Immunotherapy in Cancer

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- Evolution of cancer and its relationship to the immune system
- How immune cells (T cells) work
- Current cancer immunotherapies and application to Prostate cancer
  - Checkpoint blockade
  - Adoptive Cell Transfer (ACT)
  - CAR-T cells
  - Making cold tumors hot

Definitions/Explanations

- Genes code for proteins and proteins are responsible for most of what goes on in a cell
- We often use the name of the gene and the protein interchangeably
- A protein can be inside a cell or on the cell surface
- Cells can “talk” to each other when a protein on one cell binds specifically to a “receptor” protein on another cell, leading to a signal being received by the second cell.
- A mutation in a gene is change in the genetic code that leads to change in its protein. This can have many different affects on the protein
- These mutation happen during cell division when the cell has to make a new copy of all of its genes – the mutations are random.

How do cancer cells arise?

- Exposure to some insult
  - Chemicals – environmental, smoking, food, work (asbestos)
  - Viruses like HPV
  - Chronic inflammation
- These insults lead to mutations in genes. The transformation of a normal cell to a cancerous one begins when the mutation occurs in a gene that control cell division (“driver mutations”)
  - Cell division is very tightly controlled by many different genes
  - Mutations can inactivate genes that prevent cell division (P53) or permanently activate genes that support cell division (cMyc, BRAF)
  - Need at least 3 or 4 of these mutations to transform a normal cell to a cancerous state (redundant regulation)

How do cancer cells arise?

- The transformation to cancer progresses when mutations in DNA repair genes (proteins) happens
  - Mistakes happen during replication of the genome when cells are dividing but very rare
  - Cancer cells divide a lot so more mistakes can happen
  - In normal cells, lots of genes are involved in repairing these mistakes
  - But these repair genes can also be mutated (inactivated) leading to accumulation of even more mutations
- Result of mutations
  - Can kill the cancer cell
  - Can enhance survival and aggressiveness

Cancer and the immune system

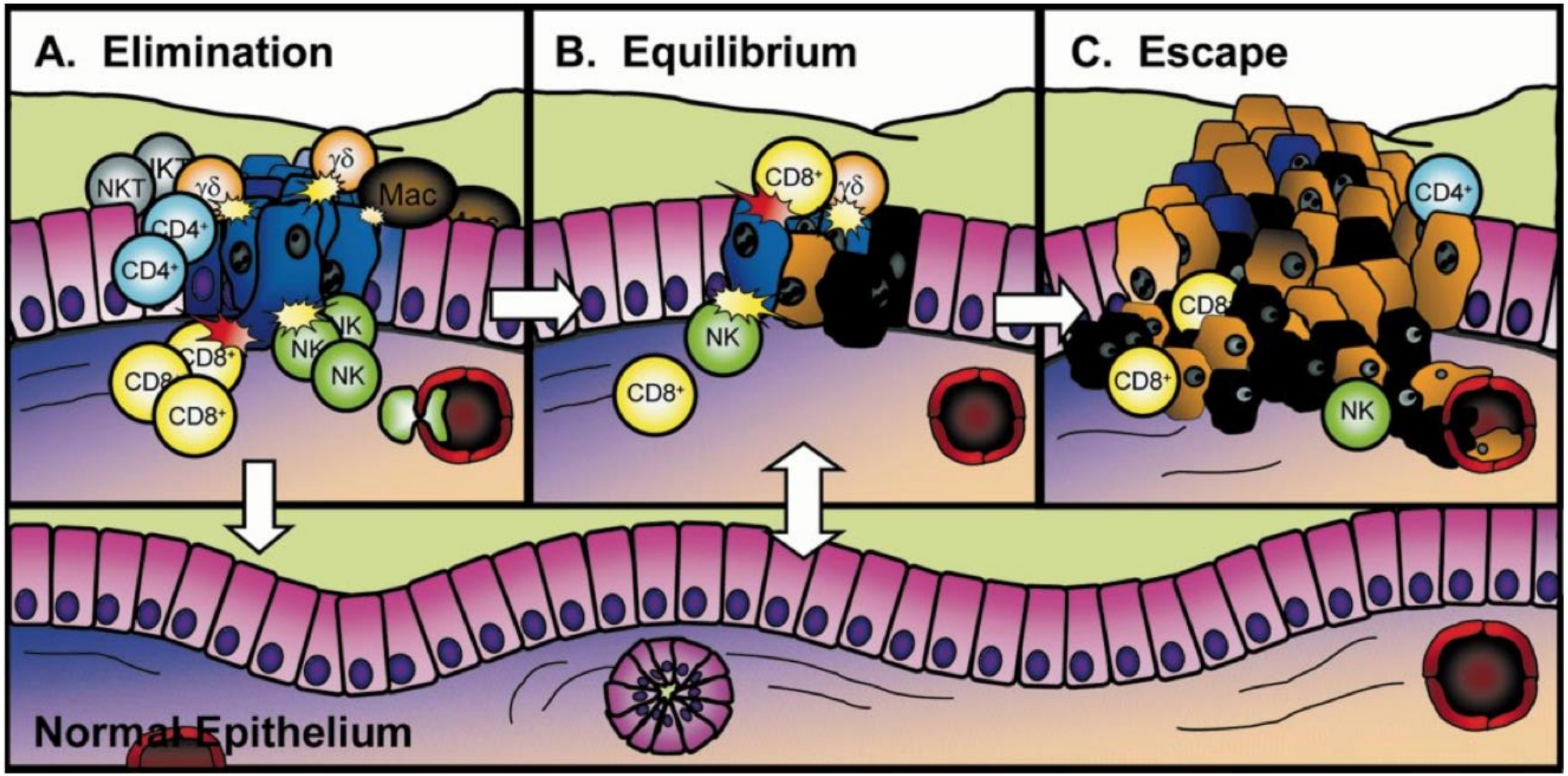


Figure 3 The three Es of cancer immunoediting: elimination, equilibrium, and escape. As indicated by the arrows, the immune system may eliminate the tumor in either the elimination or equilibrium phases, returning the tissue to normal.

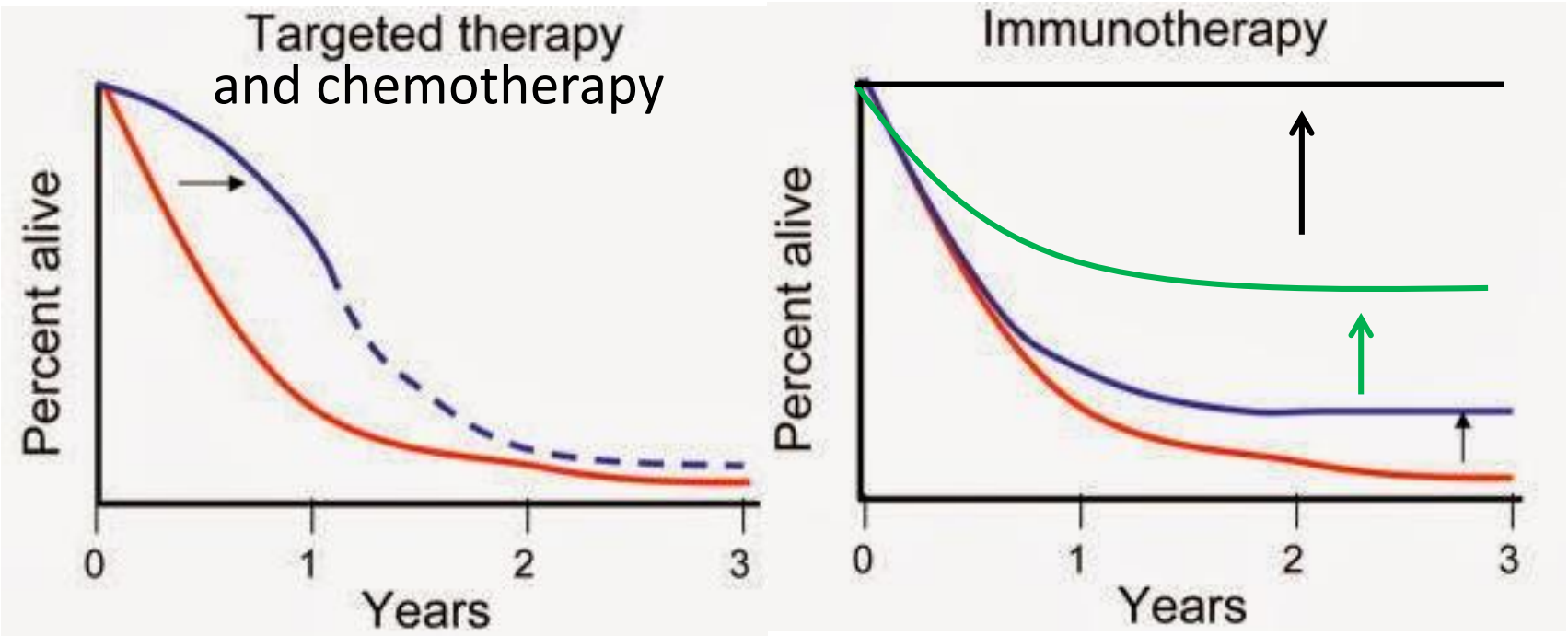
Robert D. Schreiber
Annual Review of Immunology, 2004

Modalities of cancer treatment

- Surgery – potentially curative
- Radiation – potentially curative
- Chemotherapy –Target cell division, but hard on normal cells that are dividing (stem cells, immune cells etc.)
  - Not curative
- Inhibitors of normal and mutated genes that drive cancer cells (Androgen receptor, estrogen receptor, HER2Neu, B-Raff, etc.)
  - Less side effects but also not curative
- Immunotherapy – engage the immune system to kill cancer cells. Can be highly specific, but auto-immune effects can arise.
  - In general, the only approach that can provide a cure in stage 4 metastatic disease.

