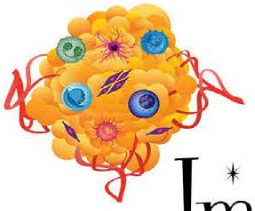
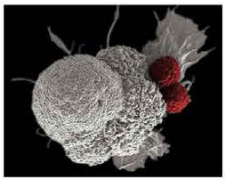


The Tumor Microenvironment 3: Immunotherapy & Immuno-Oncology



Definition: immunotherapy is a form of disease treatment (including, but not necessarily limited to, cancer) that co-opts certain parts of the immune system to help fight disease

This can be accomplished using two main strategies:

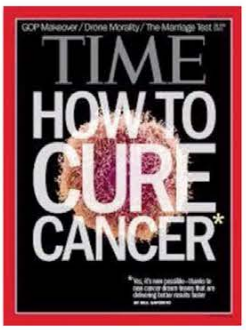
"Active Immunotherapy" = stimulating or fine-tuning the patient's own immune system to work harder or better in order to fight disease (e.g., immune checkpoint inhibitors)

"Passive Immunotherapy" = supplying the patient with exogenously-engineered immune system components that supplement or substitute for the existing immune system in order to fight disease (e.g., CAR-T cell therapy)

A. What is Immunotherapy? What are its strengths and limitations?

1) the *idea* of harnessing the immune system to help facilitate cancer therapy has been around a long time, however any sort of practical application has only been possible for the last 25 years or so, after technologies to ramp up production of immune molecules were developed (e.g., hybridoma technology to generate large numbers of mono-clonal antibodies, first accomplished in the early 1980's)

Cancer immunotherapy came into its own in 2013, when many high profile scientific journals annointed it "Medical Breakthrough of the Year" **and the hype has continued ever since.**



a] the appeal of immunotherapy approaches was obvious, that is, that by their very nature, immune molecules already have (or can be specifically engineered to contain) built-in "targeting"

b] the downside was also fairly obvious: that the immune molecules would have to be highly potent (somewhat problematic), highly specific for their intended target (more problematic), don't cause anaphylaxis in the recipient (mostly under control...emphasis on mostly) and highly "penetrable" so as to easily access the intended target (most problematic, especially given that many immune molecules are large and bulky)

c] another major downside - identified much more recently - is that ***the tumor's microenvironment is immunosuppressive*** (and that this is facilitated by multiple components in the microenvironment)

B. Why (and how) is the tumor microenvironment immunosuppressive?

1. Why? To evade detection and destruction by the host's immune system

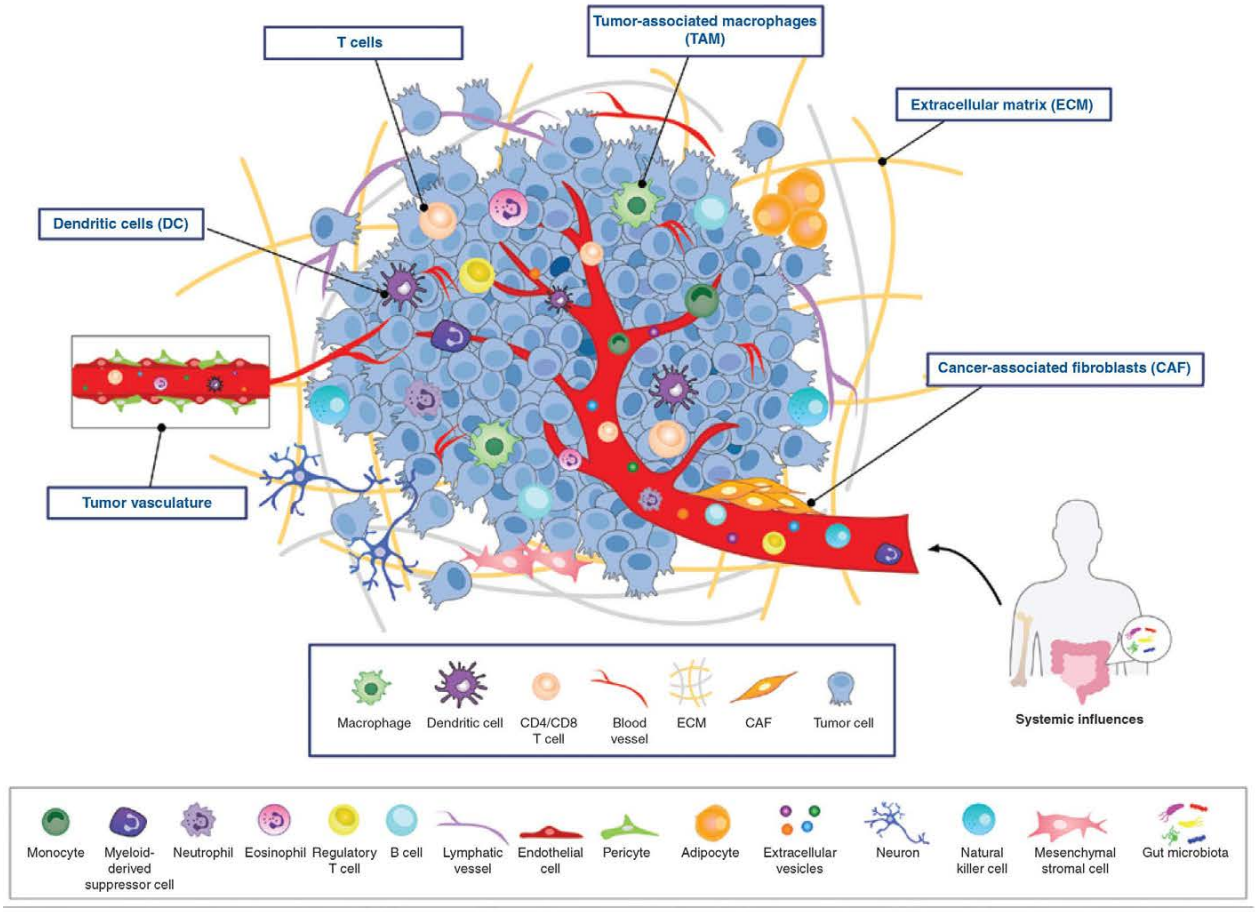
2. How? A (very) complicated interaction between various cells, proteins (cytokines and cell surface antigens and receptors in particular), acellular elements that surround the tumor cells, and the tumor cells themselves

a) immunosuppressive cell types include: **regulatory T-cells** (**Tregs** - see below), **cancer-associated fibroblasts** (**CAFs**), **tumor-associated macrophages** (**TAMs** - these have two different phenotypes: "Type 1" = immunostimulatory and more normal-like; and "Type 2" = immunosuppressive, angiogenesis-promoting and more tumor-favoring), **dendritic cells**, and **myeloid- and/or stroma-derived suppressor cells (MDSCs)**

b) immunosuppressive cytokines include: **VEGF**, **TGF-β** and various **interleukins** and **prostaglandins** (not a coincidence that many of these also participate in tumor angiogenesis)

c) **EMERGING SCIENCE**: tumor cells also secrete neurotropins that attract **neurons** to the tumor, and once entering the tumor microenvironment, they release **norepinephrine**... which further facilitates immune system evasion, tumor cell proliferation and angiogenesis

Cancer Discov 2021;11:933–959



3. Note that technically, ***many of these cells and proteins have multiple, normal functions – some that even promote immune activity – but that the tumor has co-opted them to support immunosuppression***

C. In order to understand how tumors maintain an immunosuppressive microenvironment and how cancer immunotherapy attempts to overcome this, you first have to understand (to a first approximation, anyway) how the immune system works normally...it's complicated!

Components of the Immune System

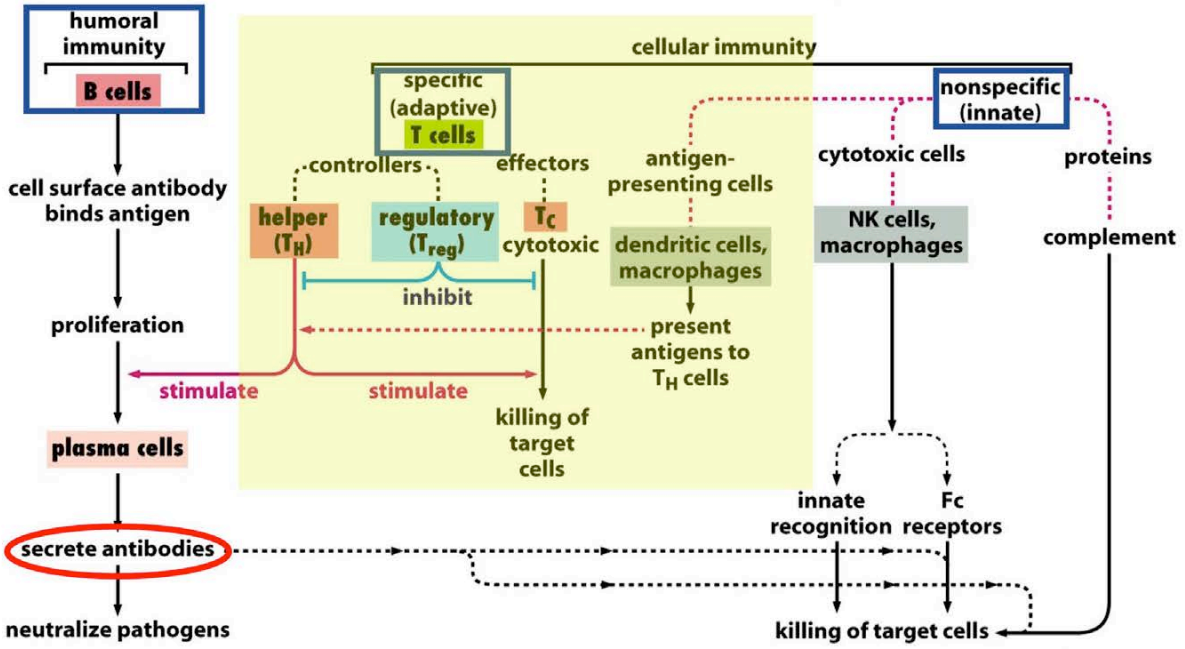
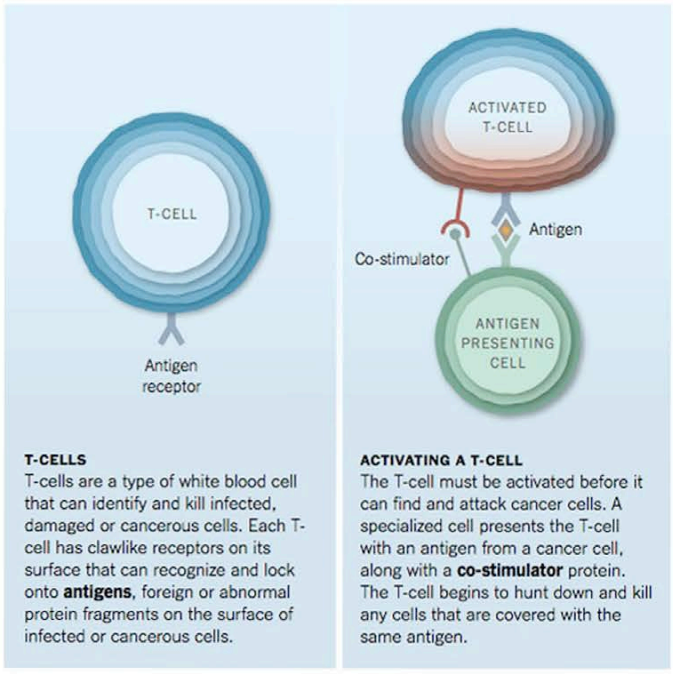


Figure 15.14 *The Biology of Cancer* (© Garland Science 2007)

1) but let's focus on those parts of the immune system currently being studied in the context of cancer (yellow box, above), that is, components that either directly or indirectly can recognize, attack and kill cancer cells, OR conversely, can prevent this from happening

