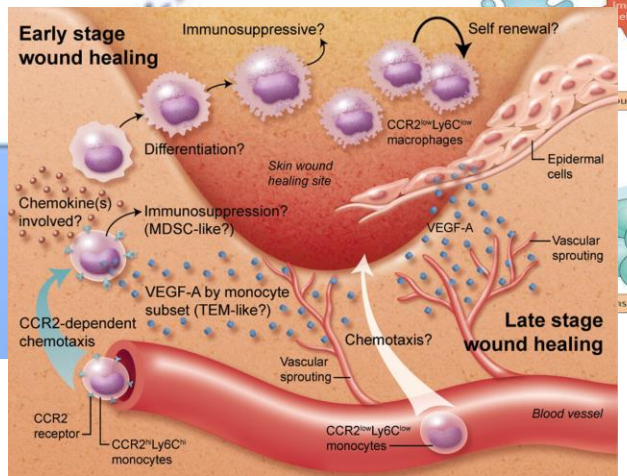

Treg differentiation
CTL exhaustion or suppression
Kynurenine
Tryptophan
VEGF-A
PGE2
Pro-tumorigenic polarization of TAMs and TANs
TGF-β
IDO
Hypoxia
Tumor-induced vessel remodelling
High IFP
Low pH
CTL exhaustion or suppression
Inhibit DC maturation
High immune checkpoint expression
TIM1
CTLA-4
PD-1
TCR nitration
MDSC expansion
Treg recruitment
CCL-28
PGE2
IL-10
VEGF-A
nCCL-2
Monocyte recruitment
CLEVER-1
FasL
ICAM1, VCAM1
CTL apoptosis
Immature dendritic cell (DC)
Differentiated DC
Monocyte-derived suppressor cell (MDSC)
Tumor-associated macrophage (TAM)
Tumor-associated neutrophil (TAN)
Monocyte
Fibroblast
Regulatory T cell (Treg)
Cytotoxic T cell (CTL)
Apoptotic CTL


Antigen presenting cell or other cell
PD-L1
Bind
PD-1
3a
Recruitment


DC homing
Antigen uptake
DC activation
FPR1
ANXA1 release
ANXA1
LRP1
CALR
CALR exposure
DC maturation
DC recruitment
P2RX7
Immature DC
P2RY2
ATP
ATP release
HMGB1
TLR4
HMGB1 release
Immunogenic cell death
Type I IFN release
CXCL10 release
αβ T cell
γδ T cell
IFNγ
T cell recruitment
IL-1β
IL-1β production
IL-17
IL-17 production
T cell priming
Nature Reviews | Immunology