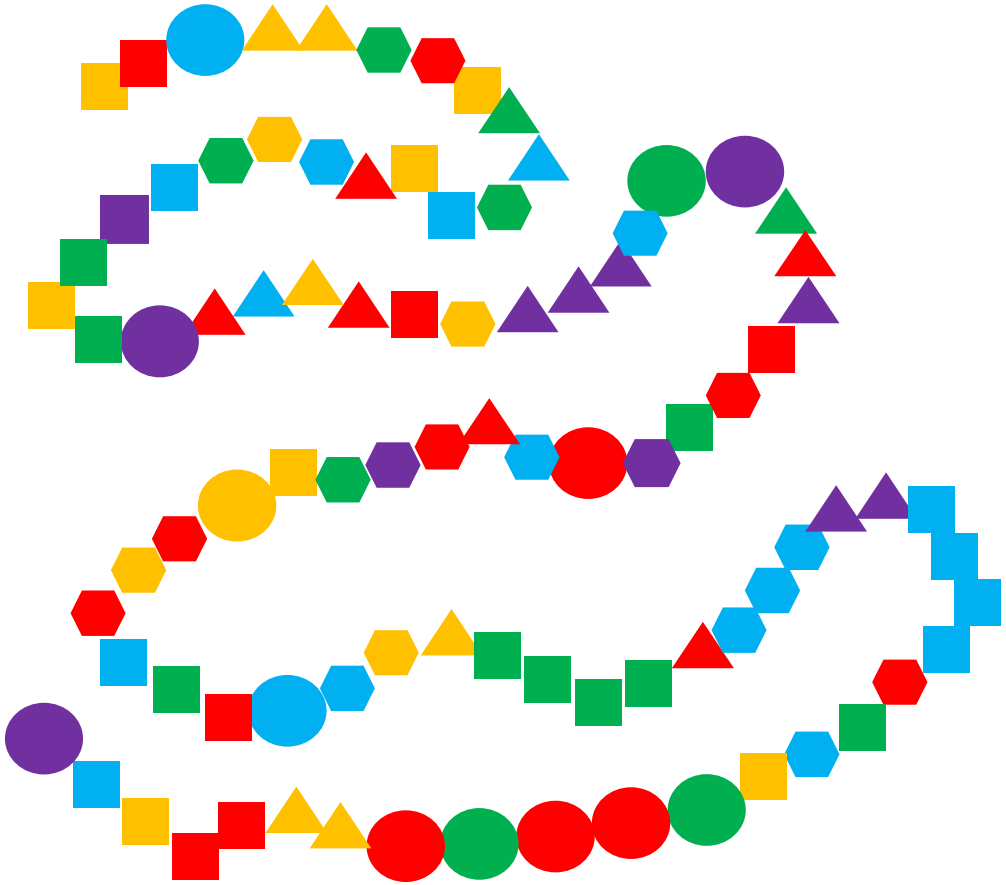
Benefits of immunotherapy

"shifting the curve"



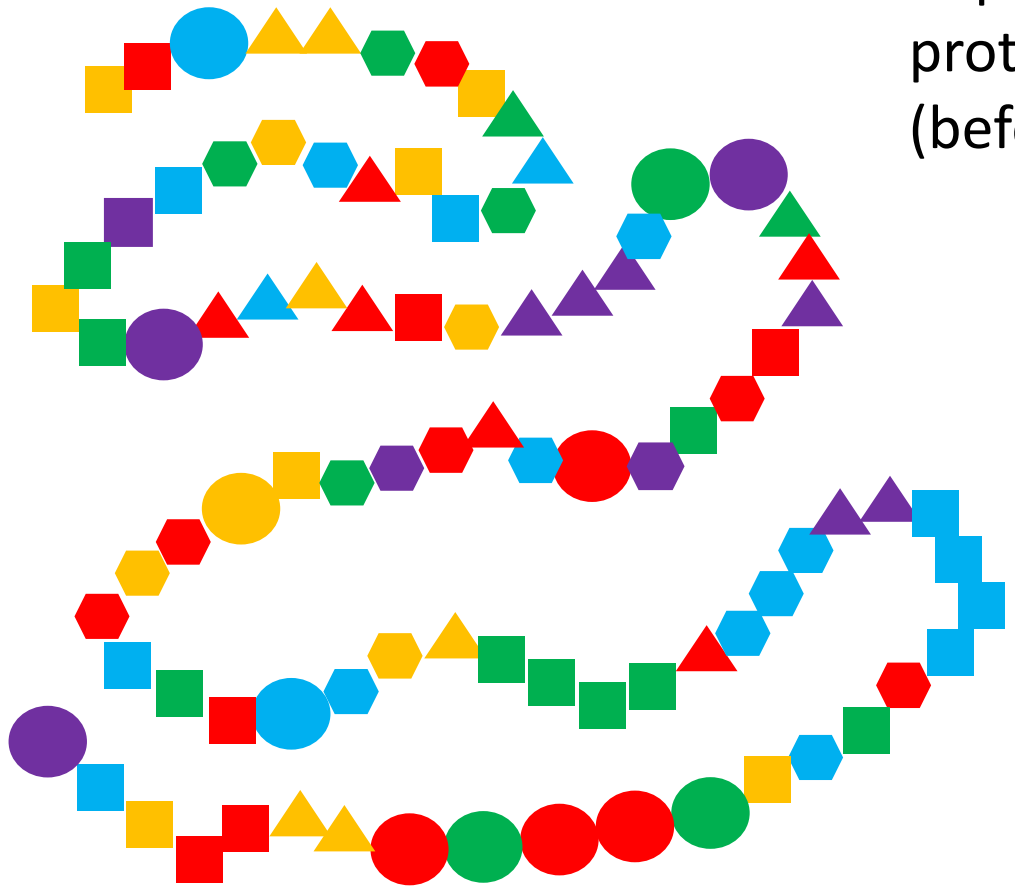
How do T cells recognize tumor cells?

What is a protein?