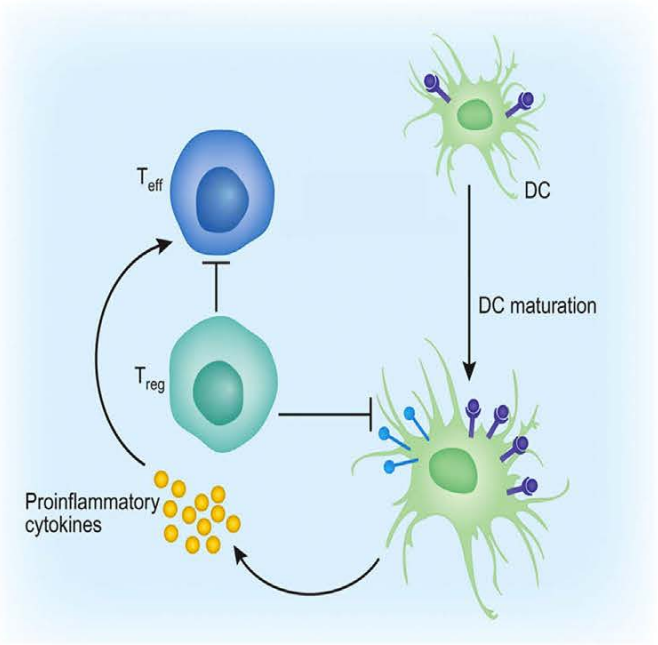


T-lymphocytes are the ones that identify and attack cancer cells, and kill them

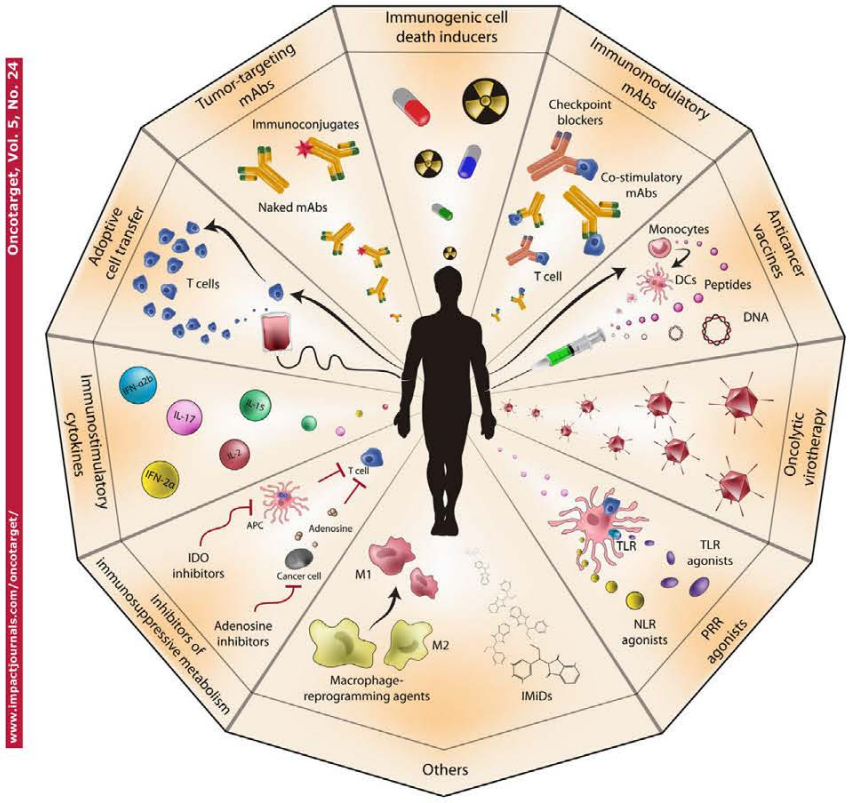
Dentritic cells and macrophages have to activate the T-lymphocytes first before they can function as cancer cell killers; they do this by expressing both the cancer antigen(s) the T-cells will hone in on, as well as a co-stimulatory cell surface protein that acts as the "on" switch

However, rather than risk the T-lymphocytes overreacting and staring to attack "self", there also needs to be a way to turn off the immune response.

This is accomplished by regulatory T-cells in particular, and to a lesser extent, by the dendritic cells and macrophages themselves



1. What are the general immunotherapy approaches?



Anticancer immunotherapy. Several anticancer immunotherapeutics have been developed during the last three decades, including tumor-targeting and immunomodulatory monoclonal antibodies (mAbs); dendritic cell (DC)-, peptide- and DNA-based anticancer vaccines; oncolytic viruses; pattern recognition receptor (PRR) agonists; immunostimulatory cytokines; immunogenic cell death inducers; inhibitors of immunosuppressive metabolism; and adoptive cell transfer. 1MT, 1-methyltryptophan; APC, antigen-presenting cell; IDO, indoleamine 2,3-dioxigenase; IFN, interferon; IL, interleukin; IMiD, immunomodulatory drug; NLR, NOD-like receptor; TLR, Toll-like receptor.

a) what can facilitate the success of most of these approaches by helping to overcome the tumor's immunosuppressive microenvironment?

a. liberate tumor antigens by killing some cancer cells (e.g., radiation)

b. stimulate immune system to better react to the tumor antigens (e.g., immune checkpoint inhibitors)

c. prevent tumor cells from over-compensating for the heightened immune response (e.g., add immunostimulatory cytokines, to compete against the tumor's immunosuppressive ones)

a] **Non-Specific Immunotherapies/Adjuvants** -

1. non-specific immunotherapy involves administering immune system stimulating agents (not whole immune cells) in the hopes of boosting existing immune system function...bearing in mind that these can stimulate all sorts of immune responses, and not necessarily only those you are interested in

2. one class of generalized immunostimulants are **cytokines**, certain types of which are known to increase the production of immune cells (and bone marrow cells in general, e.g., EPO, pegfilgrastim) and/or enhance their function in eradicating cancer cells

a) the most commonly-used immunostimulant cytokines are the ***interleukins*** (IL-2 in particular, but also IL-7, 12 and 21) and the ***interferons*** (in particular, INF-α)

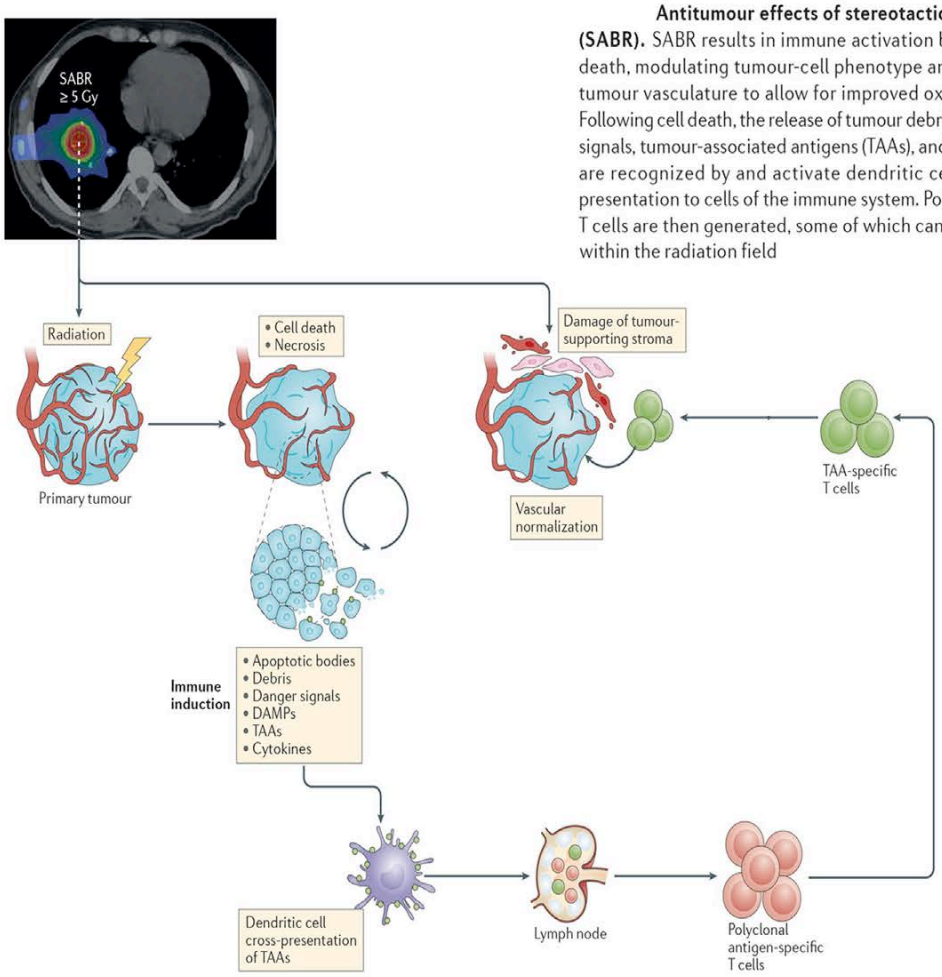
3. most studies of cytokines in the context of cancer therapy are still considered experimental – some haven't even been used in humans yet – however *INF-α is FDA-approved for human use against several types of leukemias and lymphomas, melanoma, kidney cancer and Kaposi's sarcoma*

4. other types of generalized immunostimulants that aren't cytokines include BCG (Bacille Calmette-Guérin), KLH (Keyhole limpet hemocyanin) and IFA (incomplete Freund's adjuvant); these are highly immunogenic compounds that literally "over-stimulate" the host's immune system (to everything, hopefully including the cancer cells); *BCG was the very first type of immunotherapy approved for human use in the treatment of cancer, especially early stage bladder carcinoma*

5. Ionizing radiation as a non-specific immunotherapeutic  **Hot Topic**

a) ***Given that radiotherapy kills large numbers of tumor cells, and that some of them eventually die in such a way that they release their contents into the extracellular space, do all these tumor cell fragments/proteins/antigens prime the patient's immune system?***

b) **A growing body of evidence suggests the answer is yes, *especially* when the dose is really high, such as would be the case with SRS/SBRT**

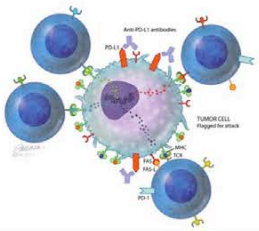


Antitumour effects of stereotactic ablative radiotherapy (SABR). SABR results in immune activation by inducing tumour-cell death, modulating tumour-cell phenotype and normalizing aberrant tumour vasculature to allow for improved oxygen and drug delivery. Following cell death, the release of tumour debris with associated danger signals, tumour-associated antigens (TAAs), and inflammatory cytokines are recognized by and activate dendritic cells, promoting antigen presentation to cells of the immune system. Polyclonal antigen-specific T cells are then generated, some of which can attack tumours located within the radiation field

Bearnstein *et al.*, Nat Rev Clin Oncol, Vol 13, August 2016

Historically though, this doesn't happen very often, most likely because of the immuno-suppressive tumor microenvironment, which would tend to blunt any immune response. What might help?

- A tumor with a high mutational burden, meaning a wider variety of potential antigens.



- Vascular normalization, which would better allow immune cell access to the tumor.
- Adding one or more immune checkpoint inhibitors (see below), in the hopes of overcoming the local immunosuppression.

1. ***If anti-tumor immunity mostly DOESN'T develop during or after radiotherapy, how did we ever figure out the few instances when it DOES?***

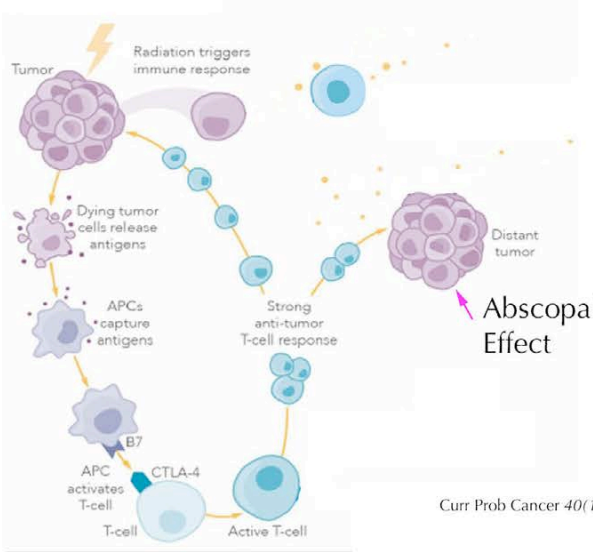
ANSWER: the ***abscopal effect***, where treatment of a primary tumor causes a "reaction" in metastatic disease at a distant, unirradiated site

Mole (1953) was one of the first to describe an abscopal effect for radiotherapy, and to suggest that it could be the result of a systemic immune response.

Although only (very) rarely observed clinically since that time, a couple of observations are noteworthy:

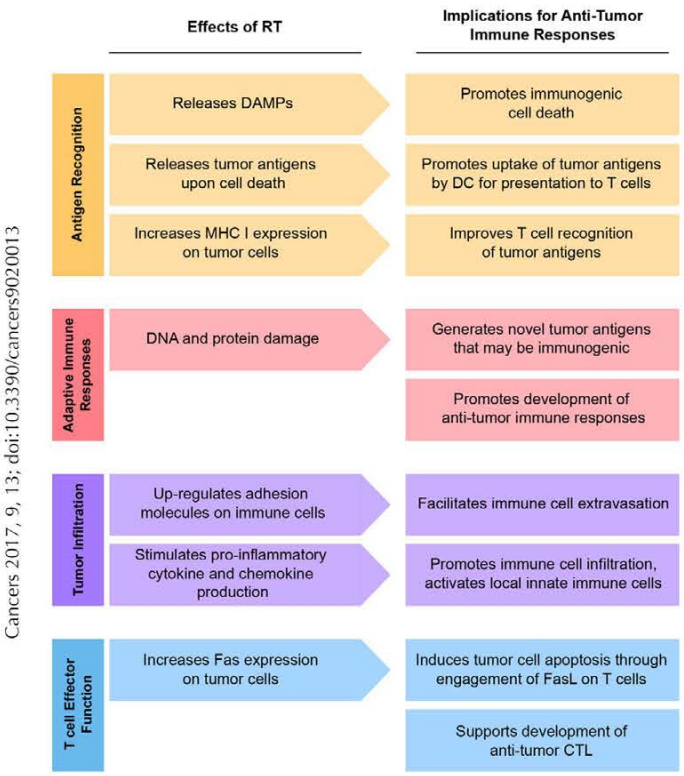
1. it's more likely to occur after higher radiation doses than lower ones; and

2. it's more likely to occur when radiotherapy is combined with an immune checkpoint inhibitor