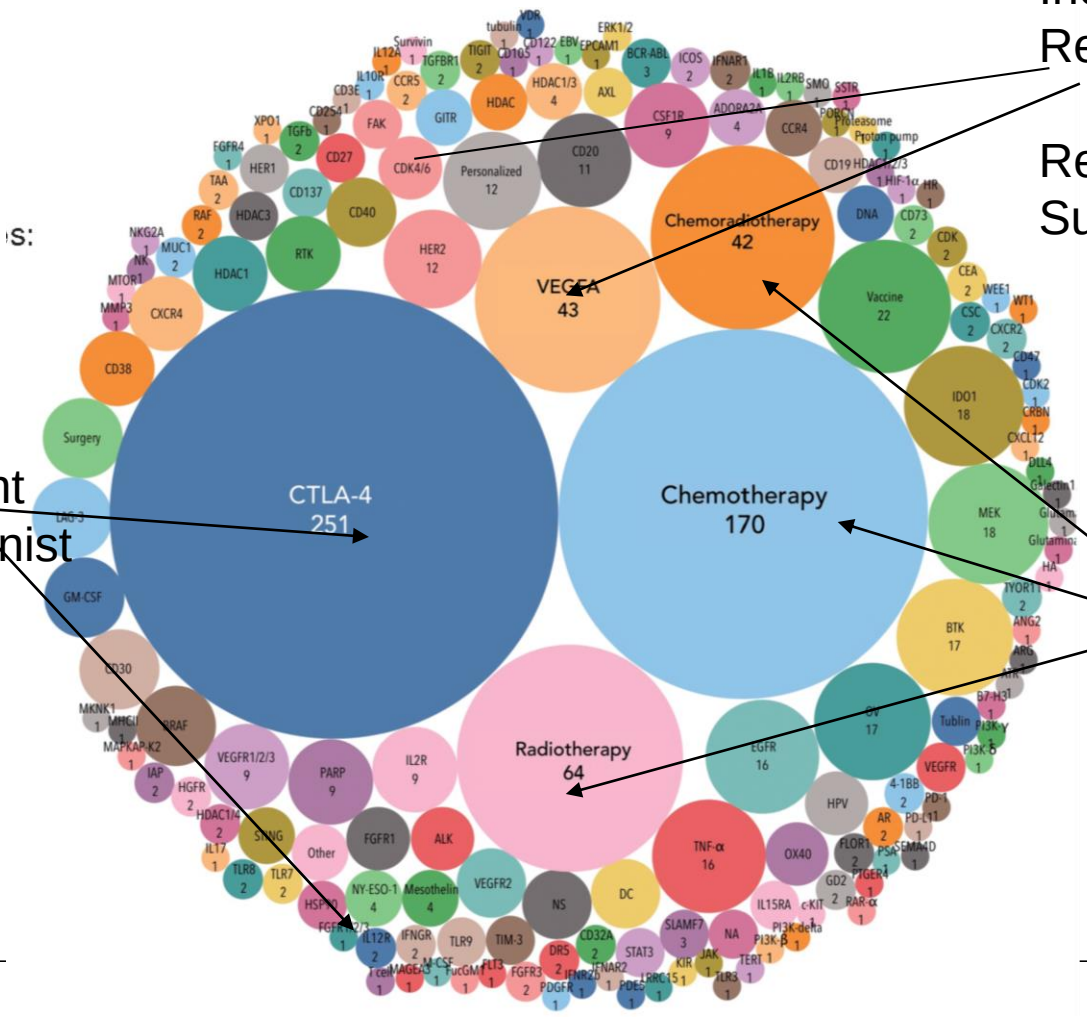

Early stage wound healing
Immunosuppressive?
Self renewal?
Differentiation?
CCR2lowLy6Clow macrophages
Skin wound healing site
Epidermal cells
Chemokine(s) involved?
Immunosuppression? (MDSC-like?)
VEGF-A
Vascular sprouting
VEGF-A by monocyte subset (TEM-like?)
CCR2-dependent chemotaxis
Chemotaxis?
Late stage wound healing
Vascular sprouting
CCR2 receptor
CCR2hiLy6Chi monocytes
CCR2lowLy6Clow monocytes
Blood vessel

Increase T-cell Recruitment

Reduce Immuno Suppressive env

Multi-checkpoint Agonist/antagonist

Immunogenic Cell death/ synergy



No combination talk is complete without….

“Microbiome”

- 70% of T-cells are in the gut
- Gut microbiome influences T-cell immunity
- Gut (and other) microbiome influences innate and adaptive immunity.

- Why? (unknown)
  - ?training/keeping T cell’s diverse?
  - ?Commensal bacteria and humans have co-evolved?

Mechanism Unknown - but

- Recent abx associated with worse outcomes
- Probiotics associated with less diversity and worse outcomes (?why were they on probiotics)
- ?poor diet associated with less diversity and worse outcomes?

Rationale for

- Diet studies
- Fecal microbiota transplant studies
- “Poop pill studies”
- No clinical indication yet

My rationale for:

judicious use of antibiotics (i.e. not every UTI needs cipro..)

Less likely to use prophylactic antibiotics

Avoid probiotics

Summary

- Our immune system has evolved largely to get rid of viruses and other infections
- Body has many needs to be able to control immune system both at specific times in development and in specific niches.
- Cancers may hijack these control systems
- Therapies act to restore the balance

If: Then

If T cell is there, recognizes cell as foreign, and has intact machinery, then can eradicate tumour

If/Then

- If T cell is present, but is turned off by PD-L1 on tumour cell then turn back on (anti-PD1)

If/Then

If T cell is present, but Tumour cell doesn't present antigen THEN increase presentation or work around using epigenetics OR CAR-T therapy.

If/Then

If T cells are present but doesn't recognize MHC/antigen complex as foreign THEN

Increase antigen expression (chemotherapy/radiation/epigenetics)

If/Then

If T cells are present, and could recognize antigen that is presented, but are “turned off by stromal elements”, then:

Block stromal elements or reduce their attraction to tumour (anti-VEGF, chemokine inhibitors)

If/Then

If T cells aren’t even present:

Increase activation/circulation T cells (CTLA4)

Increase attraction from tumour (activate STING or activate immunogenic cell death)

Reduce barriers to T-cells (anti-VEGF)

If/Then

- If T cells are not present, or there is not enough diversity, or they ‘don’t work well’

Train them up with some microbiome replacement therapy.

Next Steps

- Need better correlative to predict who needs what

- Need early assessments to determine who it is working in and who needs something extra.

Other issues

- We don’t know a lot
- PD-L1/PD-1 have non-canonical effects
- PD-L1 exosomes