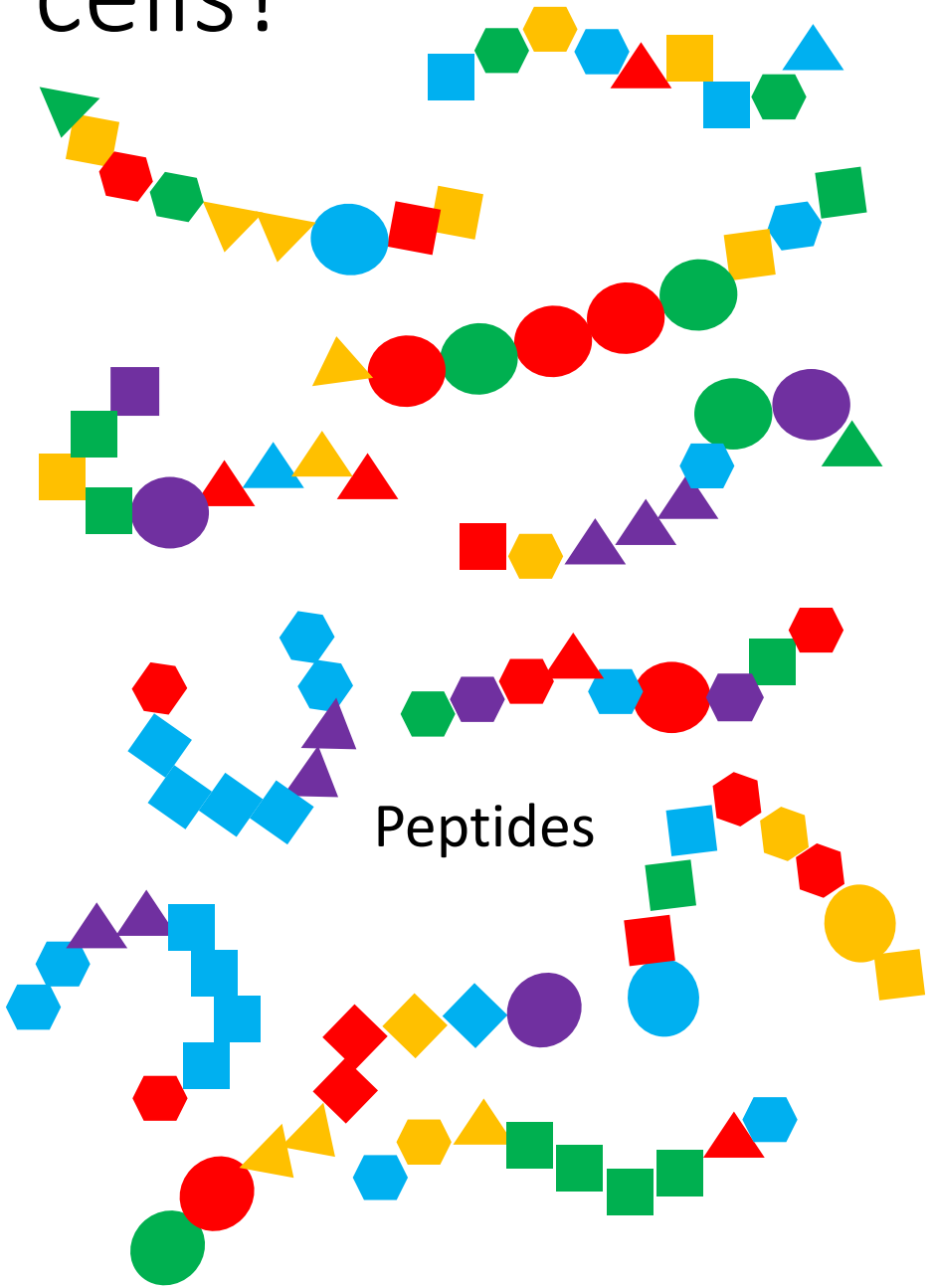


- Proteins are made of a linear array of about 20 different amino acids
- These are represented here by a combination of 5 colors and 4 shapes
- Each gene codes for a unique sequence of amino acids that can very in size from less than 100 to many 1000s
- The linear array of amino acids spontaneously folds into a shape that is determined by the sequence.

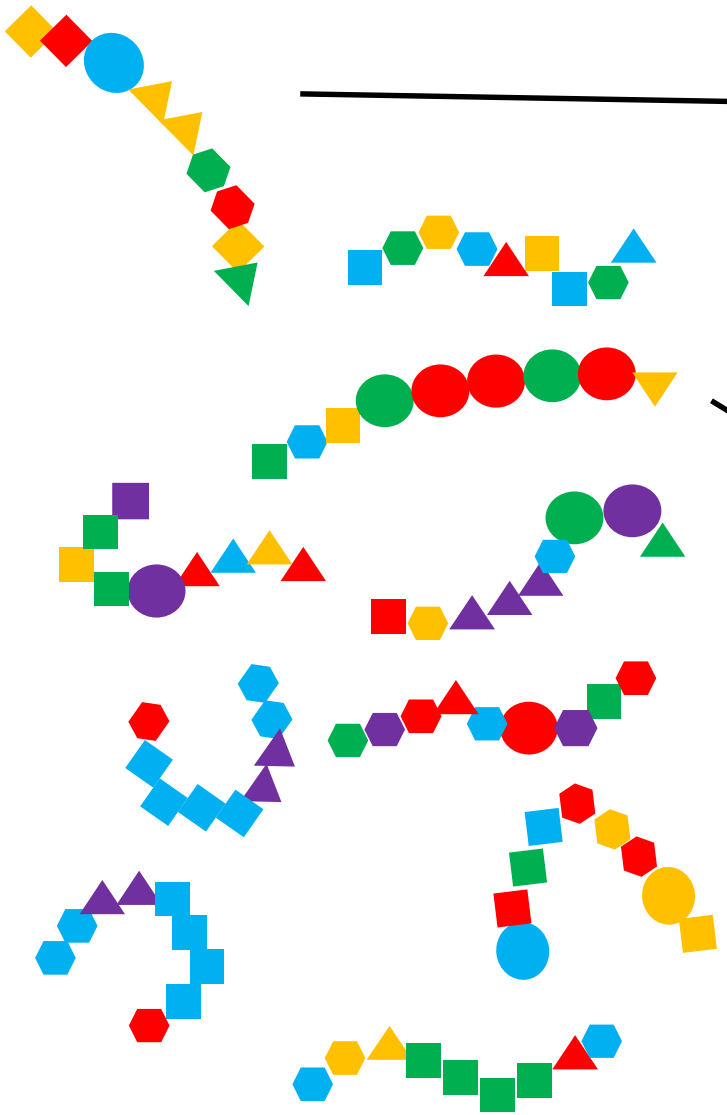
How do T cells recognize tumor cells?



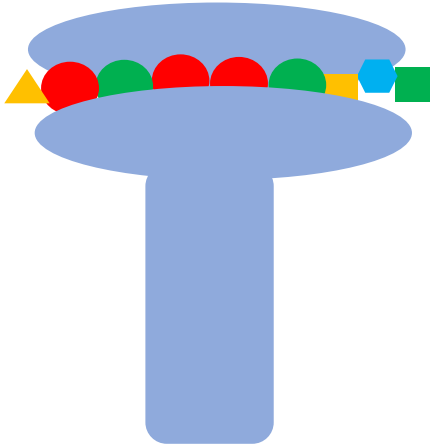
A portion of each new protein is chopped up (before it folds)

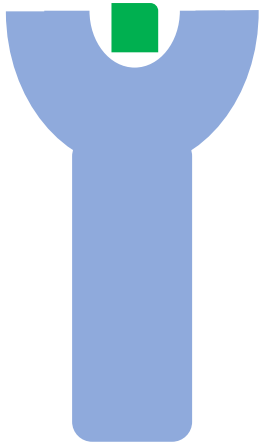


How do T cells see tumor cells?