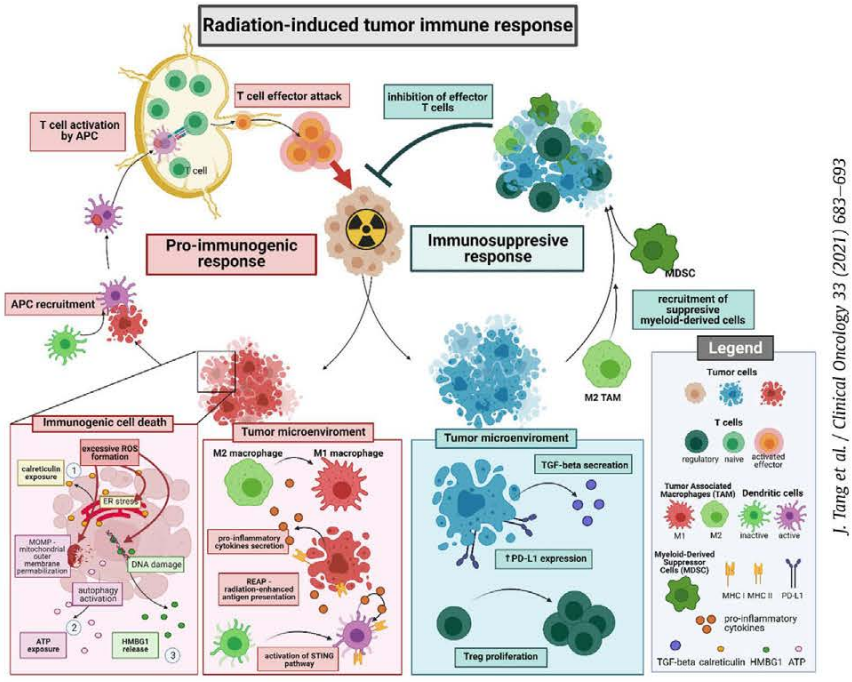


Curr Prob Cancer *40(1)*: 25-37, 2016

c) ***HOW does radiation act as an immunomodulator, besides causing a massive release of tumor antigens?***

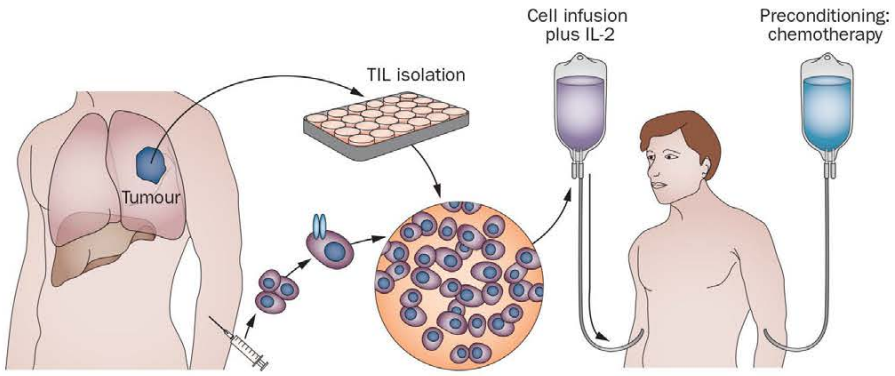


d) ***Never forget however that while we're busy trying to exploit radiation's immunostimulatory effects, if anything, it's better know as an immunosuppressant***



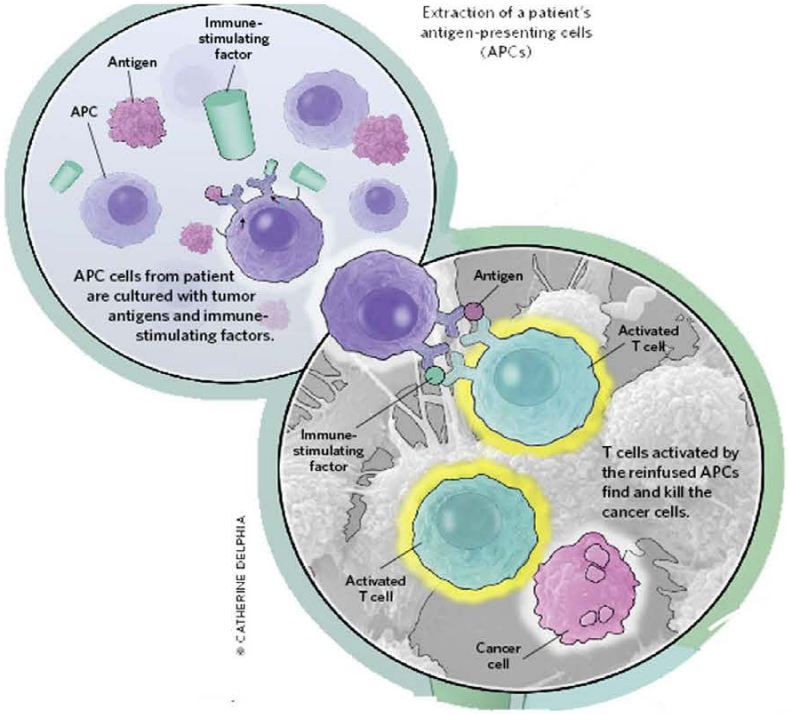
Radiation-induced tumour immune response. Pro-immunogenic response: radiation therapy induces immunogenic cell death with the release of ATP and high mobility group protein B1 (HMGB1) in the serum and calreticulin translocation to cell membrane, which attracts tumour-infiltrating T cells and recruitment of antigen presenting cells (APC). Immunosuppressive response: ablative radiation therapy can also induce a compensatory immunosuppressive response by promoting infiltration and/or reprogramming of tumour-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC) to anti-inflammatory M2 phenotype.

b] **Adoptive Cell Therapies** - types of immunotherapy that use the patient's own immune cells to attack cancer, either by collecting them and simply expanding their numbers, or by genetically engineering them (via gene therapy) to enhance their activity



Adoptive immune cell transfer techniques:

1. **Tumor-infiltrating lymphocytes** (TILs) are collected from a tumor biopsy specimen, isolated in the lab, expanded in numbers, and infused back into the patient (who has already been lympho-depleted, usually using chemotherapy)

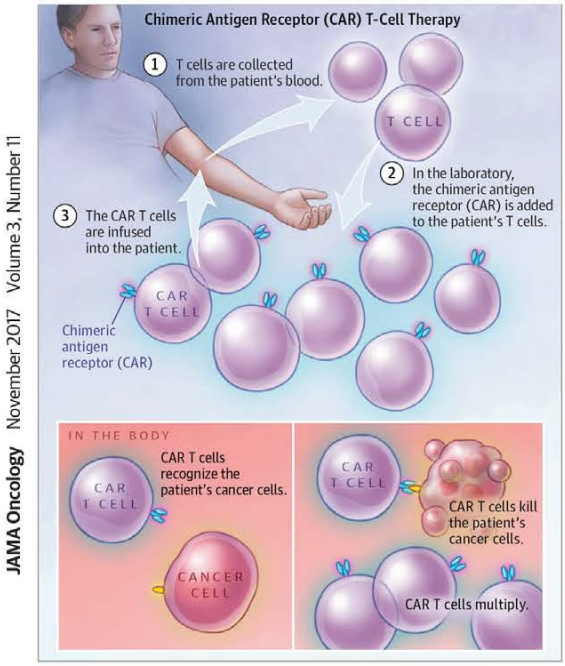


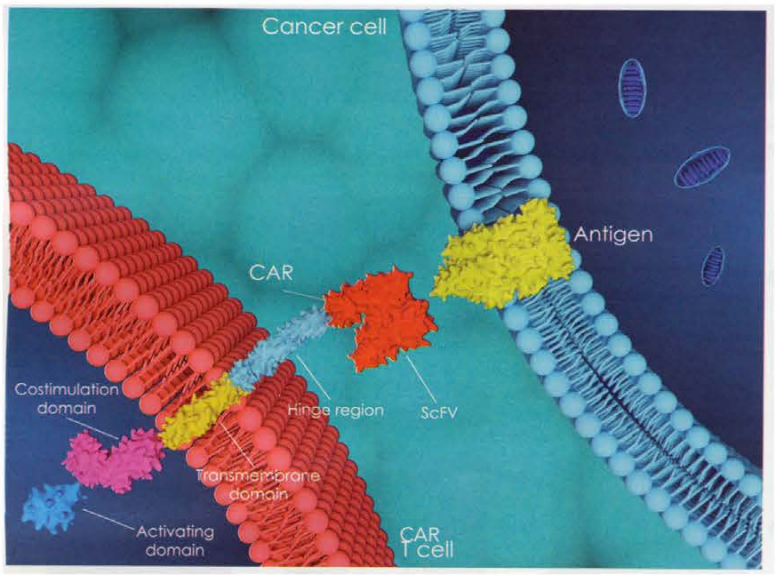
2. The patient's **dendritic cells** (i.e., APCs, antigen presenting cells) are collected from the peripheral blood, cultured and expanded *in vitro* in the presence of tumor antigens (either tumor-specific peptides/proteins or a whole tumor cell lysate; this could come from the individual patient's tumor, or else a mixture of other patients' tumor cells of the same type).

The activated dendritic cells are then infused back into the patient and they go on to activate the patient's T cells, which in turn go on to attack the tumor.

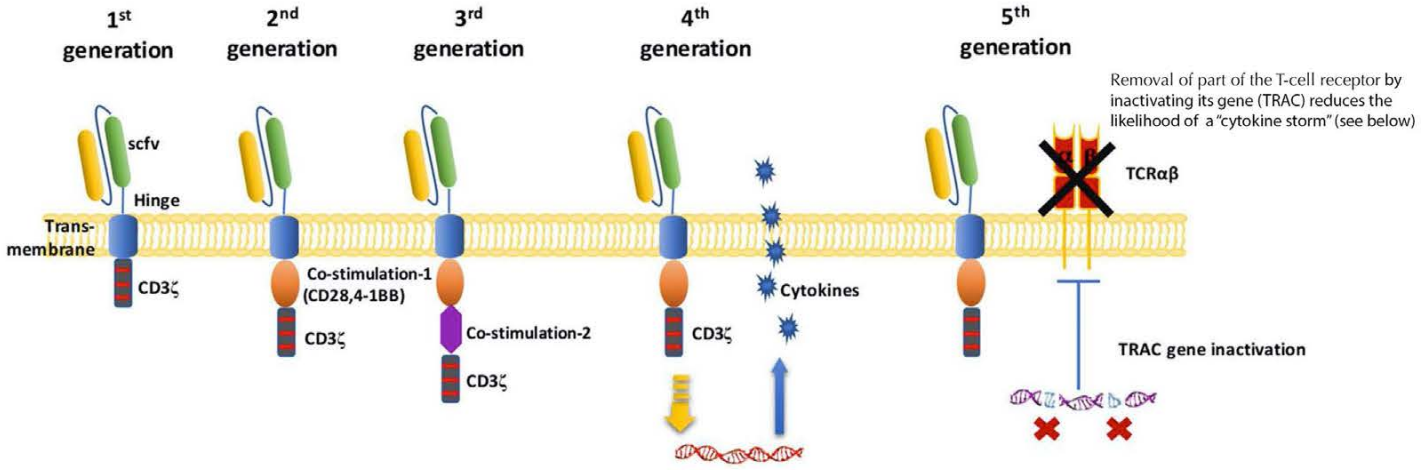
3. **CAR-T Cell Therapy** (another variation on the same general theme)

a) i*n CAR-T cell therapy, the patient's lymphocytes are harvested, but instead of being primed with tumor antigens, engineered genes are introduced that provide them with cell surface receptors that recognize tumor antigens ("chimeric antigen receptors" or CARs); these genetically engineered lymphocytes are expanded in vitro, infused back into the patients, and directly go on to attack cancer cells*





a) CARs are constantly being refined and updated in the hopes of increasing their activity and specificity and/or reducing toxicity



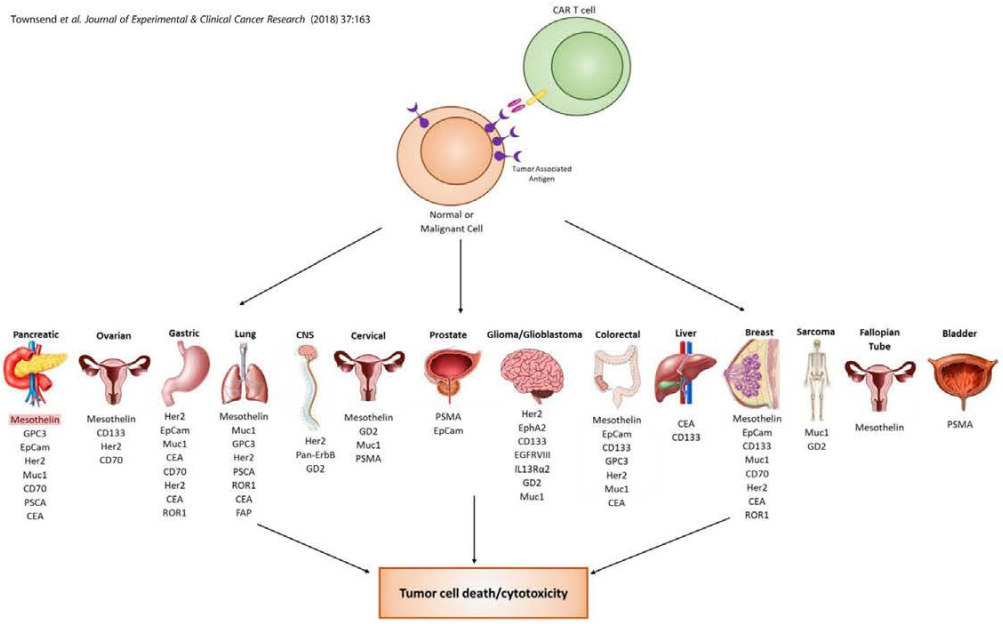
Schematic diagram of the CAR-T cell structure. In the first generation of CARs, there was only one intracellular signal component CD3ζ. The second generation of CAR added one costimulatory molecule on the basis of the first generation. Based on the second generation of CARs, the third generation of CAR added another costimulatory molecule. Fourth-generation of CAR T cells can activate the downstream transcription factor to induce cytokine production after the CAR recognizes the target antigens. The fifth-generation of CARs, based on the second generation, uses gene editing to inactivate the TRAC gene, leading to the removal of the TCR alpha and beta chains. Front Immunol 10:2250. doi: 10.3389/fimmu.2019.02250

b) CAR-T cell therapy is now FDA-approved for the treatment of certain leukemias and lymphomas (note: not solid tumors); the first agent, ***tisagenlecleucel (Kymriah™)*** was approved in August, 2016 – which targets the CD19 antigen found on most B-cell lymphomas – and others have followed, e.g., **axicabtagene ciloleucel (Yescarta™)**