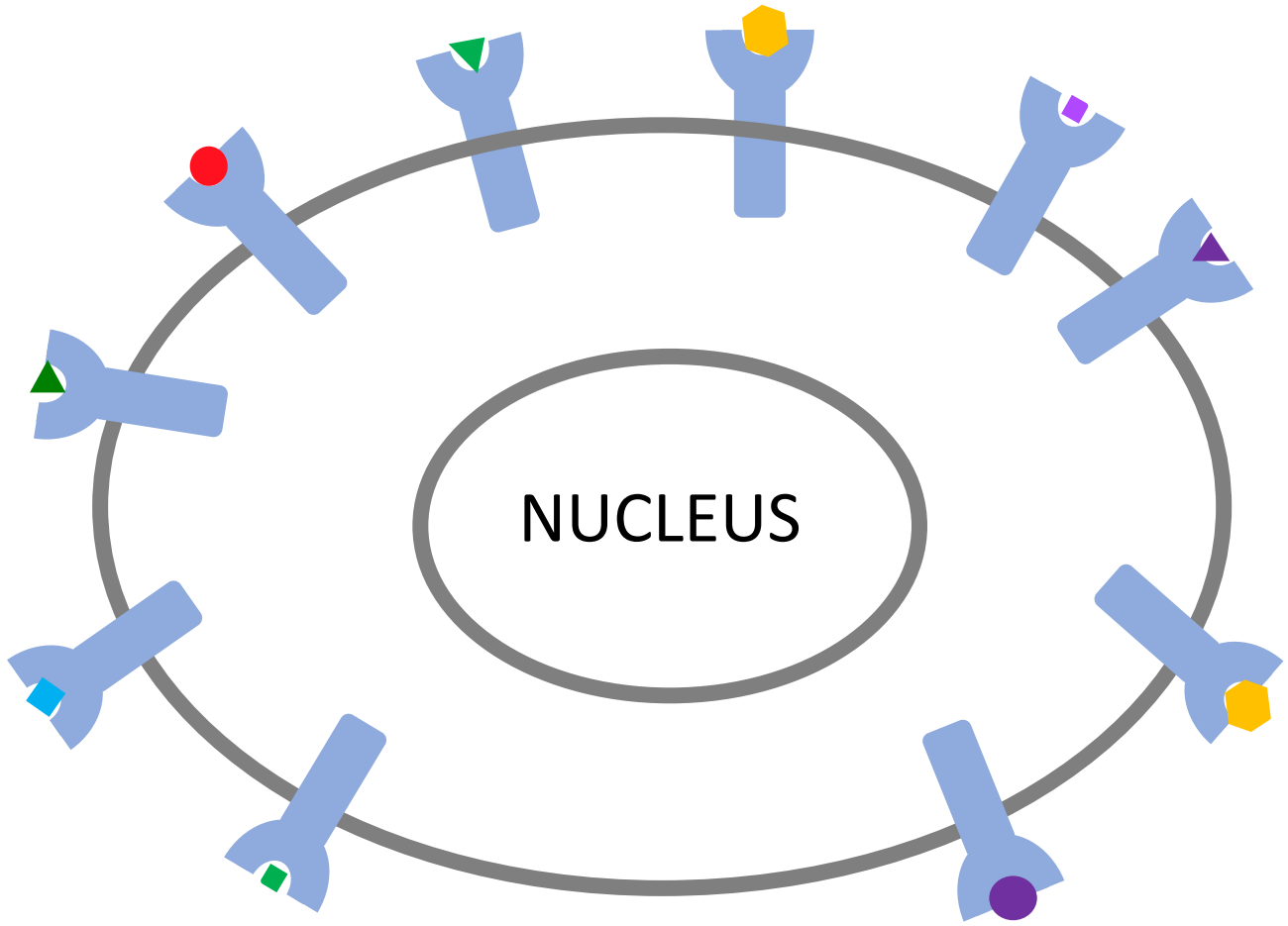
MHC molecule + peptide



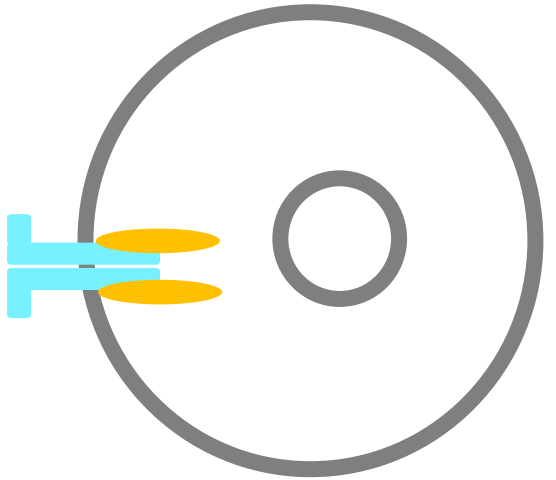


- Peptides are loaded on to MHC proteins and are transported to the cell surface
- Not every peptide fits, there are rules

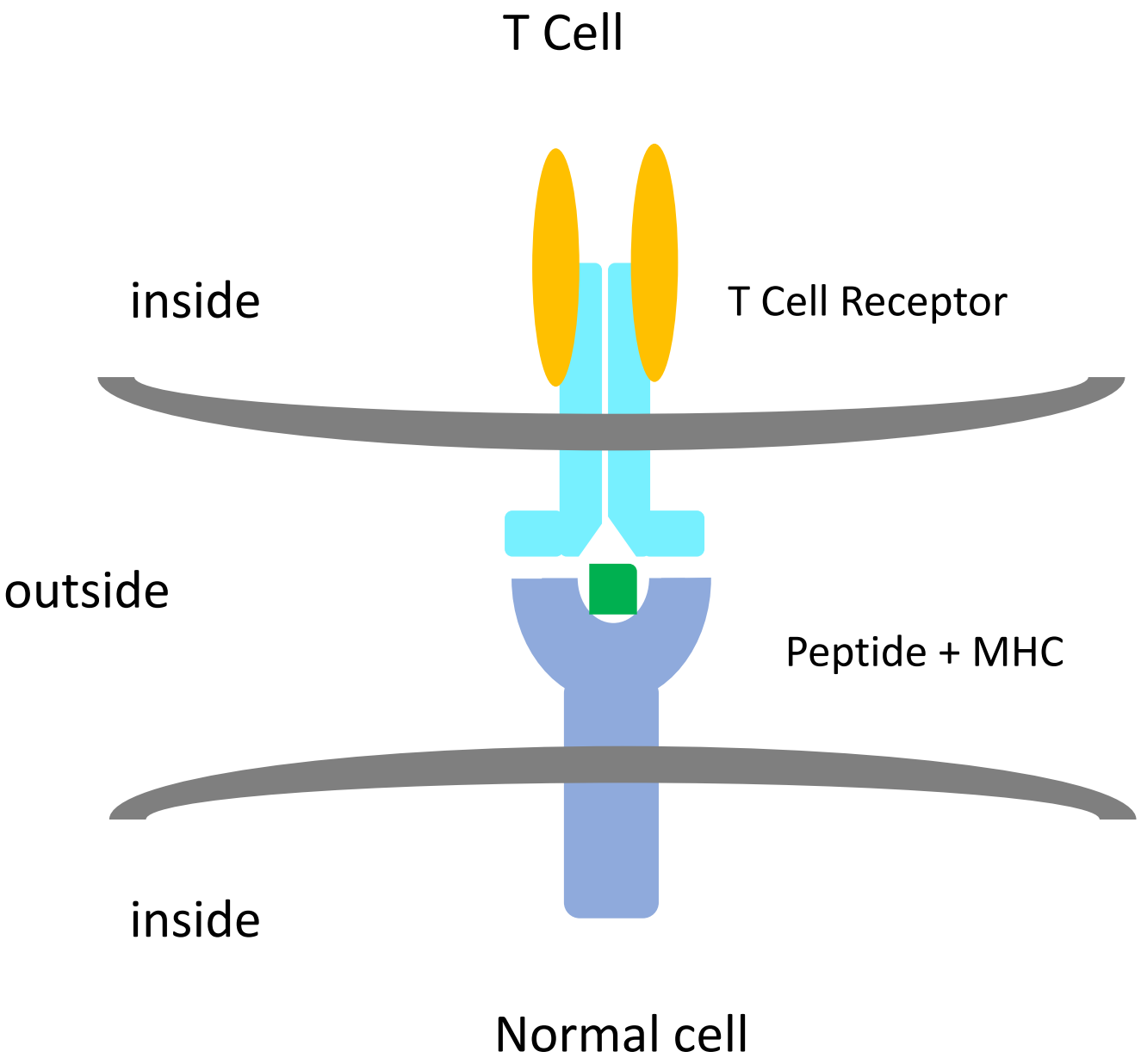
NORMAL
CELL



T CELL

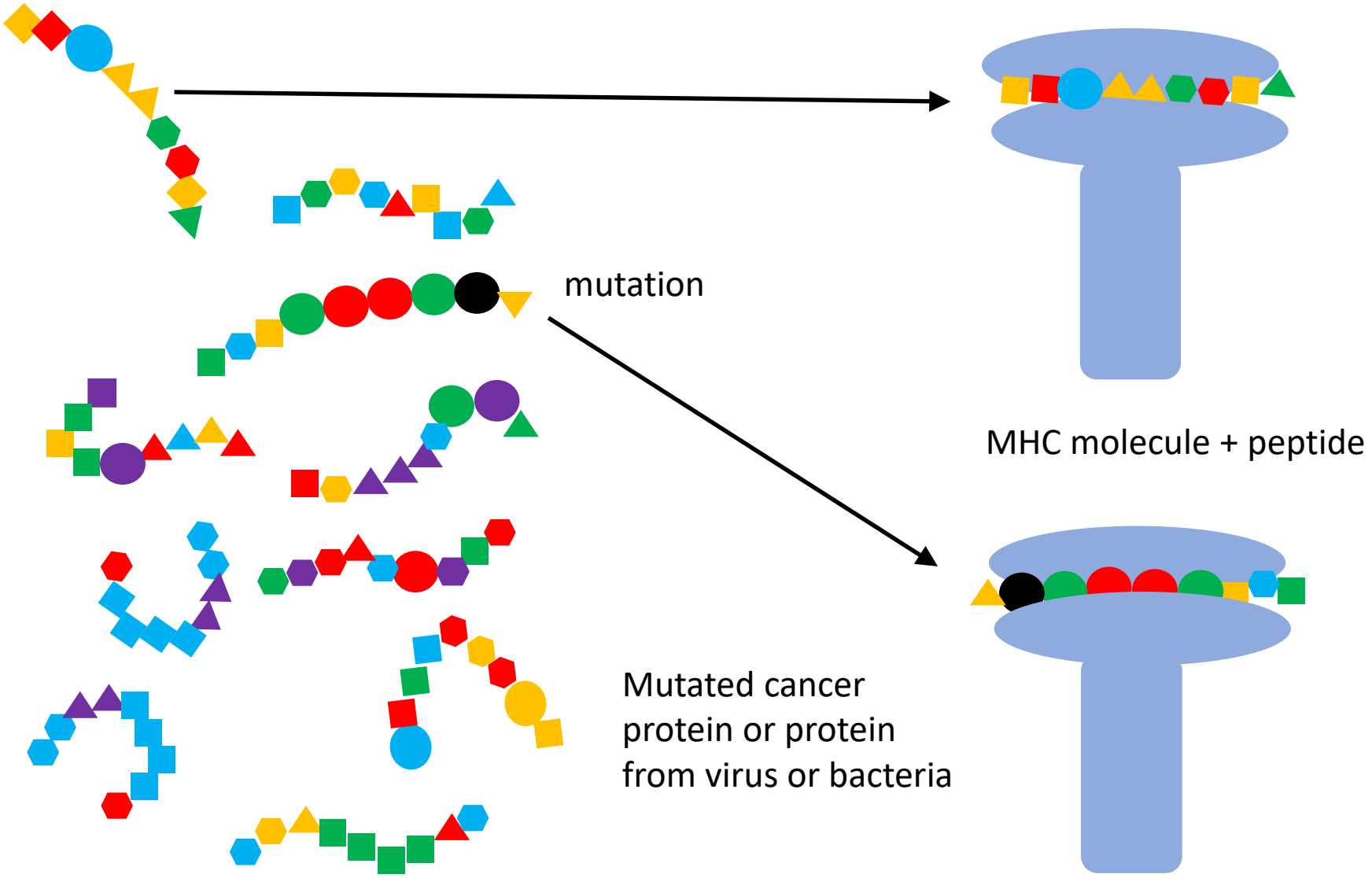


T CELL Receptor (TCR)



- The T cell receptor recognizes and binds to both the whole peptide and the MHC protein
- Every T cell makes only one kind of TCR
- T cells can’t “see” normal MHC-peptide complexes

What happens with tumor cells?

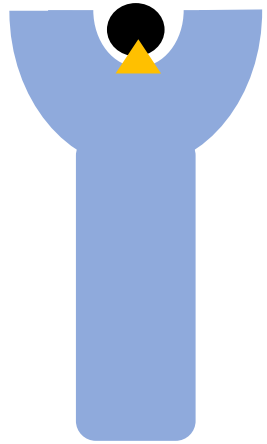


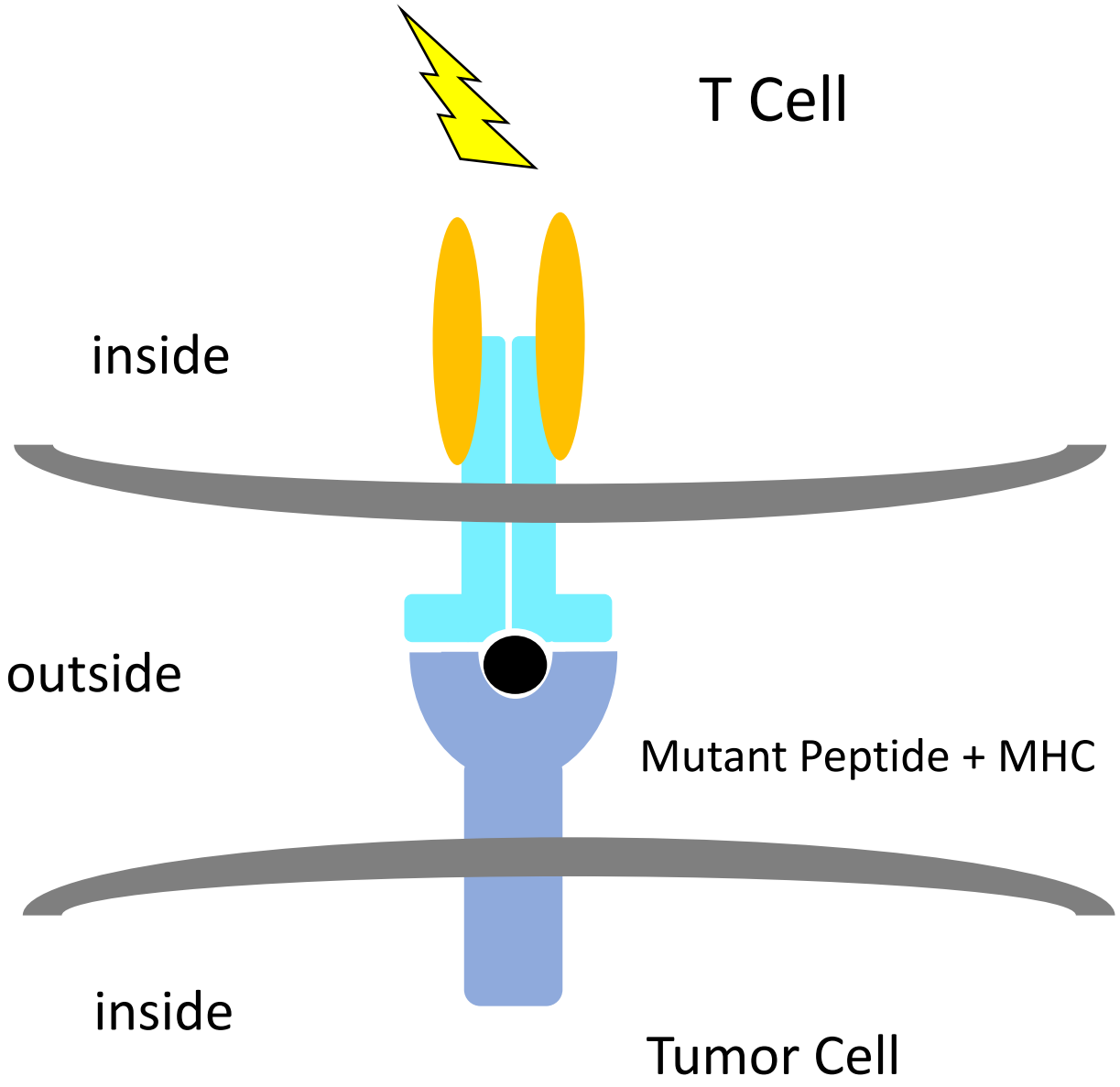
mutation

Mutated cancer protein or protein from virus or bacteria

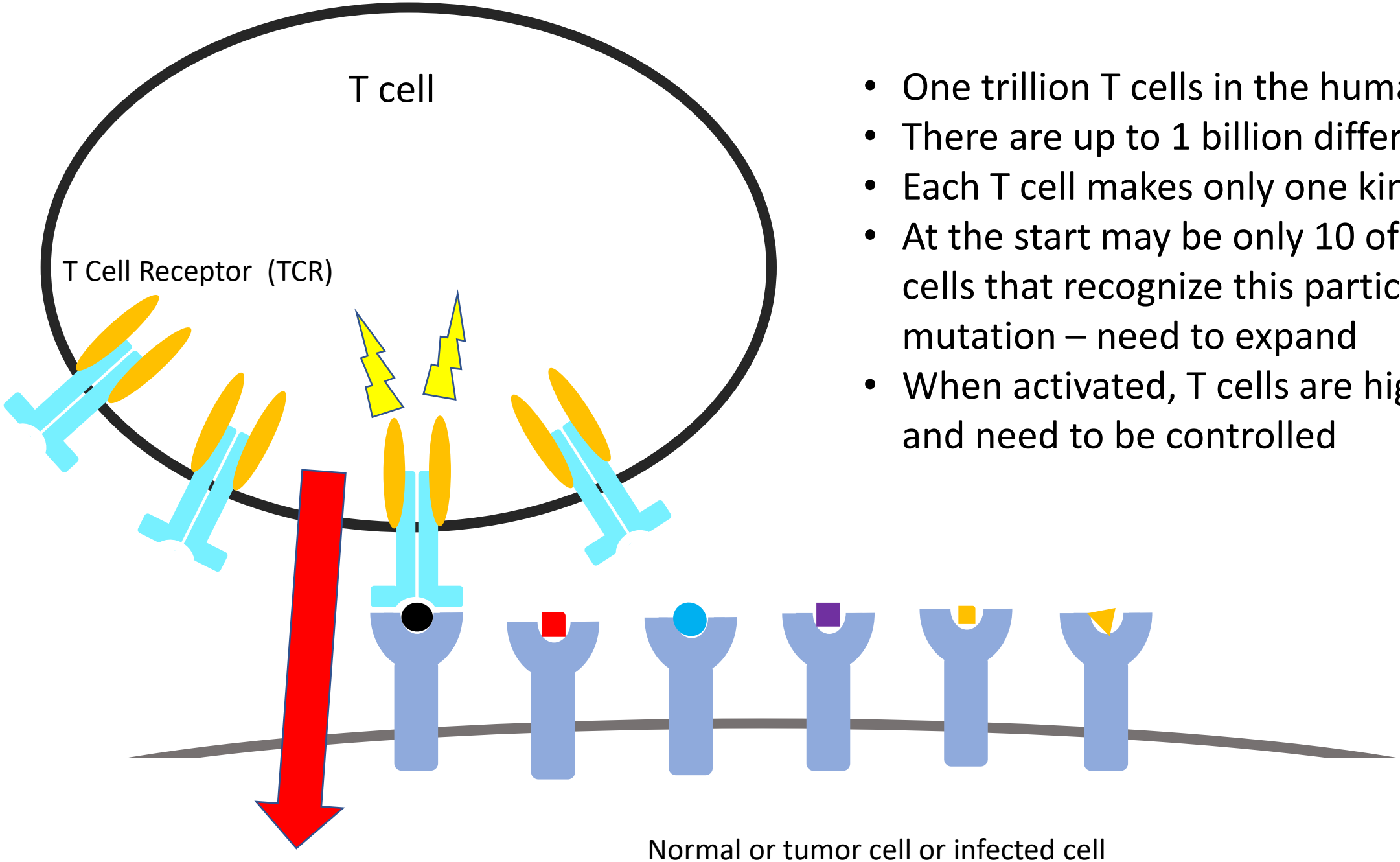
MHC molecule + peptide

- Peptides are loaded on to MHC proteins and are transported to the cell surface
- Not every peptide fits, the rules still apply
- The more mutations, the greater the chance that one will fit the MHC protein





- The stronger the binding the stronger the signal and the more activated the T cell
- When activated, T cells divide every 8 hours. One cell can make two million cells in a week
- The cells also become more “cytotoxic” – make more of the proteins used to kill the target cell
- What happens if MHC is mutated in the tumor cell?