1. as of early 2021, there were more than 500 clinical trials involving CAR-T cell therapies for hematological malignancies, e.g., lymphomas and mutliple myeloma in particular, and less often, leukemias

2. **Will CAR-T cell therapy work with solid tumors, where immune cell access can be a big problem, never mind the immunosuppressive tumor microenvironment?**

a) as more (mostly) tumor-specific biomarkers are identified for particular tumors, they can serve as templates for designing CAR-T cells, and that is already happening (e.g. mesothelin)



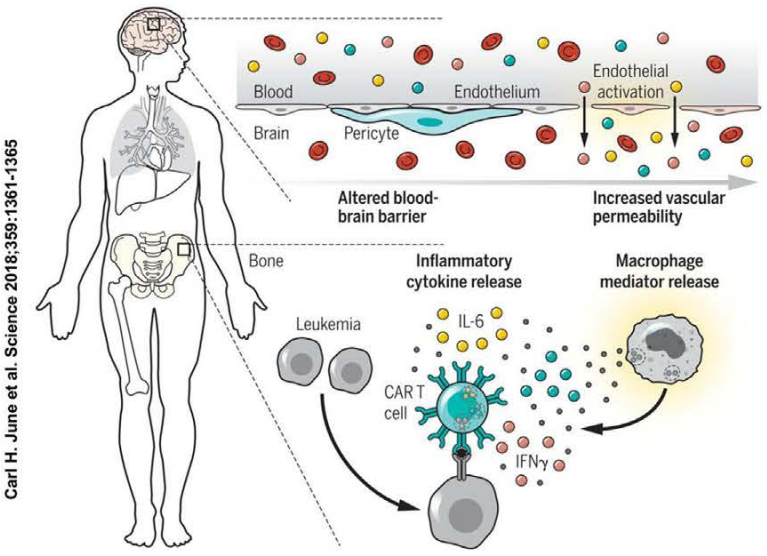
Biomarker targets for solid malignancies. Over 14 different organ types are currently being targeted using a variety of different biomarkers. Many biomarker targets have expression in several different cancer types

b) even if there were a way to normalize the tumor vasculature to allow better access of CAR-T cells and to overcome tumor immunosuppression, ***most remain skeptical at present that CAR-T cell therapy will work for solid tumors (emphasis on "at present")***

c) So what's the biggest downside of CAR-T cell therapy? Answer: *there's not an easy way to shut down the CAR-T cells once they are activated; this leads to multiple possible side effects, many of them quite serious, and not infrequently, fatal*

Note: A drug approved in 2018, **tocilizumab** (Actemra®), helps mitigate CRS

CAR T cell therapy is associated with cytokine release syndrome and neurotoxicity



Neurotoxicity
Delirium
Aphasia
Seizures
Cerebral edema
Intracranial hemorrhage

Hemodynamic instability
Tachycardia
Hypotension
Capillary leak syndrome

Organ dysfunction
AST and ALT elevation
Hyperbilirubinemia
Respiratory failure

Monoclonal Antibodies - at present, the most widely used type of cancer immunotherapy, but are a little different than other approaches in that the antibodies are produced fully outside the body and don't depend on the patient's own immune system for their activity (i.e., they are a form of "passive" rather than "active" immunotherapy)

1. **therapeutic monoclonal antibodies are used either alone ("unconjugated"), or are complexed with a toxin of some type, be it a chemical agent or a radionuclide ("conjugated")**

a) unconjugated antibodies work by one or more of the following mechanisms to destroy cells bearing the target antigen(s):

- by complement-mediated cytotoxicity
- by the blocking of receptor-ligand interactions and the resulting signaling pathway(s)
- by induction of apoptosis

1] **the first anticancer, unconjugated antibody approved for human use by the FDA was Rituximab (in November, 1997), used for treatment of recurrent or refractory B-cell lymphoma of low grade; it targets the CD20 protein, a cell-surface antigen present on healthy B lymphocytes and on 95% of B-cell lymphomas** (note that Rituximab is not truly tumor-specific)

2] many other therapeutic monoclonals have been developed and approved since that time; e.g., herceptin, cetuximab, bevacizumab, etc.

b) **the use of conjugated antibodies has a somewhat different "philosophy"; in this case, the antibody's immunoreactivity** (i.e., does it elicit an immune response and cause destruction of the cell, like unconjugated antibodies do) **is of secondary importance compared to its ability to target the conjugated toxin directly to the cancer cells and kill them that way**

1] different classes of toxins can be conjugated to monoclonal antibodies:

- plant or bacterial toxins (e.g., ricin, diphtheria toxin, or pseudomonas toxin)
- chemotherapy agents (e.g., doxorubicin)
- radionuclides (e.g., β- or α-emitters with short half lives) - discussed later

Monoclonal antibodies approved for cancer therapy as of 2020.

Monoclonal Antibody	Cancer Targeted	Target Molecule	Type of mAb
Alemtuzumab	CLL	CD52	Humanized IgG_1
Bevacizumab	Colorectal, lung cancer	VEGF	Humanized IgG_1
Blinatumomab	ALL	CD19	Mouse
Cetuximab	Colorectal cancer	EGFR	Chimeric IgG_1
Daratumumab	Multiple myeloma	CD38	Human IgG_1
Elotuzumab	Multiple myeloma	SLAM7	Humanized IgG_1
Gemtuzumab	AML	CD33	Humanized IgG_4
Ibritumomab tiuxetan	NHL	CD20	Mouse
Ofatumumab	CLL	CD20	Human IgG_1
Panitumumab	Colorectal cancer	EGFR	Human IgG_2
Pertuzumab	Breast cancer	Her-2/neu	Humanized IgG_1
Ramucirumab	Gastric cancer	VEGFR2	Human IgG_1
Rituximab	NHL, CLL	CD20	Chimeric IgG_1
Tositumomab	NHL	CD20	Mouse
Trastuzumab	Breast cancer	HER-2/neu	Humanized IgG_1

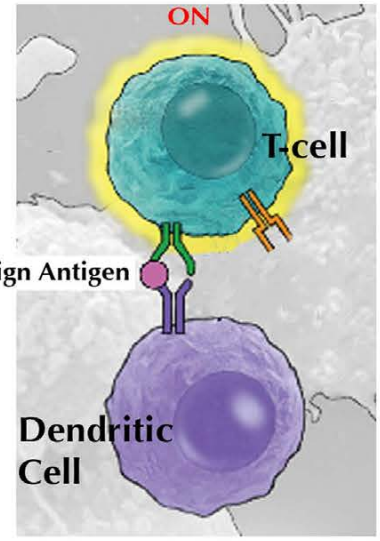
Abbreviations: ADCC, Antibody-dependent cell-mediated cytotoxicity; *ALL,* Acute lymphocytic leukemia; *AML,* acute myelogenous leukemia; *CLL,* chronic lymphocytic leukemia; *EGFR,* epidermal growth factor receptor; *IgG,* immunoglobulin G; *NHL,* non-Hodgkin lymphoma; *VEGF,* vascular endothelial growth factor.

Immune Checkpoint Inhibitors are Unconjugated Monoclonal Antibodies

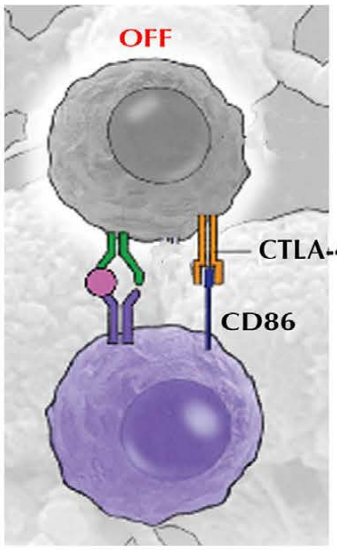
Immune checkpoint inhibitors are antibodies raised against cell surface antigens or receptors that under normal conditions turn T-cells on and off; they allow these immune responses to be fine-tuned to therapeutic advantage

CTLA-4 and **PD-1** are receptors on the surface of T-cells that, when bound to their appropriate ligands (normally located on dendritic cells and/or macrophages) *inactivate* the T-cells, which turns off the immune response

a) ***without*** **the binding of the ligands to their receptors, T-cells remain active and attack cells bearing foreign antigens**



The dendritic cell presents the foreign antigen to the T-cell, and primes it to attack other cells bearing that antigen.

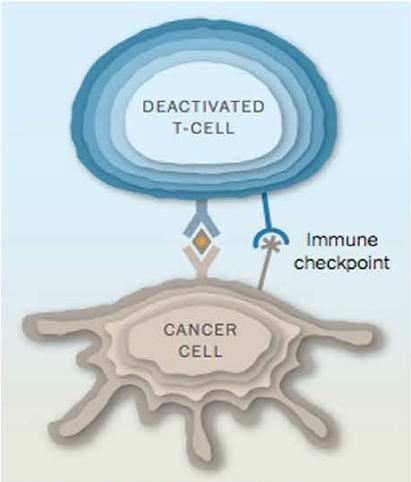


When the job is done, the dedritic cell expresses ligand CD86, which binds to the CTLA-4 receptor on the T-cell and turns it off.

There are many other receptor-ligand interactions that can activate or inactivate T cells, also mediated by dendritic cells, tissue macrophages or Tregs

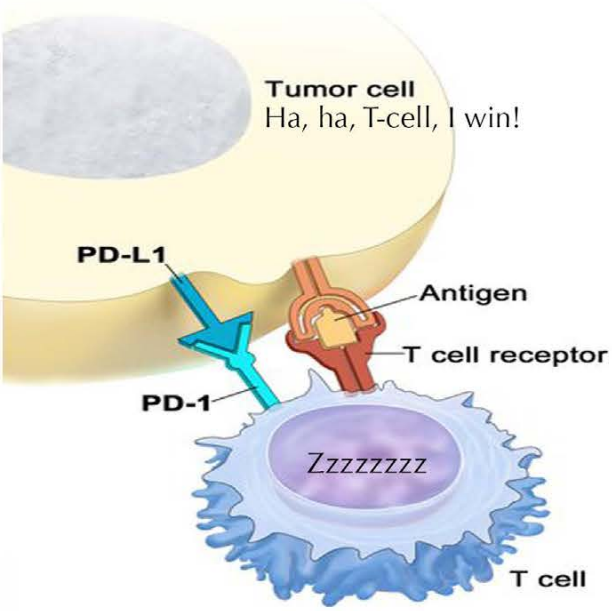
Unfortunately, tumor cells have learned how to express some of the "inactivate" ligands, which is how they are able to evade immune system recognition and T cell activation.

Other cells in the tumor's microenvironment, and in stem cell and metastatic niches, can also express inhibitory ligands.



CANCER AND CHECKPOINTS
Cancer cells can avoid destruction by taking advantage of a switch on the T-cell called an **immune checkpoint**. The checkpoint can shut down the T-cell and suppress the immune response, allowing the cancer to grow undisturbed.

PD-L1/PD-1 binding inhibits T cell killing of tumor cell

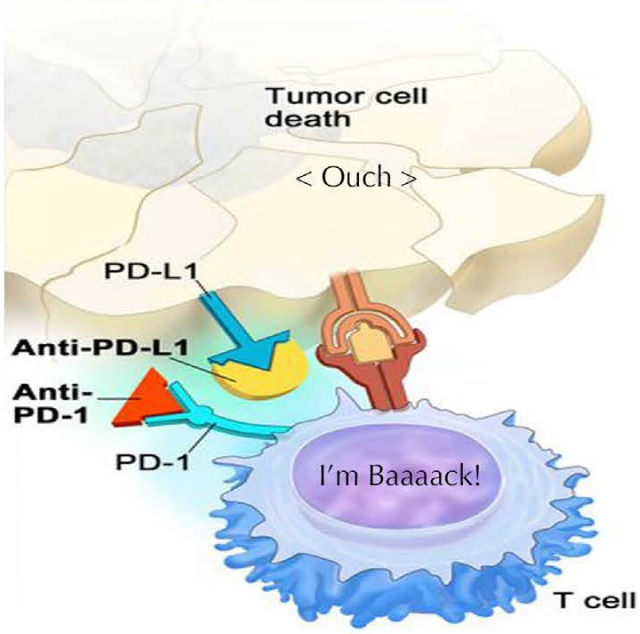


Thus, newer immunotherapy techniques using monoclonal antibodies have been developed to block the T cell receptors for these "inactivate" molecules, keeping T cells active and primed to attack tumor cells.