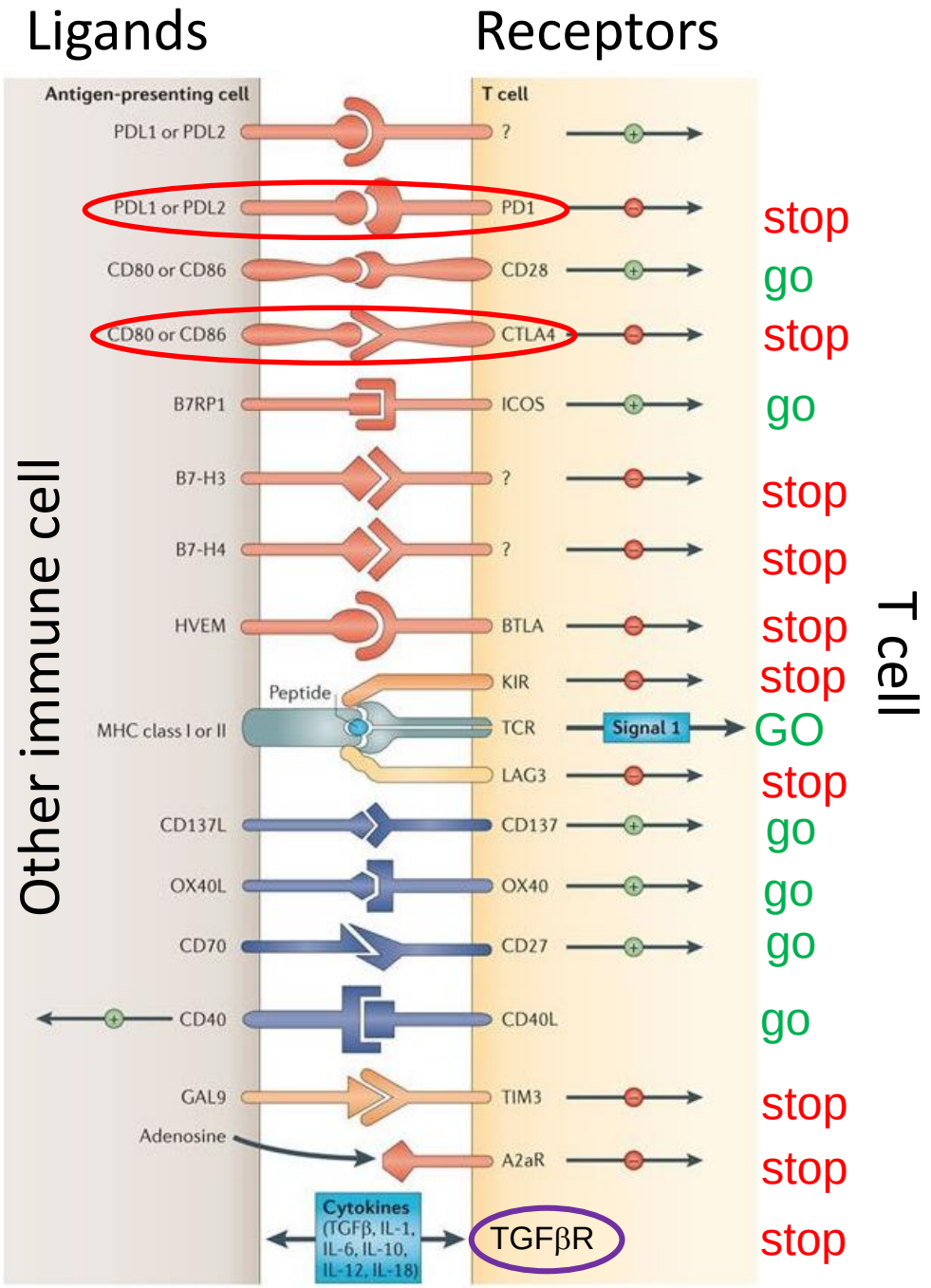
- One trillion T cells in the human body
- There are up to 1 billion different TCRs
- Each T cell makes only one kind of TCR
- At the start may be only 10 of the T cells that recognize this particular mutation – need to expand
- When activated, T cells are highly toxic and need to be controlled

Control of T Cell responses

- Need to be “quiet” while moving to the right place in the body.
- Need to be highly active at the sight of trouble (infection, cancer)
- Need to be deactivated when infected or cancerous cells are gone and to need promote healing (TGFβ growth factor that stimulates repair, but also helps end T cell response)
- Need to be removed to make space for other immune responses

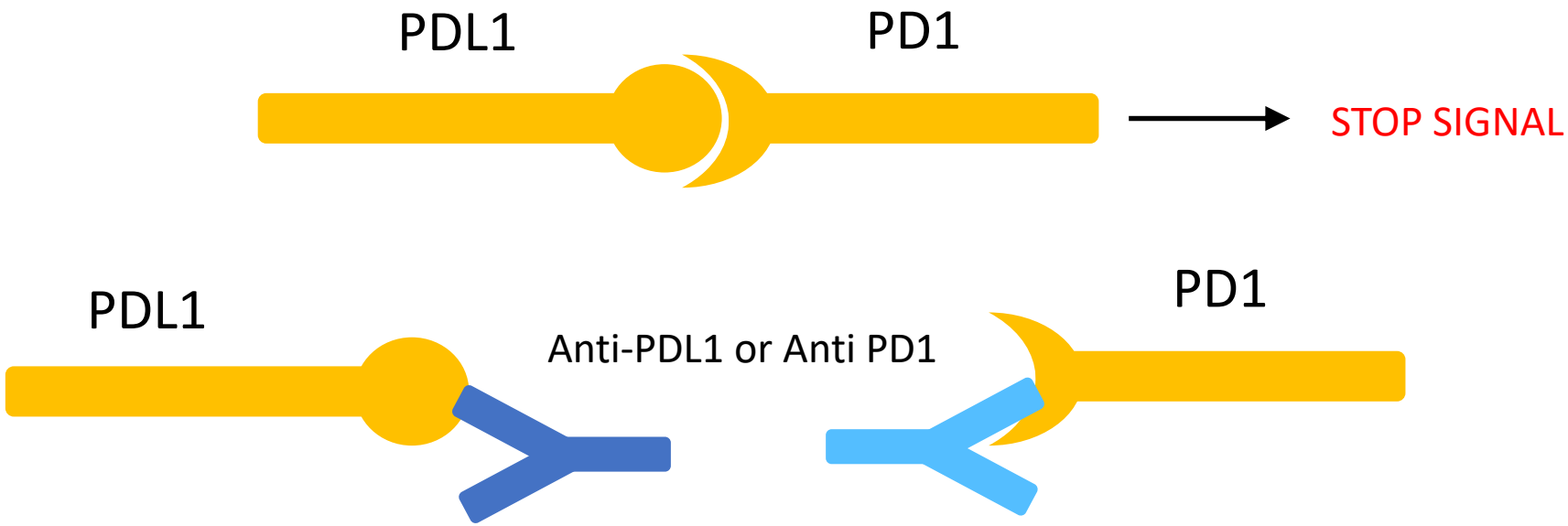
Regulation of T Cell activity

- Go signals help start the T cell activation
- Stop signals usually come from accessory immune cells, often called checkpoint inhibitors
- They also can be made by the tumor cells themselves (remember mutations)
  - PDL1 for example
- In a chronic situation like a tumor, Stop signals accumulate and together shut down but not necessarily kill the T cells (exhaustion)
- Other tumor tissue related factors
  - Abnormal blood vessels
  - Acidic pH
  - Low oxygen



Checkpoint immunotherapy

- Reactivation of a suppressed T cells, in place, in the tumor
- Patients are infused with an antibody that binds to either the ligand or receptor to block the inhibitory signal.



Checkpoint immunotherapy

- This leads to the activation of the T cells residing in the tumor and renewal of tumor destruction
- Works well (approved) in tumors with high mutational load such as melanoma, lung, and bladder cancers (nine total), but not prostate cancer (but data is changing)
- Overall response rates (stable disease, partial response, complete response) in melanoma is about 50-70% and the complete response rate is about 20%

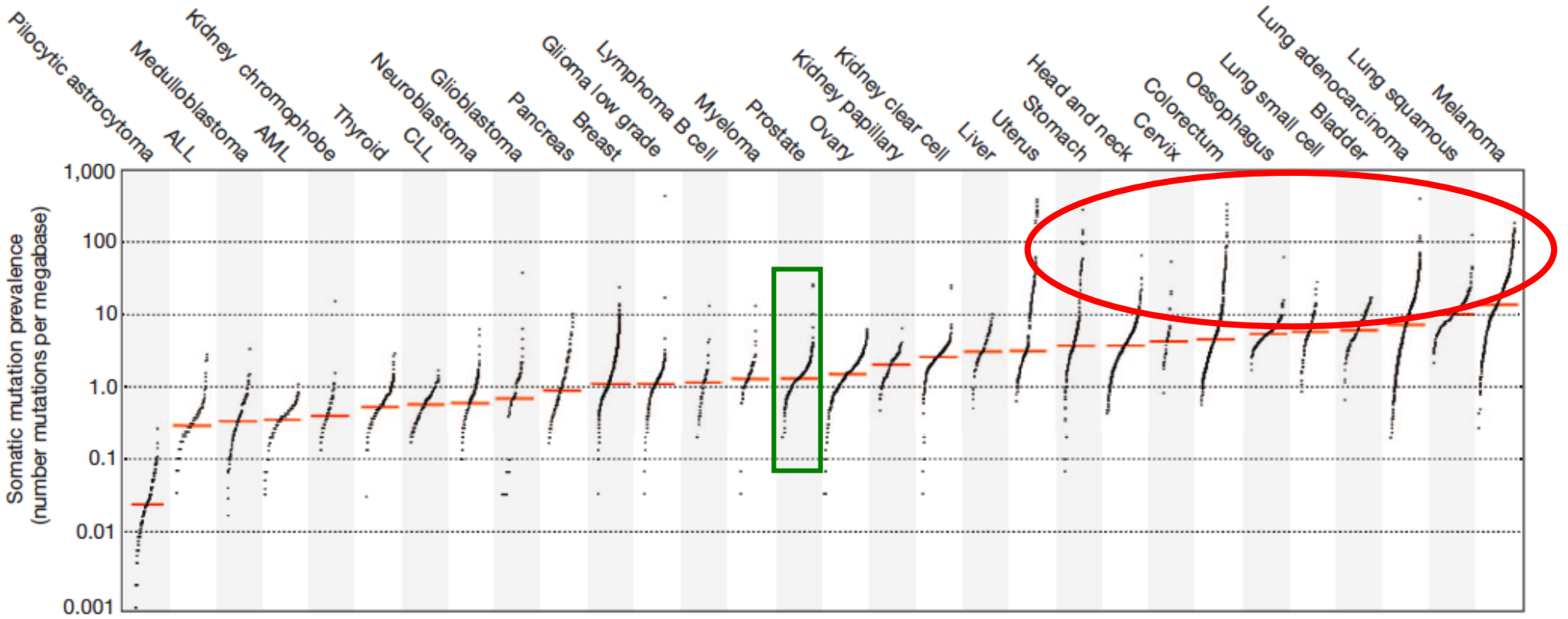
Who is getting treated?

ARTICLE

doi:10.1038/nature12477

Signatures of mutational processes in human cancer

Alexandrov, *Nature*, (2013)



The more mutations, the greater chance of the “right” mutations

Checkpoint immunotherapy

- The only approved checkpoint therapies are for the PD1 and CTLA4 pathways. Different agents approved for different cancers
  - CTLA4 – Yervoy
  - PD1 – Opdivo, Keytruda, Cemiplimab (under development)
  - PDL1 – Imfinzi, Tecentriq, Bavencio
- CTLA4 and PD1 can be used in combination because they target different pathways, can have better results, but more toxicity
- Not approved in Prostate cancer but many trials underway, mostly in mCRPC setting
- These trials include new combinations of therapies.

Limitations of checkpoint inhibitors

- Must have an existing T cell response, even if supressed
- Both CTLA4 and PD1 blockade induce severe side effects that can force patients off treatment. These resemble autoimmune diseases and can be quite severe. However in most cases the condition is manageable and transient.
- As much as half of patients do not respond (although all were initially terminal (cup partly full) – we need to do better.
- There are many mechanisms of failure which vary from patient to patient (antigen loss, presence of other inhibitory proteins (e.g. TGFβ)

CheckMate 650 clinical trial: CTLA4 (Yervoy) and PD1 (Optdivo) blockade in prostate cancer

- 62 castration-resistant (mCRPC) patients total, in two groups by prior treatment
  - Group 1 (32 patients) had received androgen ablation
  - Group 2 (30) had received androgen ablation followed by chemotherapy
- In group 1, 25% (8/32) patients showed an objective response with two complete responses (no evidence of disease)
- In group 2, 10% (3/30) patients showed a response with two complete responses
- Responses were better with soft tissue disease than with boney mets
  - In bone metastases, the bone is degraded releasing TGFβ that inhibits T cells
- Toxicity was severe, grade 3-5 in half the patients, four deaths (two in each group)

CheckMate 650 continued

- So although Prostate cancer is a “cold tumor” in general and not expected to respond to immunotherapy, 11 of 62 patients (18%) had an objective response. These patients will be followed to see how durable the response is.
- It is likely better to get checkpoint blockade before one starts chemotherapy
- Toxicity is a problem and further trials will explore changing dose level and dose schedule to try to avoid the more severe side effects.
- It may be possible to screen for patients that are likely to respond (biomarkers)
  - Emmanuel Antonarakis
- Prior to treatment, responders had one or more of the following
  - Higher tumor mutational burden (>75 mutations detected)
  - Higher PDL1 in the tumor
  - Mutations in DNA damage repair and homologous recombination repair

Combinations with Checkpoint inhibitors in prostate cancer

- Enzalutamide- tumor cell death (stimulates the immune system) and generation new T cells
- PARP inhibitors (inhibition of DNA repair enzymes) – induce cancer cell death
- Any other treatment that induces tumor cell death including radiation therapy
- Vaccination

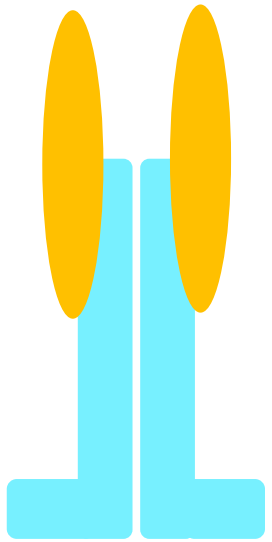
Adoptive Cell Therapy: expand T cells from the tumor in culture and give back to the patient

- Perform surgery to remove the tumor
- Isolate the T cells from the tumor
  - physical and enzymatic methods
- Grow the T cells in culture in an incubator by stimulating then to divide
- Expand to 0.5 to 1.0 billion cells
- Use chemotherapy drugs to deplete the T cells in the patient (transient)
  - “Make space” so that support systems for T cells in the body will be available to the expanded TIL
- Infuse the expanded TIL back into the patient – works well in melanoma
- Has not been tried in prostate cancer patients

CAR T Cell Therapy

- CAR is a chimeric antigen receptor. (chimera is a mythological beast that has a lion's head, a goat's body and a snake's tail)
- Use genetic engineering to combine the TCR and an antibody

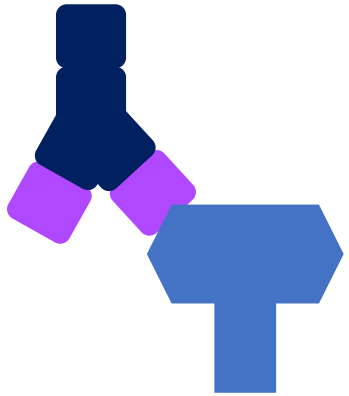
TCR



Recognizes and binds
Peptide MHC

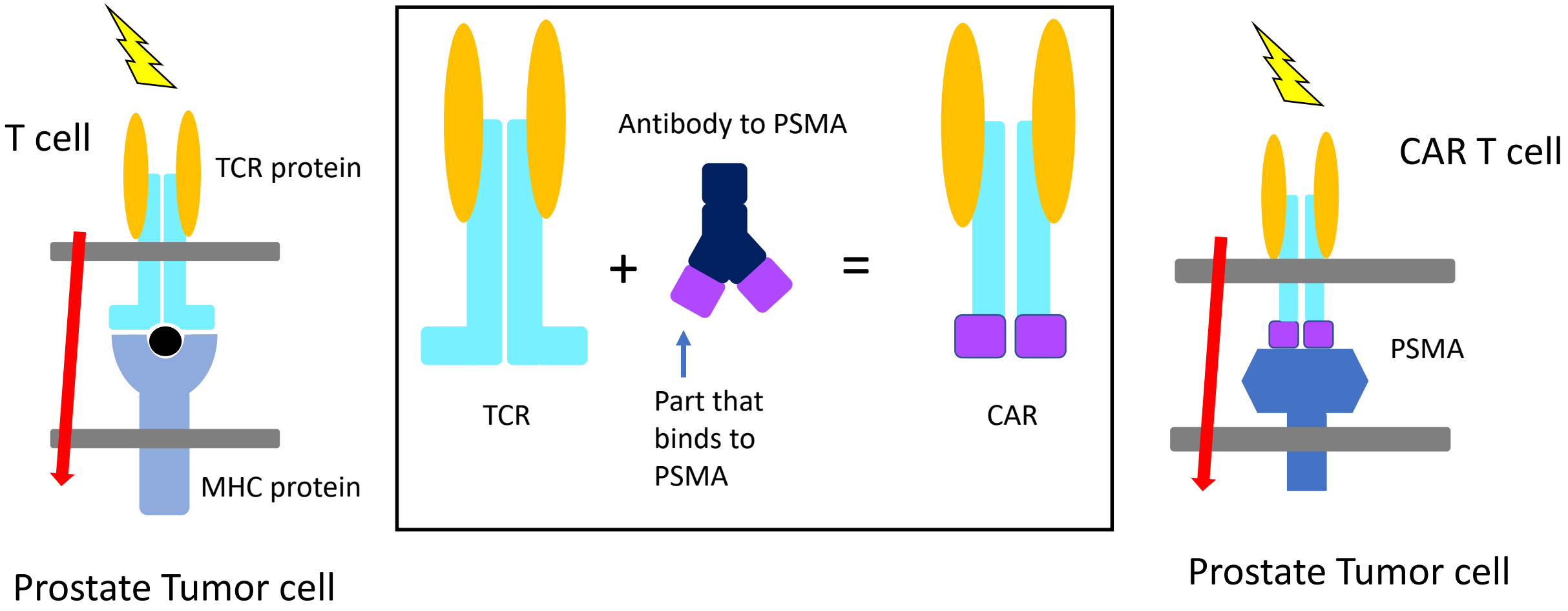
+

Antibody



Recognizes and binds
Protein antigen like PSMA

CAR T Cell Therapy in Prostate Cancer redirect the T cell.