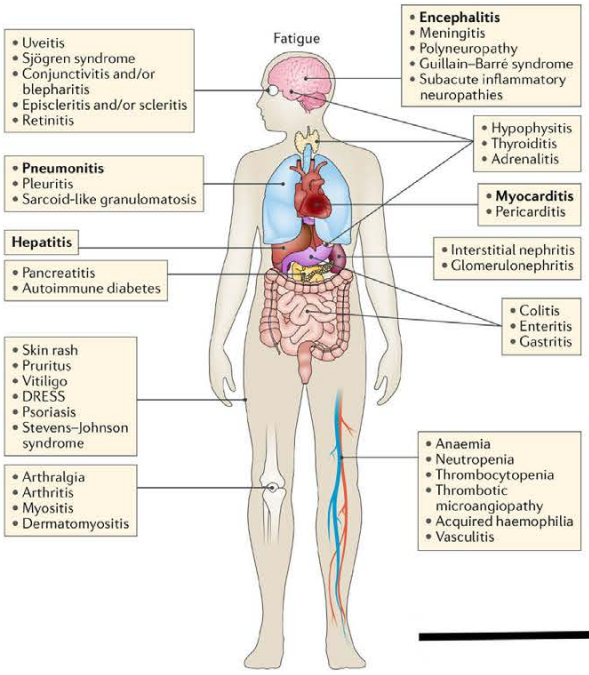
One monoclonal (**ipilimumab - Yervoy®**) blocks the CTLA-4 receptor, which is used normally to shut off T cell activity. Other monoclonals (**pembrolizumab - Keytruda®** and **nivolumab - Opdivo®**), block the PD-1 receptor, thereby preventing tumor cells expressing PD-L1 from shutting down the immune response.

Atezolizumab (Tecentriq®), **avelumab (Bavencio®)** and **durvalumab (Imfinzi®)** target the PD-L1 ligand.

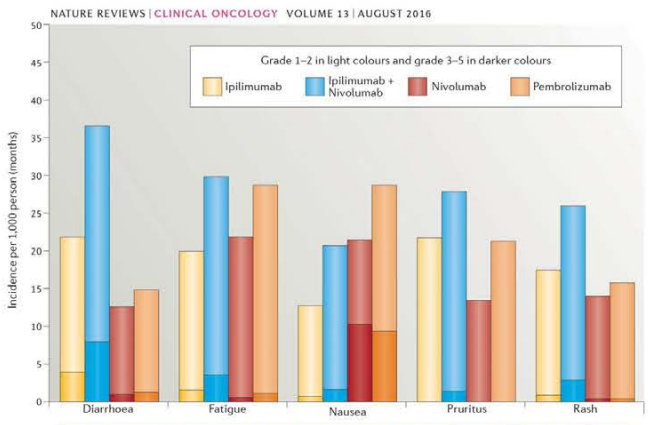
Blocking PD-L1 or PD-1 allows T cell killing of tumor cell



Big problem #1: ***An overactive immune system can cause many different types of side effects, quite a few of which can be severe or even fatal and limit the use of these drugs***

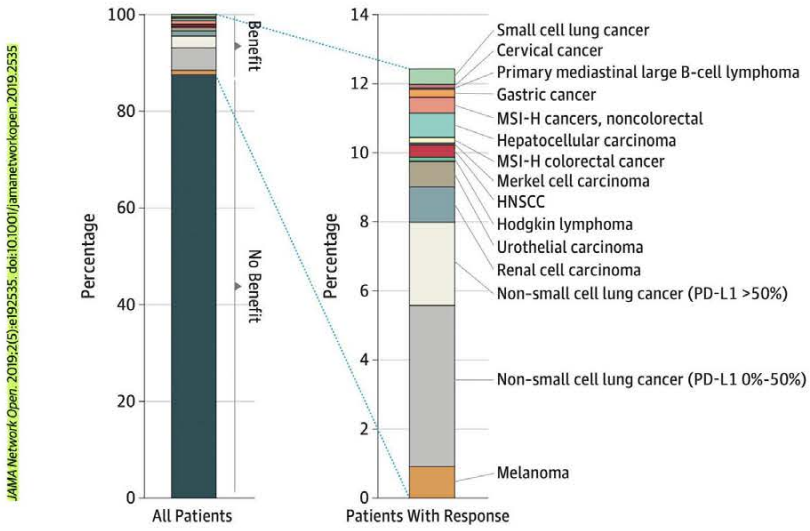


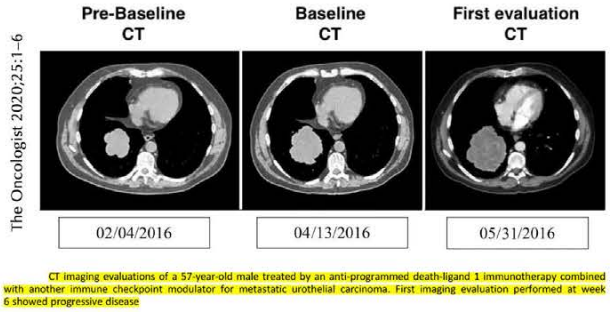
| The spectrum of irAEs by affected organ or organs.



| **The most common adverse events in patients treated with ipilimumab, pembrolizumab, nivolumab, or ipilimumab plus nivolumab.** Incidence per 1,000 person-months.

Big problem #2: ***Checkpoint inhibitors only work in a small subset of patients (and we don't yet know why)***

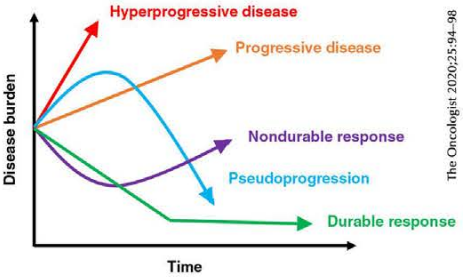




CT imaging evaluations of a 57-year-old male treated by an anti-programmed death-ligand 1 immunotherapy combined with another immune checkpoint modulator for metastatic urothelial carcinoma. First imaging evaluation performed at week 6 showed progressive disease

To date, the tumors most likely to respond to immunotherapy are melanoma, non-small cell lung cancer and renal cell carcinoma. However those responders only make up a little less than 15% of all cancer patients.

To further complicate matters, a small subset of the responders respond the wrong way, i.e., treatment with checkpoint inhibitors elicits **hyperprogression** of the tumor, causing most patients to die within 3-4 months.

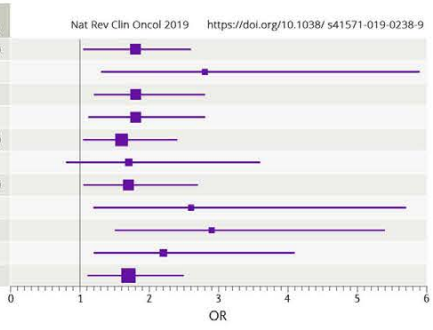


Potential outcomes after initiation of immunotherapy with immune checkpoint inhibitors for the treatment of various cancers over time.

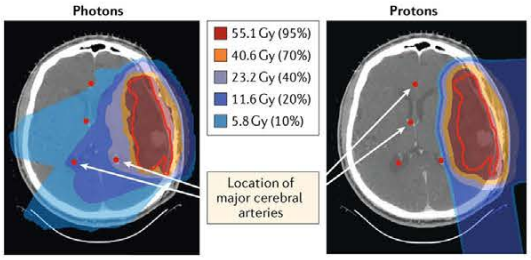
Finally, one caveat for studies in which radiotherapy is combined with immunotherapy:

1. while the hope is that radiation can act like an *in situ* vaccine and stimulate an immune response, it can also be immunosuppressive in that it can readily kill radiosensitive lymphocytes; therefore, if it's possible to avoid irradiating too many blood cells, it could be worth it!

Study	Indication	n	OR (95% CI)
Grossman et al., 2011	High-grade glioma	96	1.8 (1.05–2.6)
Mendez et al., 2016	High-grade glioma	76	2.8 (1.3–5.9)
Rudra et al., 2018	High-grade glioma	210	1.8 (1.2–2.8)
Liu et al., 2017	Nasopharyngeal cancer	413	1.8 (1.1–2.8)
Davuluri et al., 2017	Oesophageal cancer	504	1.6 (1.05–2.4)
Campian et al., 2013	NSCLC	47	1.7 (0.8–3.6)
Tang et al., 2014	NSCLC	711	1.7 (1.05–2.7)
Cho et al., 2016	SCLC	73	2.6 (1.2–5.7)
Balmanoukian et al., 2012	Pancreatic (resectable)	53	2.9 (1.5–5.4)
Wild et al., 2013	Pancreatic (unresectable)	101	2.2 (1.2–4.1)
Chadha et al., 2017	Pancreatic (unresectable)	177	1.7 (1.1–2.5)



One possible example: Radiation-induced lymphopenia is associated with inferior survival across several tumor types (even without adding immunotherapy). Odds ratio >1 means worse outcome.



One interesting suggestion: Use proton radiotherapy for the purposes of avoiding irradiating too many major blood vessels (and therefore, too many blood cells). Same could be said of FLASH radiotherapy, because the entire treatment would be completed so quickly that there'd be almost no blood flow involved.

What's next for checkpoint inhibitors?

1. Combinations - with each other, with radiotherapy, with chemotherapy, and with anti-angiogenics – for both primary and metastatic disease – and how best to accomplish this

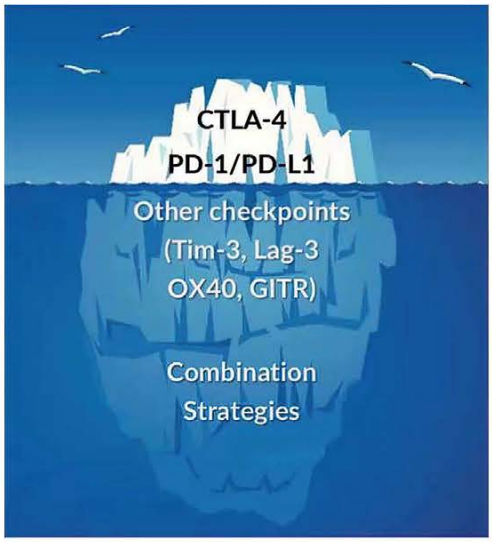
Main ongoing phase III trials with ICI/RT combinations in advanced NSCLC.

Cancers 2021, 13, 678. https://doi.org/10.3390/cancers13040678

Trial Name	Study Phase	Enrollment	Stage	Experimental/Control Arm	Status
NCT03867175	Phase III	116	III	Consolidative immunotherapy with pembrolizumab +/− SBRT after first line systemic therapy	Recruiting
NCT03774732 NIRVANA-lung	Phase III	688	III/IV	Anti PD1 +/− RT 15 d after the beginning of ICI, 18 Gy	Recruiting
NCT03391869 LONESTAR	Phase III	116	IV	Local consolidation therapy: 14 d after nivolumab + ipilimumab for ICI-naive patients with metastatic NSCLC	Recruiting

Main ongoing clinical trials with ICI/RT combinations in advanced HNSCC.

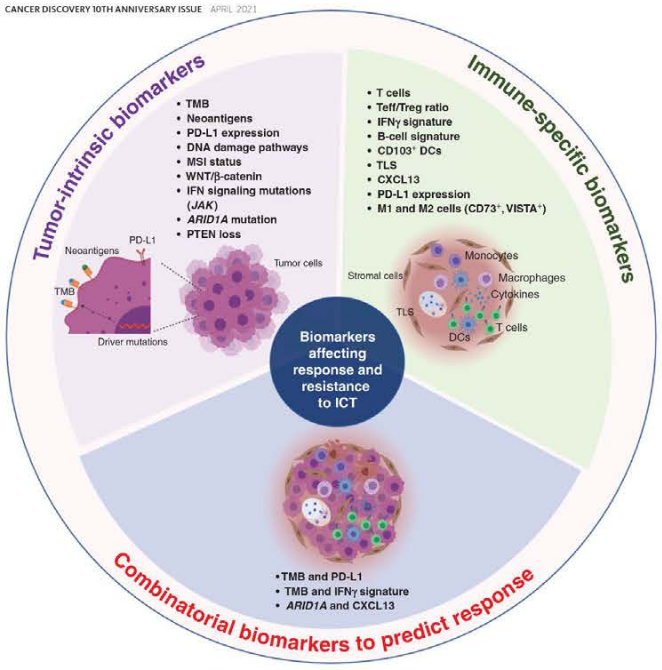
Trial Name	Study Phase	Enrollment	Stage	Experimental/Control Arm	Status
NCT03380394	Phase II	122	III	RT + pembrolizumab vs. RT + cisplatin	Recruiting
NCT03673735	Phase III	650	III	RT + cisplatin +/− durvalumab HPV-negative HNSCC only	Not yet recruiting
NCT02999087	Phase III	688	III	RT + cisplatin vs. RT + cetuximab vs. RT + cetuximab + avelumab	Recruiting
NCT03349710	Phase III	1046	III/IV	RT + cetuximab +/− nivolumab vs. RT + cisplatin +/− nivolumab	Recruiting
NCT03426657	Phase II	120	III	RT + durvalumab + tremelimumab	Not yet recruiting



2. Reducing side effects

Yin-Yang Model of Factors That Lead to Toxicity and Response in Patients Treated With Immune Checkpoint Inhibitors.