CAR T Cell Therapy

- The antibody is used for recognition of a specific normal protein on the cell surface that triggers the TCR signaling and destruction of the target cell
- But need a protein that is found only on the target (tumor) tissue and not anywhere else or off-target cells/tissue will be affected.
- CAR T cell have been used very successfully in blood cancers like lymphoma and leukemia because they have unique normal proteins on their cell surfaces AND you can live without the normal non-cancerous cells
- It is much harder to find a unique surface protein on solid tumors
- However Prostate has some fairly unique proteins like PSMA and PSCA and we can live without our prostates (as many of us can attest).

Current CAR-T Cell trials in Prostate cancer

Trial Number	Trial Phase	Target	Disease Stage	Sponsor	Study Start Date
NCT03873805 #	I	Prostate Stem Cell Antigen (PSCA)	CRPC	City of Hope	May 2019
NCT03089203 #	I	Prostate Specific Membrane Antigen (PSMA)	CRPC	University of Pennsylvania	March 2017
NCT03013712	I/II	EpCAM (adhesion protein) Found in many cancers	CPRC	Chengdu Medical College, China	January 2017
NCT01140373 *	I	PSMA	CPRC	Memorial Sloan Kettering	June 2010
NCT02744287	I/II	PSCA	CPRC	Moffit Cancer Center, Tampa	November 2016

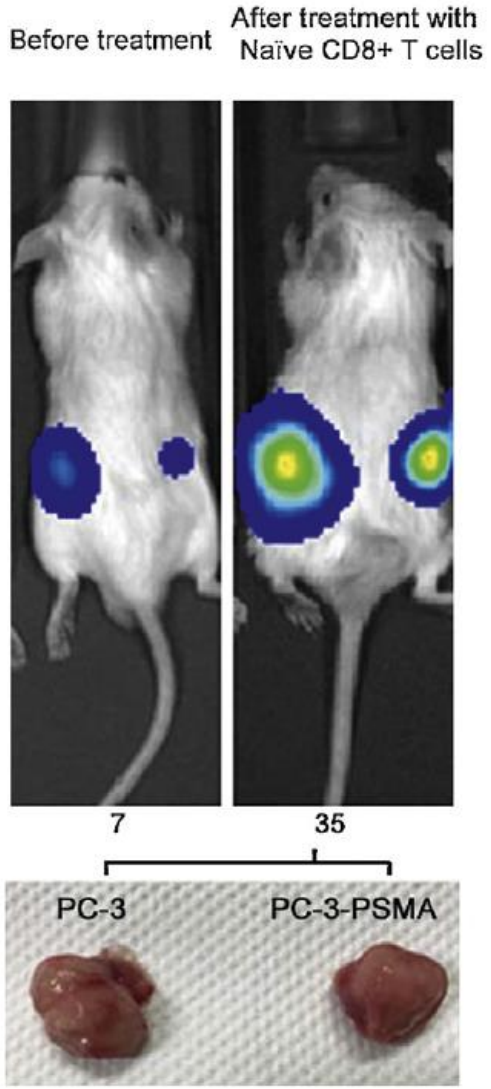
insensitive to TGFβ
* closed

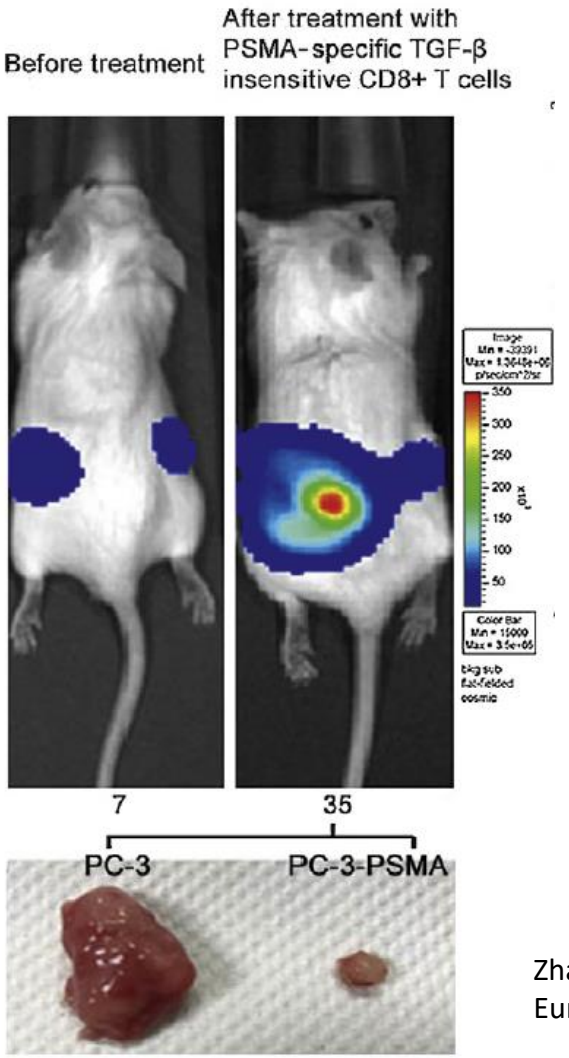
Preclinical data for PSMA CAR-T cell Therapy

- Human tumor cell line, PC3
  - Sub-line that makes PSMA
  - Sub-line that does not make PSMA

- Use mice with no immune system so human tumor cells and T cells can survive
- Implant PSMA(+) tumor on one flank and PSMA (-) tumor on the other flank

- Non specific T cells from Prostate cancer patient
- PSMA CAR-T cell made from same cells that also are not affected by TGFβ.





Zhang, Q et al. (2018) European Urology vol 75:

Trial NCT03089203: Carl June

- Cohort 1: 20-60 million CAR T cells
- Cohort 2: 200-600 million CAR T cells
- Cohort 3: 20-60 million cells with lympho-depletion (make space)

- Not much happened in cohorts 1 and 2 (a few transient responses)

NCT03089203 – Cohort 3

- patient 1: bone only disease and rising PSA, 40 ng/ml at treatment
  - Abiraterone
  - Sipuleucel-T
  - Abiraterone again
- PSA to zero at day 19 after treatment
- Wait and see what happens

Making cold tumors hot: ProstAtak

- Borrowing from cold sore (Herpes infection) therapy .
  - Cyclovir is a drug that exist in two forms Pro-cyclovir and cyclovir
  - Cyclovir kills cells, Pro-cyclovir does not
  - When you treat a cold sore with pro-cyclovir, the Herpes Virus has an enzyme called TK that converts Pro-cyclovir to cyclovir and the infected cell dies
  - Normal cells do not have this enzyme, so are not harmed.
- Make a virus (adenovirus) that contains a gene for the herpes TK gene (ProstAtak).
- Inject the prostate with the virus and prostate cancer cells are infected (not exclusively)
- Give the patient pro-cyclovir and infected (preferentially)) cancer cells die
- The presence of viral proteins and DNA and the dead and dying tumor cells are highly stimulatory to the immune system
- Tumor specific T cells recruited to the tumor

ProstAtak

- NCT01436968: phase III trial in progress
  - High risk patients with local disease
  - Radiation + ProstAtak
- NCT02768363. phase II: no new patients
  - Localized disease
  - Active surveillance
- One viral/immunologic prostate cancer therapy that targets local disease stages. Something to do before mCRPC

Summary

- Lots of interest and clinical trials for the immunotherapy of prostate cancer
- Many different approaches and combinations to be tried
- Complex problem so immunotherapy will not necessarily work for every one
- Need to tailor the therapy to the individual patient.

Vaccines

- Typically subcutaneous
- Start an immune response away from the suppressive environment of the tumor
- Can include multiple prostate specific proteins PSA, PSMA, PSCA at the same time
- Include danger signal stimulators to enhance the immune response
- Many versions in clinical trials (Prostvac), but most show modest benefit by themselves
- However all are now being tried in combination with Checkpoint inhibitors- step on the gas away from the tumor, take off the brakes inside the tumor