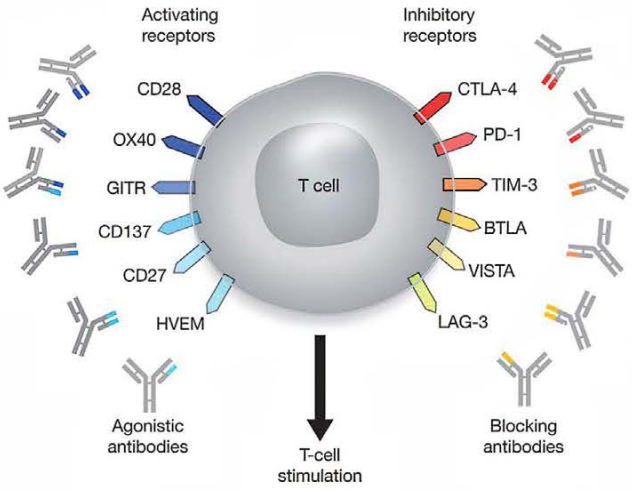
3. Figuring out why only a small subset of patients benefit (and how to predict this)



Need. Robust. Biomarkers.

Biomarkers of response and resistance to ICT. Tumor cell–specific and immune cell–specific biomarkers associated with response and resistance to ICT, and combinatorial biomarkers that may predict response to ICT. DC, dendritic cell; MSI, microsatellite instability; Teff, effector T cell; TLS, tertiary lymphoid structure; TMB, tumor mutational burden; Treg, regulatory T cell.

4. Identifying new immunomodulatory molecular targets



5. Dealing with T-cell exhaustion

a. T-cell "exhaustion" occurs when repeated or excessive exposure to tumor antigens causes a massive up-regulation of PD-1 and CTLA-4; ***over time, T-cells become insensitive to PD-1 signaling, so lose their ability to attack and kill tumor cells*** bearing the antigens

b. this is a particular problem for "hot" tumors, i.e., those that are highly mutated and have tons of antigens to choose from, and therefore would be the best targets for the use of checkpoint inhibitors

1) such tumors are often full of immune cells too...only problem being that many of them are already exhausted

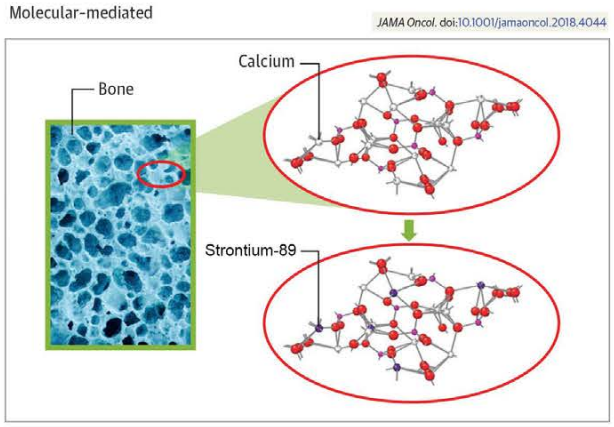
6. Dealing with drug resistance - for example, it has already been suggested that mutations in genes related to the JAK/STAT signaling pathways (which are needed for activated T-cells to produce further immunostimulatory interferons) reduce or eliminate the effectiveness of anti-PD-1 and PDL-1 therapy

Radiopharmaceuticals (aka "Molecular Radiotherapy")

A. Historical Background

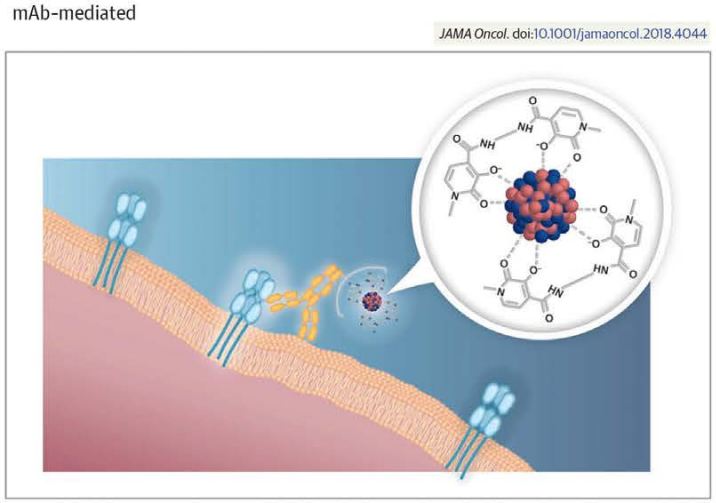
1. the "model" that drove the field of radiopharmaceuticals forward was the use during the 1980's of strontium-89 (**MetaStron**), a radioactive calcium analog, for the palliation of pain caused by bone metastases, and later, by the development and FDA approval of samarium-153 lexidronam (**Quadramet**), a radiopharmaceutical consisting of samarium-153 chelated to a bone-seeking tetraphosphonate

a) in the case of these agents, the "targeting" was achieved by their propensity to be metabolized and incorporated into bone, NOT because they were conjugated to an antibody, however their effectiveness both in relieving pain and shrinking some bone metastases provided compelling evidence that targeting a radionuclide by way of an antibody might work as well, if not better



b) side effects profile of MetaStron and Quadramet? Myelosuppression, because the drugs are incorporated into bone and incidentally irradiate bone marrow cavities in addition to the bone mets themselves; this problem was worse the longer the radionuclide's half life, both biological and physical (major reason Quadramet ultimately replaced MetaStron...half life of strontium-89 was 90 days, compared to samarium-153's of 2 days)

2. the first radiolabeled antibodies approved for radioimmunotherapy of cancer, in 2002 and 2003, respectively, were **ibritumomab tiuxetan (Zevalin)** and **tositumomab (Bexxar)**, used to treat certain types of non-Hodgkin lymphoma refractory to standard treatments; both are targeted to the CD20 cell surface antigen on B lymphocytes

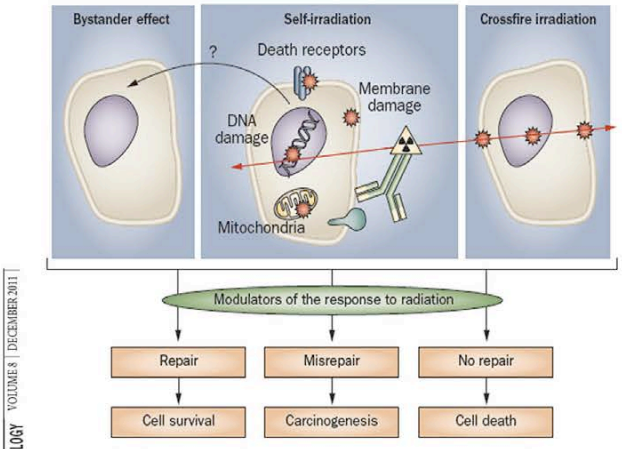


a) *Zevalin is conjugated to the radionuclide yttrium-90 (a β- and γ-emitter, half life of 2.7 days), and Bexxar to iodine-131 (β-emitter, half life of 8 days)*

an extra *advantage to radionuclide conjugates is that they can produce collateral damage by lethally irradiating other, nearby cancer cells, effectively giving them "more bang for the buck"; this has been termed the Cross-Fire Effect, and depends on a number of factors including the type of radionuclide used (i.e., an α- versus β-emitter), its concentration, activity, half life and the average dose rate, and the uniformity of its distribution in the target tissue (usually not uniform)*

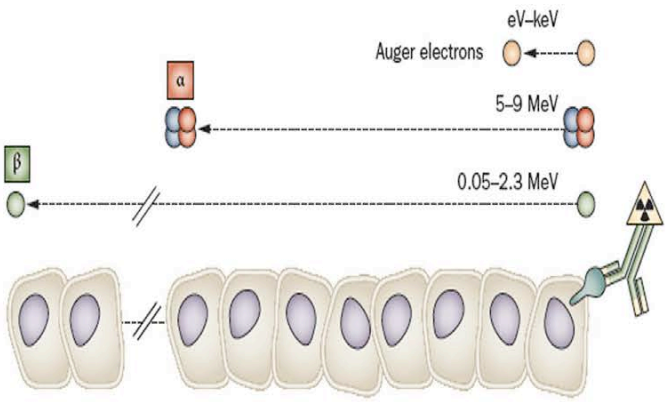
List of Radionuclides for Radiotherapy and Imaging

Sealed sources		Origin	Energy	Half-life
^{226}Ra	Radium	Uranium decay (^{238}U)	0.83 MeV gamma	1601 years
^{222}Rn	Radon	Uranium decay (^{226}Ra)	0.83 MeV gamma	2.7 days
^{198}Au	Gold	Neutron bombardment	0.411 MeV gamma	2.7 days
^{192}Ir	Iridium	Neutron bombardment	0.38 MeV gamma	74 days
^{137}Cs	Cesium	Fission by-product	0.662 MeV gamma	30 years
^{131}Cs	Cesium	Fission by-product	30 keV X-ray	9.7 days
^{125}I	Iodine	Neutron bombardment	28 keV X-ray	60 days
^{103}Pd	Palladium	Neutron bombardment	21 keV X-ray	17 days
^{60}Co	Cobalt	Neutron bombardment	1.25 MeV gamma	5.26 yrs
Unsealed sources		**Origin**	**Energy**	**Half-life**
^{223}Ra	Radium	Uranium decay (^{235}U)	6 MeV alpha	11.4 days
^{153}Sm	Samarium	Neutron bombardment	810 keV beta−	47 hr
^{177}Lu	Lutetium	Neutron or proton bombardment	490 keV beta−, 210 keV gamma	6.7 days
^{131}I	Iodine	Fission by-product	606 keV beta−, 364 keV gamma	8 days
^{90}Sr	Strontium	Fission by-product	546 keV beta−	29 yrs
^{90}Y	Yttrium	Daughter elution (^{90}Sr)	940 keV beta	50 days
^{89}Sr	Strontium	Neutron bombardment	583 keV beta−	50.5 days
^{32}P	Phosphorous	Neutron bombardment	695 keV beta−	14 days
Imaging nuclides		**Origin**	**Energy**	**Half-life**
^{123}I	Iodine	Proton bombardment	159 keV gamma	13 hr
^{111}In	Indium	Proton bombardment	208 keV gamma	2.8 days
^{99m}Tc	Technetium	Daughter elution (^{99}Mo)	140 keV gamma	6 hr
^{64}Cu	Copper	Daughter elution (^{64}Zn)	653 keV beta+	12.7 hr
^{18}F	Fluorine	Proton bombardment	630 keV beta+	110 min
^{15}O	Oxygen	Proton bombardment	1.73 MeV beta+	2 min
^{11}C	Carbon	Proton bombardment	960 keV beta+	20 min
^{3}H	Tritium	Neutron bombardment	19 keV beta−	12 yrs
^{68}Ga	Gallium	Daughter elution (^{68}Ge)	1.9 MeV beta+	68 min



NATURE REVIEWS | CLINICAL ONCOLOGY VOLUME 8 | DECEMBER 2011

Radiobiology of radioimmunotherapy. As a consequence of the application of radioimmunotherapeutic agents, targeted cells die owing to self-irradiation, cross-fire irradiation, or bystander effects. Radiation-sensitive targets in cells include the DNA, cytoplasm, and the membrane lipids ('lipid peroxidation'). Growth factors, death receptors, and several enzymes can also be activated by radiation and trigger downstream signaling pathways. The cellular response to radiation may be modulated by therapeutic combinations of different drugs. Depending on whether cells can repair radiation-induced lesions, the outcome is either cell survival, carcinogenesis, or cell death.



Track length of alpha-particles, beta-particles, and Auger electrons relative to the cell diameter. The energy of particles released through radioactive decay covers several orders of magnitude (from few eV to 2.3 MeV). The track path-length varies between particles and corresponds to the diameter of a single cell for Auger electrons, to several cell diameters for alpha-particles, and to up to one hundred cell diameters for beta-particles.

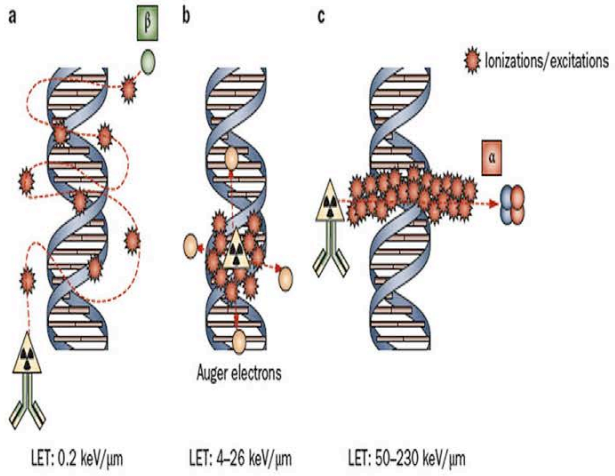
B. OK, so what about the radiobiology of radiopharmaceuticals? In fact, what about the dosimetry? Come to think of it, what dose are we even delivering?

1. frankly, there are no easy answers to these questions, because so many different factors would influence the results

a) reasonable guesses:

1] the doses locally (at the cellular level) are probably really, really high...although the average dose rate is probably fairly low, which should reduce the biological effectiveness of the low(er) LET β-emitters, but not the high LET α-emitters

2] figuring out the actual dosimetry (micro- or macro-) is REALLY tricky, especially since there will be so-called "direct dose" to the target cell and "cross-fire dose" from the nuclides bound to neighboring cells...and that different cells have different numbers of conjugated antibodies bound to them based on how many surface antigens they possess...and that all the emissions from the radionuclides have different ranges...and that the dose rate decreases over time...and that the cells aren't necessarily all the same size or packed at the same density in the tumor tissue...and that the antibodies also have a biological half life as well as their nuclides having a physical one...and that the antibodies likely can't get to all the places they should be getting



Patterns of cellular damage caused by radiation sorted by LET. **a** | Radionuclides undergoing beta-decay emit low LET radiation that produces sparse ionizations and excitations within DNA along a contorted track, resulting in individual DNA lesions that are easily repairable. **b** | Cascades of Auger electrons (with intermediate LET). Auger electrons emitters can be used for labeleling of DNA base analogs and emit cascades of electrons that produce densely localized ionizations and excitations within DNA, inducing poorly repairable damage. In the context of radioimmunotherapy, ionizations localize mainly at the cell membrane if the antibody binds to cell-surface antigen. However, ionizations are found mainly in the cytoplasm if antibodies are taken up by the cell. **c** | Alpha-particles with high LET produce densely localized ionizations and excitations along a linear track, resulting in locally, multiple damaged sites that are poorly repairable.

C. Future Directions: **Theranostics** *(aka, agents that allow you to see what you treat)*

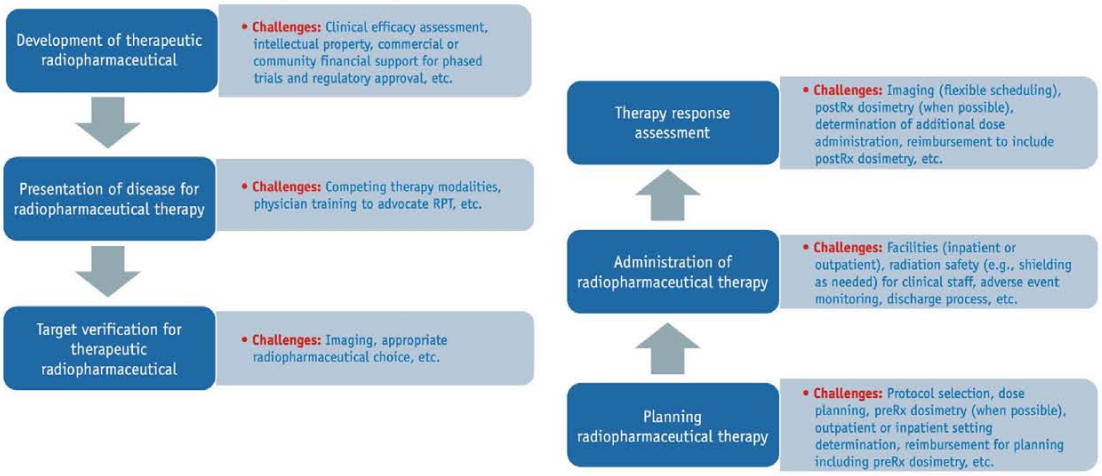
1) theranostics (def.) = *an approach to personalized medicine in which an element of disease diagnosis can also serve a therapeutic function*

a] one of the earliest example of this was the HercepTest® immunohistochemical assay that identified breast cancers that specifically overexpress the HER-2 cell surface receptor; the same antibody used for this diagnostic application then served as the model for trastuzumab, the targeted therapeutic for HER-2 positive breast cancer

2) many theranostics are conjugated monoclonal antibodies, however there are also ones that are small molecule inhibitors, metabolic intermediates, nanoparticles, quantum dots, gold or other metal conjugates, etc.

3) currently, most theranostics in use are complexed with a radionuclide for the imaging and therapeutic components, but this is not an absolute requirement, i.e., in theory, the entire electromagnetic spectrum could be used, at least for the diagnostic part

4) at present, what stands in the way of theranostics becoming more mainstream?



Lancet Oncol 2020; 21: e146–56

Summary of challenges associated with implementation of radiopharmaceutical therapy (RPT) and a treatment-planning approach to its delivery.

Appendix Materials: Immunotherapy

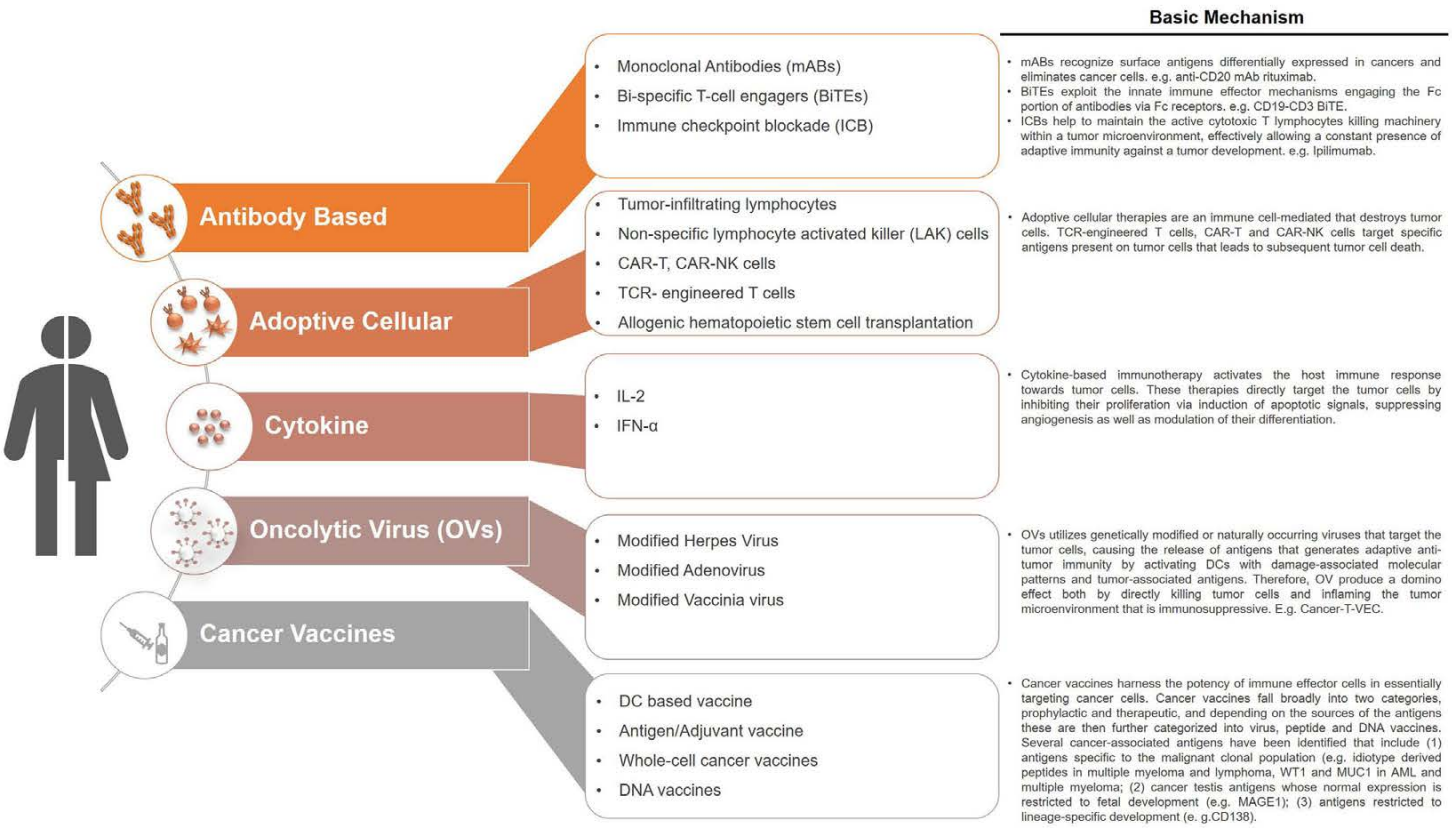
G.W. Tormoen et al Advances in Radiation Oncology

Mechanisms of immunosuppression in the tumor microenvironment

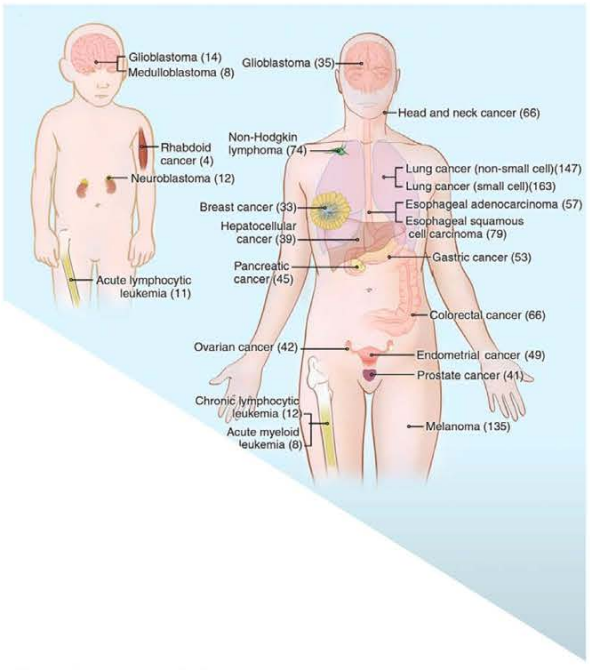
Mediator	Mechanism of immunosuppression
Cell surface proteins	
Programmed death-ligand 1	Induce T-cell tolerance/anergy after ligation with programmed cell death protein 1 on T cells
CTLA-4	Inhibit activation of naïve T cells
	Enhance regulatory T cell function
↓Major histocompatibility complex I	Avoid detection by effector CD8 T cells
↓FAS	Avoid FAS ligand–mediated cell killing
↓TRAIL	Avoid TRAIL-mediated cell killing
CD39/CD73	Convert extracellular immunostimulatory adenosine triphosphate to immunosuppressive adenosine
Secreted cytokines	
Transforming growth factor beta	Inhibit T cell priming and infiltration
	Suppress effector cell cytotoxicity
Vascular endothelial growth factor	Inhibit dendritic cell maturation
	Enhance programmed cell death protein 1/programmed death-ligand 1/2 expression
	Enhance interleukin-10 secretion
Interleukin-10	Inhibit major histocompatibility complex II expression on antigen presenting cells
	Suppress M1 cytokine secretion
	Suppress iNOS (inducible Nitric Oxide Synthase)
	Induce T cell anergy
Metabolic pathways	
Indoleamine-2,3 dioxygenase	Convert tryptophan to kynurenine
	Inhibit T cell proliferation
Adenosine	Inhibit T cell proliferation and activation
Hypoxia	Inhibit effector T cell function
	Promote prostaglandin E2 synthesis
Lactate	Inhibit effector T cell function
Arginase	Degrades L-arginine needed for cytotoxic iNOS production
Prostaglandin E2	Inhibit effector T cell function
	Suppress M1 cytokine secretion
	Recruit myeloid-derived suppressor cells

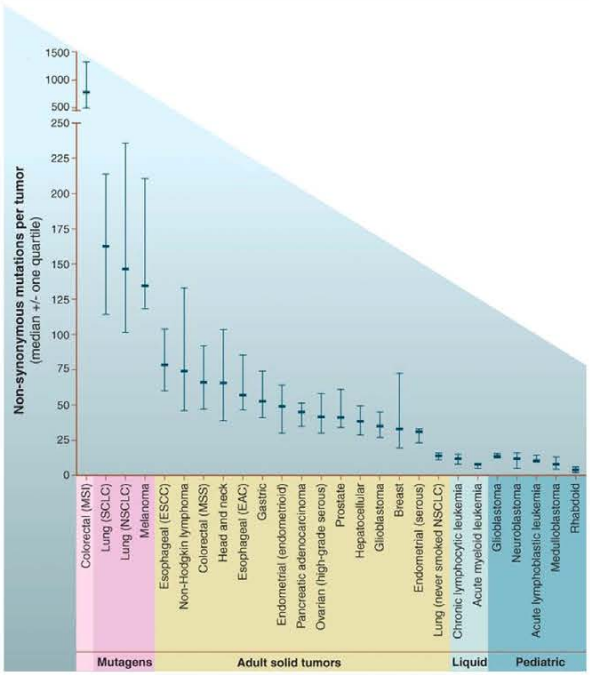
Abbreviations: CD = cluster of differentiation; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand.

Different Types of Immunotherapy Summarized



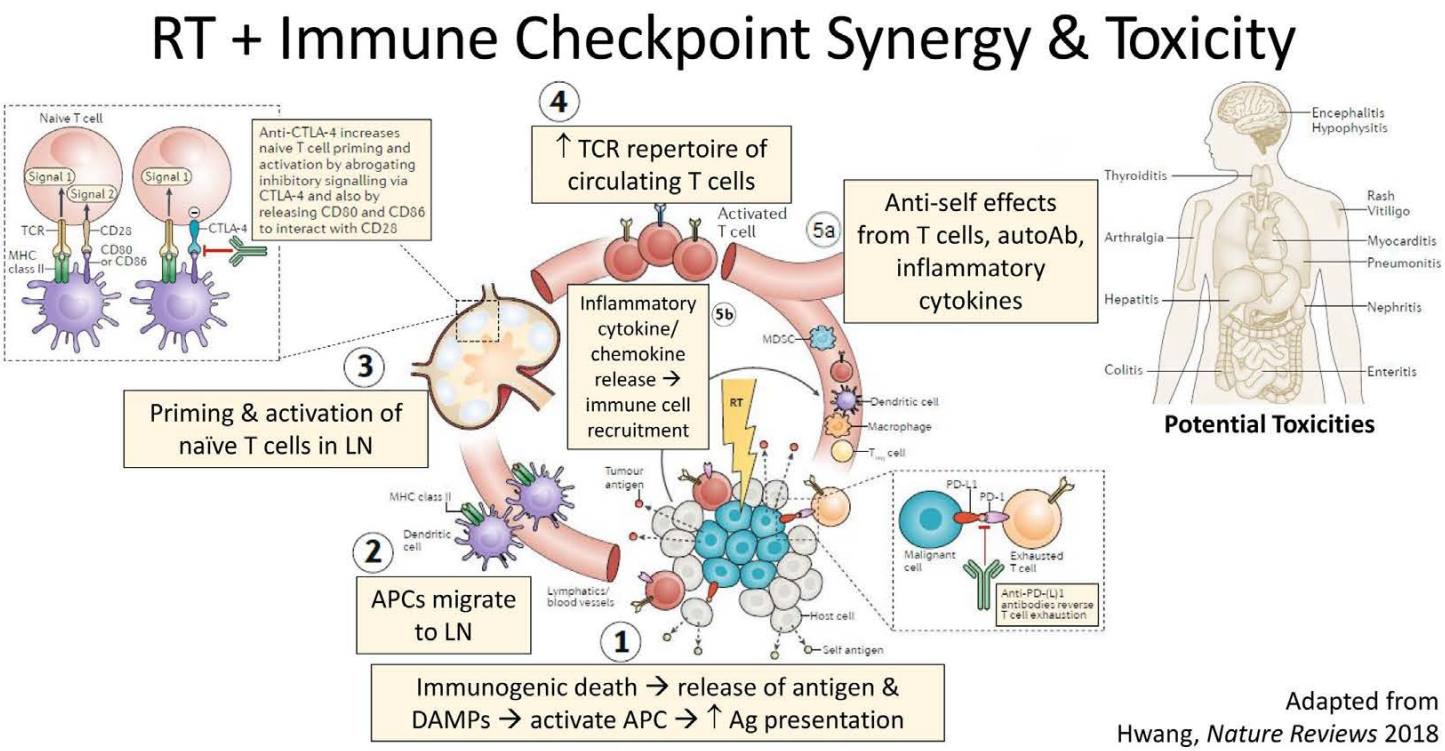
Immunotherapy seems to show greater efficacy the more mutations the cancer cells contain





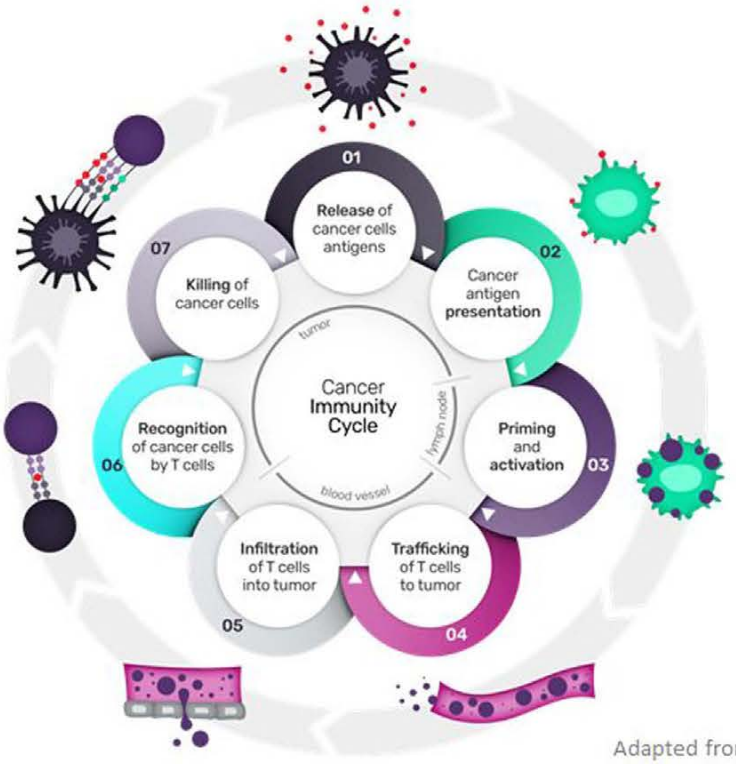
Vogelstein B et al. *Science* 2013

Deeper dive into how radiotherapy can act as an immuno-stimulant "vaccine"



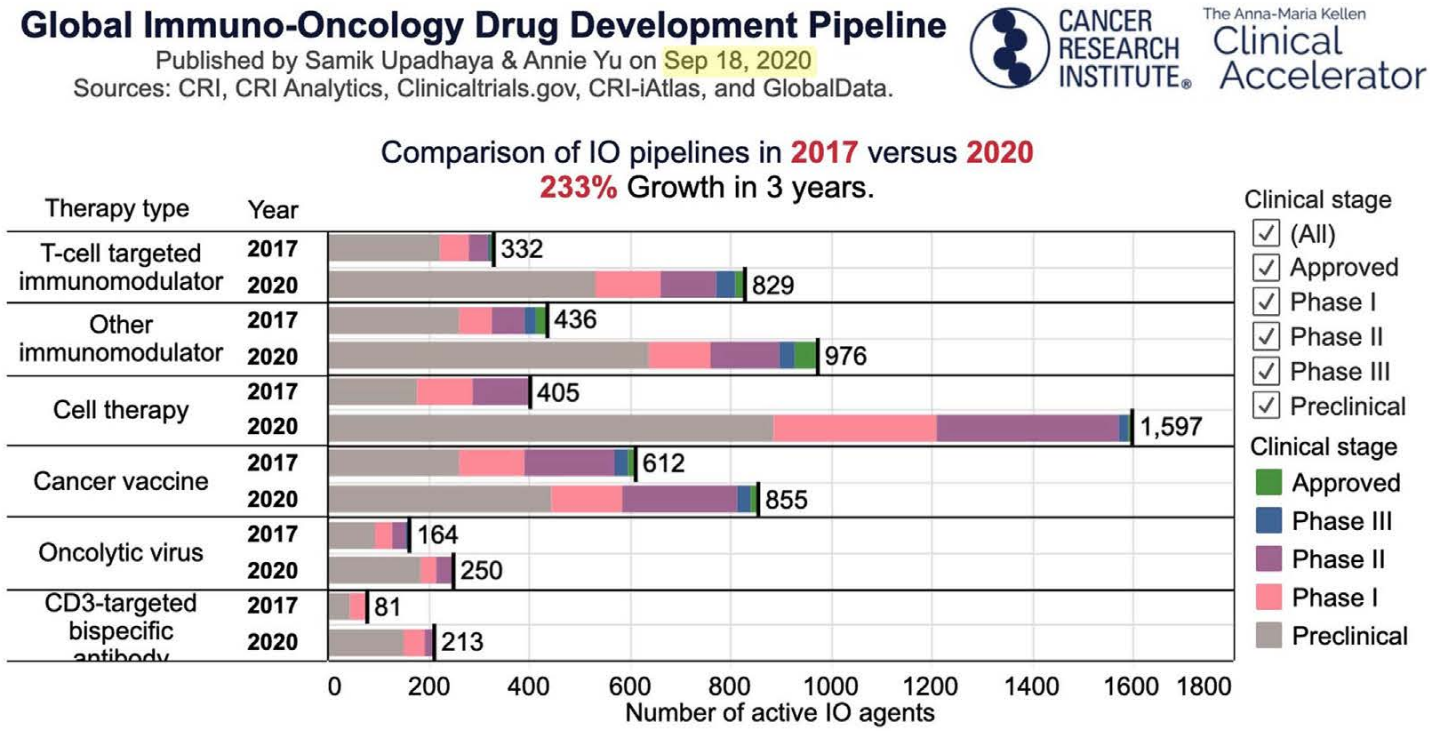
Adapted from
Hwang, *Nature Reviews* 2018

The Cancer Immunity Cycle - different molecular events in the process translate into different opportunities where immunotherapy could make a difference

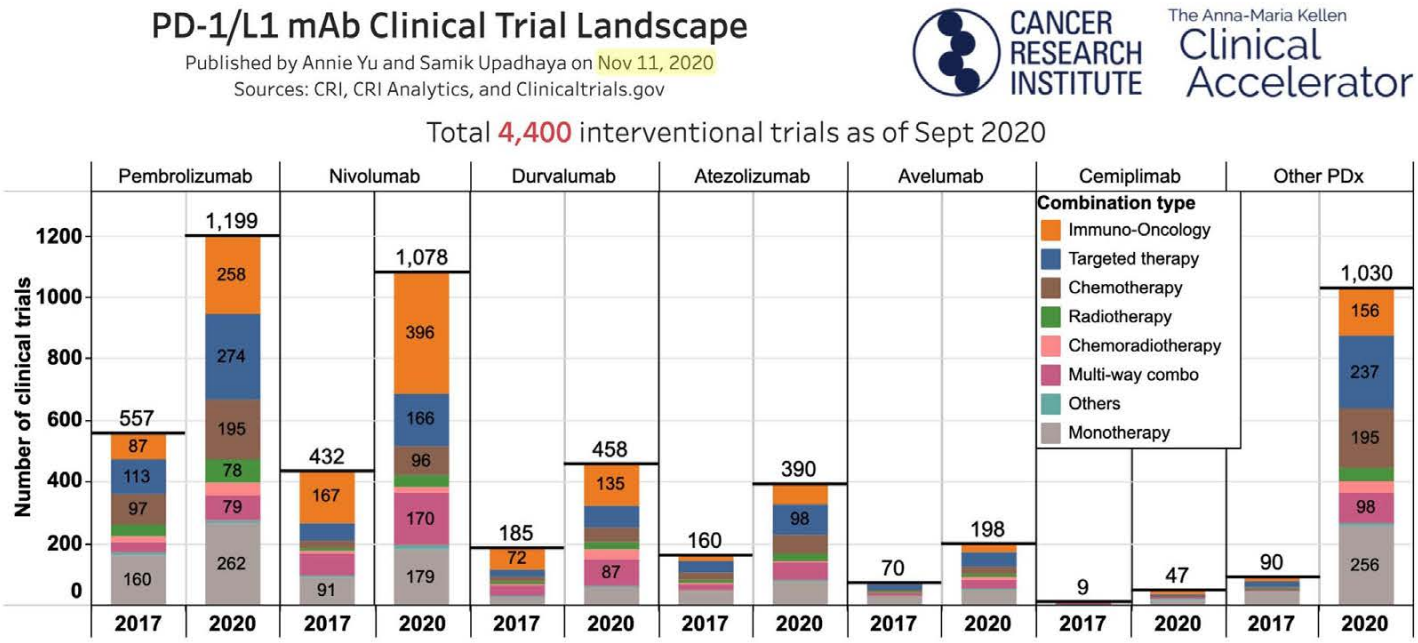


Adapted from
Chen and Mellman, 2013

Current status of clinical trials for different types of immunotherapies



Ongoing clinical trials of immunotherapy in combination with other modalities



Antibody Naming Conventions

Identifiers are used as infixes preceding the -mab suffix, for example:

-omab = mouse
-ximab = chimera
-zumab = humanized
-umab = human

The general disease state or target (infix 1) is next forward, the following is a partial sampling:

viral = vir-
immune = lim-
cardiovascular = cir-
interleukins = kin-
tumor = tum-

Although alternative infixes exist for specific tumor types, the breadth of targets for one antibody is often wider than originally conceived. In practice, therefore, most cancer antibodies have been assigned the general -tum- infix. The target or disease infix is combined with the source infix and suffix, as follows:

-tum- -u- -mab = panitumumab (i.e., "anti-tumor human antibody")

When combining, the last consonant of the target/disease syllable may be dropped for ease of pronunciation, for example:

-cir- -xi -mab = abciximab
-lim- -zu -mab = daclizumab
-tum- -zu -mab = trastuzumab

If the antibody is radiolabeled or conjugated to a chemical or toxin, this conjugate is identified with a separate word or chemical designation (e.g., chemical conjugate: gemtuzumab ozogamicin).

Antibody toxins include the "-tox" suffi x as part of the name selected for the toxin (e.g., zolimomab aritox, in which aritox indicates ricin α-chain).

For radiolabeled products, the word order is as follows: name of the isotope, element symbol, isotope number, and name of the mAb (e.g., Tc^{99m} biciromab). A separate name is also assigned if a linker/chelator is used to conjugate the antibody to a toxin or isotope (e.g., In^{111} satumomab pendetide), or for pegylated antibodies (enlimomab pegol).